International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Development and Validation of Stability Indicating by UV Spectrophotometric Method for Determination of Ketoconazole in Both Bulk and Marketed Dosage Formulation

Megha V. Hange[1], Owais Tamboli[2], Mahaveer Tathed[3], Tamamnna Shah[4], P. N. Sable[5], Rajnandini Yewale[6]

[1]Assistant Professor, Siddhi College Of Pharmacy
[2,3,4,6]Student, Siddhi College Of Pharmacy
[5]Principal, Siddhi College Of Pharmacy

ABSTRACT

Title: Development and Validation of Stability indicating by UV Spectrophotometric method for Determination of Ketoconazole in both bulk and marketed dosage formulation

Background: Fungal infections have become a major public health problem and are growing in number and severity over several decades. Ketoconazole is a broad-spectrum antifungal agent that belongs to BCS class II drug.

Aim: In this study, a simple, rapid, accurate, precise and sensitive UV-visible spectrophotometric method has been developed and validated for the determination of ketoconazole in Pharmaceutical Tablet Formulation

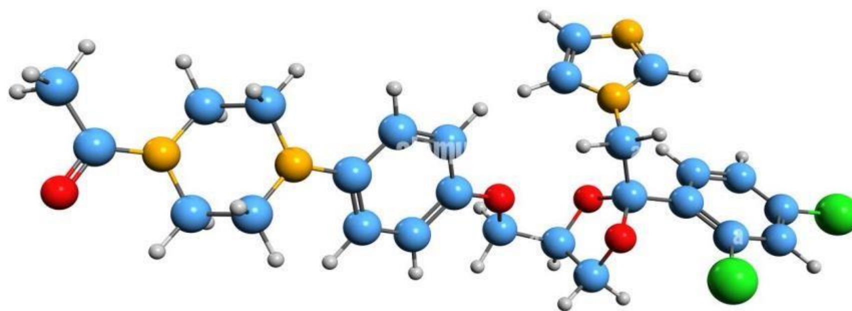
A UV spectroscopic method which is simple, accurate and rapid is developed for the determination of ketoconazole in pharmaceutical formulations as active substance in tablets, shampoos and cream. The absorption maxima of ketoconazole solutions in methanol are recorded at 208 nm. The linearity for ketoconazole in methanol with correlation coefficient values higher than 0.999 is found in the range of 0.005–0.025 mg/ml at 208 nm, R^2 = 0.9996 and 0.05–0.25 mg/ml at 296 nm, R^2 = 0.9996. The limit of detection (LOD) and the limit of quantification (LOQ) accounts to 0.000597 mg/ml and 0.00181 mg/ml at 208 nm and 0.00647 mg/ml and 0.01963 mg/ml at 210 nm, respectively. The intra and interday assay is within 2% relative standard deviation. The obtained results for tablets, cream and shampoos are in good agreement with their respective product, label claims. The developed method can be successfully applied for the purpose routine analysis of ketoconazole in pharmaceutical formulations.

Keywords: Antifungal drug, Formulations, Ketoconazole, UV spectroscopy

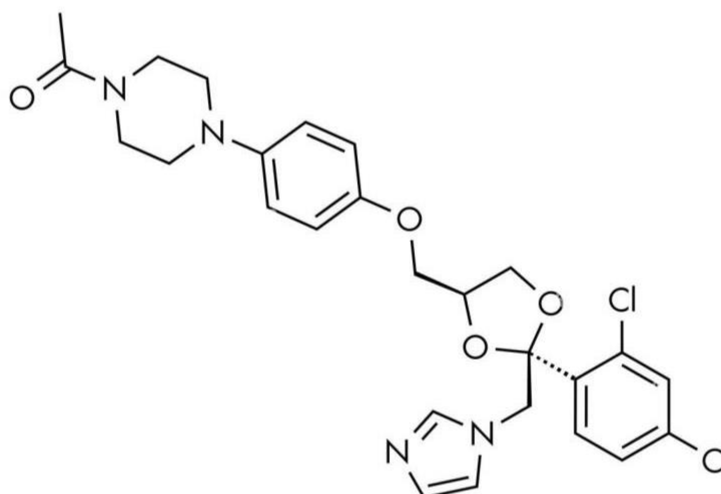
INTRODUCTION

Ketoconazole (cis-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1ylmethyl)-1,3-dioxolan-4-yl] methoxy] phenyl] piperazine) is a lipophilic imidazole derivative appears as white to off white crystalline powder.The drug is practically insoluble in water, soluble in strong bases and sparingly soluble in strong

acid. Ketoconazole is a weak base with pKa values of 6.51 and 2.94, contains two nitrogen atoms in the five-membered azole ring.



3-D Structure of Ketoconazole



Ketoconazole is a chiral drug administered as a racemic (1:1) mixture of enantiomers of the cis configuration. It is a broad-spectrum antifungal imidazole agent that has been shown to be efficient in the treatment of human systemic fungal infections, against Candida species, Cryptococcus neoformans, histoplasmosis, tinea corporis, tinea cruris, tinea manuum and tinea pedis, onychomycosis, seborrheic dermatitis, possessing anti-inflammatory and some antibacterial activities with topical and systemic action.

Ketoconazole undergoes very easily on chemical degradation. The stressed degradation of ketoconazole drug substance into imidazole and acetamide radical species were performed under acid, base, thermal, photo and oxidative stress conditions. The potency losing and forming harmful degradation products are the most common consequences of the degradation of the drug.

Ketoconazole was formulated into several pharmaceutical forms through various routes of administration such as: tablets, topical creams, ointments, gels and antidandruff shampoo .The role of ketoconazole as tablet has been limited to few indications due to the variable bioavailability, liver toxicity and inhibition of steroid biosynthesis. Nowadays, no specific evidence of development of resistance or cross-resistance of fungi to ketoconazole used in cosmetic dandruff shampoo at concentrations up to 2 %.

The mechanism of attacks and changes in the structure and function of the cell membrane permeability is the most important factor in the ketoconazole action of the fungi. The primary mechanism of action of ketoconazole as an azole derivative is the inhibition of sterol 14-α-demethylase, a microsomal cytochrome P450 dependent enzyme system which converts lanosterol into ergosterol required for fungal cell membrane synthesis. Ketoconazole prevents the converting of the lanosterol in ergosterol and disrupts the integrity of membrane-bound enzymes and fungal cell membranes. This ketoconazole action increases membrane permeability and the leakage of small ions, amino acids and proteins from the fungi, leading to cell death.The use of the ketoconazole as a drug essential in pharmaceutical formulations highlights the requirement for its determination and quantification with appropriate analytical methods. This paper gives an overview of the analytical techniques that are available and nowadays have been used for determination of ketoconazole in pharmaceutical and biological samples.

THEORY OF UV SPECTROSCOPY

Overview of UV spectroscopy: UV spectroscopy, sometimes referred to as ultraviolet-visible spectroscopy, is a method used to investigate how matter interacts with light in the visible and ultraviolet portions of the electromagnetic spectrum. It involves calculating how much light at different wavelengths a sample absorbs.

UV spectroscopy measures the amount of light absorbed by a sample by passing it through a beam of UV or visible light. The resulting spectrum, known as a UV Vis spectrum, plots the absorbed light's intensity against wavelength.

In analytical chemistry, UV spectroscopy is frequently used to identify and measure compounds since various molecules absorb light at different wavelengths. The structures and operations of proteins, nucleic acids, and other biomolecules are studied using it in biochemistry as well.

Since the excitation of electrons to higher energy levels is correlated with light absorption in the UV and visible regions, UV spectroscopy can yield valuable information on the electronic structure of molecules. The generated spectra can be used to pinpoint a substance's functional group and estimate how much of it is present in a sample.

UV spectroscopy has several uses in the food and beverage business, including as quality control, environmental analysis, and medication development. UV spectroscopy is a popular, adaptable method with a wide range of real-world uses in both research and business.

APPLICATION OF KETOCONAZOLE

Serious fungal or yeast infections such as histoplasmosis (Darling's disease), blastomycosis (Gilchrist's disease), coccidioidomycosis (Valley fever, San Joaquin Valley fever), paracoccidioidomycosis (South American blastomycosis, Lutz-Splendor-Almeida disease), or candidiasis (thrush, oral thrush) can all be treated with ketoconazole. This medication functions by either eliminating the yeast or fungus or stopping its growth.

In addition, ketoconazole is used to treat people who are unable to take griseofulvin or topical medication for fungal infections of the skin (such as ringworm or athlete's foot).

USES OF KETOCONAZOLE

Ketoconazole is used to treat yeast or fungal infections. It functions by either eliminating the yeast or fungus or stopping its growth. The following conditions are treated with ketoconazole cream: athlete's foot

(tinea pedis; foot ringworm). Ringworm of the body (tinea corporis), ringworm of the groin (tinea cruris; jock itch), and athlete's foot (tinea pedis); Dermatitis seborrheic; "Sun fungus" (tinea versicolor; pityriasis versicolor); and Cutaneous candidiasis, a yeast infection of the skin.
Scaly patches on your skin or scalp are caused by seborrheic dermatitis, which is treated with ketoconazole foam or gel. Dandruff is addressed with shampoo containing 1% ketoconazole. The treatment for "sun fungus" (tinea versicolor; pityriasis versicolor) is ketoconazole 2% shampoo.
Depending on what your doctor determines, this medication may also be used for various fungal infections of the skin.

Common Application of Ketoconazole

Antifungal Topical

For fungal infections of the skin and mucous membranes, such as athlete's foot, ringworm, candidiasis (yeast infection or thrush), jock itch, and tinea versicolor, topically applied ketoconazole is typically given. Topical ketoconazole is also used to treat seborrheic dermatitis on other parts of the body and dandruff (seborrheic dermatitis of the scalp). It is possible that topical ketoconazole works in both disorders by reducing the amount of the fungus Malassezia furfur on the skin.

Systemic Antifungal

Ketoconazole is used orally in dosages of 200 to 400 mg per day in the treatment of superficial and deep fungal infections.

Hair loss

To treat androgenic alopecia, ketoconazole shampoo has been used off-label in combination with an oral 5α-reductase inhibitor like finasteride or dutasteride.
Ketoconazole's antifungal qualities were hypothesized to lower scalp microbiota, which may in turn lessen follicular inflammation, a factor in alopecia.

Breastfeeding

There aren't enough studies on women to assess the harm to the unborn child when taking this medicine while breastfeeding. Before using this drug whilst breastfeeding, balance the possible advantages against the potential disadvantages.

Cream

Ketoconazole cream is used to treat tinea corporis (ringworm; fungal skin infection that causes a red scaly rash on different parts of the body), tinea cruris (jock itch; fungal infection of the skin in the groin or buttocks), tinea pedis (athlete's foot; fungal infection of the skin on the feet and between the toes), tinea versicolor (fungal infection of the skin that causes brown or light coloured spots on the chest, back, arms, legs, or neck), and yeast infections of the skin.

LITERATURE REVIEW

Andreia Bento-Oliveira , Radosław Starosta , Rodrigo F.M. de Almeida(2024). Fungal infections are a serious health threat, especially for patients with weakened immune systems due to viral infections, cancer, organ transplants, or certain medications . Although fungal infections outbreaks are rather rare, the emergence of pathogenic fungi resistant to commonly used antifungal drugs, is occurring at unprecedented rates , which can easily change the global picture.
Banerjee Dipali , Gangwar Poonam, Mohd Vaseem Fateh(2024).The development and assessment of an ointment containing ketoconazole and minocycline exhibit potential In addressing both fungal and

bacterial skin infections. Additionally, there is room forInvestigating the efficacy of combination therapy, evaluating long-term safety, and exploring novel drug Delivery systems to further advance this ointment for clinical studies.

Shrishail Ghurghure, Shruti Satish Garad, Shubhangi Birajdar.(2023) Ketoconazole was found to have a maximum absorbance at 255.2 nm. The regression coefficient for the concentration range of 5–25 µg/ml. Ketoconazole. LOD and LOQ were determined to be 0.0225 and0.75 µg/ml, respectively. The procedure was successfully used on ketoconazole in commercial formulation, and the outcomes were in good agreement with label claims.

Saurabh Shrivastava, Suman Shrivastava, Rakesh Tiwle(2023). Fungal infections have become a major public health problem and are Growing in number and severity over several decades. The mean correlation coefficient was found to be 0.999. The accuracy was found between 98.99 and 99.32 %. The % RSD of Ketoconazole was found to be 0.10 to 0.56 for intraday and 0.13 to 0.55 for interday precision.

Shrishail Ghurghure, Shruti Satish Garad, Shubhangi Birajdar (2014) Ketoconazole was found to have a maximum absorbance at 255.2 nm. The regression coefficient for the concentration range of 5–25 µg/ml. Ketoconazole. LOD and LOQ were determined to be 0.0225 and0.75 µg/ml, respectively. The procedure was successfully used on ketoconazole in commercial formulation, and the outcomes were in good agreement with label claims.

SM Fraihat and KM Bahgat (2014). Linear calibration graphs were obtained at 4 - 30 and 5 - 35 µg/ml for the two methods at 610 and 660 nm, respectively. The regression equation was Y = 0.01X + 0.133, (R2= 0.996, RSD = 78 % and limit of detection (LOD) of µg/ml for method A. For the second method (B), it was Y = 0.008X + 0.039, (R2 = 0.995), RSD = 0.95 % and LOD = 2.3 µg/ml.

Safila Naveed, Lailoona Jawed (2014). Ketoconazole is an antifungal drug use for the treatment of many systemic and external antifungal agents. It is also used in many external fungal infection seborrheic dermatitis. The assay was done by using 3 available brands of ketoconazole in market, different dilution of 100ppm, 50ppm and 25ppm were made and analyzed, percent assay was determine by measuring absorbance.

Olga Popovska, Vesna Rafajlovska, Zoran Kavrakovski(2013). Ketoconazole is a chiral drug administered as a racemic (1:1) mixture of enantiomers of the cisconfiguration.3,4 It is a broadspectrum antifungal imidazole agent that has been shown to be efficient in the treatment of human systemic fungal infections, against Candida species, Cryptococcus neoformans, histoplasmosis, tinea corporis, tinea cruris, tinea manuum and tinea pedis, onychomycosis, seborrheic dermatitis, possessing antiinflammatory and some antibacterial activities with topical and systemic action.

Parth Kansagra, Gaurav Sanghvi, Paresh Purohit, Ashish Vachani1, Navin Sheth, Devendra Vaishnav (2012).The method exhibited high accuracy and sensitivity, in a linear range of 2-7 µg/ml. The Limit of Detection (LOD) and Limit ofQuantification (LOQ) were found to be 0.003 and 0.064 µg/ml respectively. All the concentrations were linear with the Absorbance having a correlation coefficient 0.9956. The regression equation of calibration curve was found to be y = 0.1502x +0.4168. The accuracy was found to be 99.17 ± 0.601026% with percentage recovery of 99.44 ± 0.35746%.

Shaligram S. Ranea, P. Padmajab,n (2011). Ketoconazole was oxidized with periodate, resulting in formation of KC2þ and iodate ions. After masking the excess periodate with molybdate, the iodate was treated with iodide to release iodine. The liberated iodine was transformed toICl2 species and extracted as ion-pair with rhodamine 6G into toluene for spectrophotometric measurement at 535 nm. A linear

calibration graph was obtained between 0.2136 mg/mL and1.7088 mg/mL of Ketoconazole with a molar absorptivity of 5 105 mol L1 cm-1.

NEED AND OBJECTIVES OF CURRENT EXPERIMENT

Many new medications are introduced to the market each year. Pharmaceutical solutions are developed using a single medication or a mix of medications to address unmet patient needs and enhance therapeutic results. The analytical chemist responsible for creating and verifying analytical protocols may face considerable challenges as a result of these products. In the modern world, it is imperative to take into account the product's quality and perform content analysis without extracting or separating, as these processes are costly, time consuming, and need personnel. Therefore, developing a fast, accurate, and repeatable technique for determining drug concentration in the presence of other drugs, chemicals, excipients, and other substances is essential.

An established method for analyzing the "active pharmaceutical ingredients" of many new products is lacking. This is because the pharmacopoeia did not include the drug right away once it was released onto the market. Therefore, in order to guarantee the identification, purity, potency, and effectiveness of the medications in the dosage forms, it becomes essential to develop enhanced analytical techniques for the routine assessment of these medicines. The current study is conducted to address the concerns of analytical chemists regarding new drugs and their formulations, as the ICH recommendations further emphasize the necessity for method design of novel formulations.

Therefore, the development of analytical methods is crucial to the discovery, creation, and production of pharmaceuticals, as well as to the establishment of standards for their identity, purity, and quality. This demonstrates the significance of creating novel analytical techniques for drug formulation quality assurance and control.

PLAN OF WORK

1. Choosing and acquiring the medication, ingredients, and dose type.
2. Determining the drug's solubility in various solvents.
3. The choice of analytical methods.
4. UV Spectrophotometry.

Calculating ketoconazole using UV

The following steps are included in the spectrophotometric method:

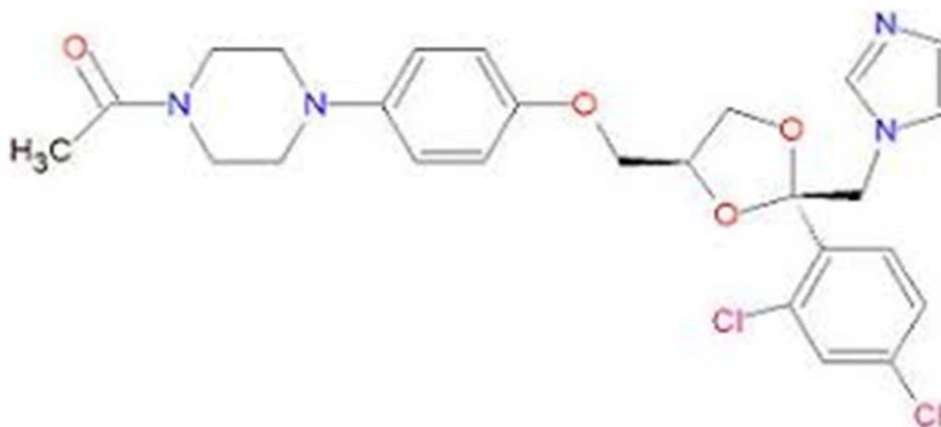
Common solvent selection.

- Spectral analysis
- Method and wavelength selection.
- Examination of marketed formulation.
- The developed method's robustness, ruggedness, linearity,
- LOD, LOQ, accuracy, and precision are all validated.

5. Data Analysis
6. Writing of Records

DRUG PROFILE

- **IUPAC NAME :** cis-1-acetyl- 4-[[(2RS,4RS) -2- (2, 4dichlorophenyl) -2- (1H-imidazol -1-ylmethyl) -1, 3-dioxolan -4- yl] methoxy phenyl piperazine.
- **Structure of Ketoconazole:**



- **Molecular formula -** C26H28Cl2N4O4
- **Molecular Weight** - 531.4 g/mol
- **Melting point -** 148-152°C
- **Solubility** - Insoluble in water Soluble in Methanol.
- **Category** - Anti fungal
- **Class** - Azole Anti fungal
- **Group** – Approved , Investigation
- **Type** – Small Molecule

▪ **Pharmacokinetics**

Absorption: Ketoconazole requires an acidic environment to become soluble in water.6 At pH values above 3 it becomes increasingly insoluble with about 10% entering solution in 1 h. At pH less than 3 dissolution is 85% complete in 5 min and entirely complete within 30 min.

Distribution : Ketoconazole has an estimated volume of distribution of 25.41 L or 0.36 L/kg.5 It distributes widely among the tissues, reaching effective concentrations in the skin, tendons, tears, and saliva.6 Distribution to vaginal tissue produces concentrations 2.

Protein binding: Ketoconazole is approximately 84% bound to plasma albumin with another 15% associated with blood cells for a total of 99% binding within the plasma.5

Metabolism: The major metabolite of ketoconazole appears to be M2, an end product resulting from oxidation of the imidazole moiety.8 CYP3A4 is known to be the primary contributor to this reaction with some contribution from CYP2D6.

Elimination: Only 2-4% of the ketoconazole dose is eliminated unchanged in the urine.5 Over 95% is eliminated through hepatic metabolism.

Half-life: Ketoconazole experiences biphasic elimination with the first phase having a half-life of 2 hours

and a terminal half life of 8 hours.
Clearance: Ketoconazole has an estimated clearance of 8.66 L/h.

MATERIALS AND INSTRUMENT :

Material : Ketoconazole was a gift sample obtained from Gufic Biosciences Ltd, Mumbai.
Reagent : All the other chemicals and reagents used were of analytical grade and procured from Sigma chemicals, Mumbai. The pharmaceutical tablets of Ketoconazole (NIZORAL 200) were purchased from a local Pharmacy shop.

INSTRUMENTATION:

Spectroscopic analysis was carried out using Double beam Shimadzu recording UV-Visible Spectrophotometer model 1900I with 10 mm path length matched quartz cells were used for analytical purposes.

Fig1 : UV- Shimadzu recording UV-Visible Spectrophotometer.

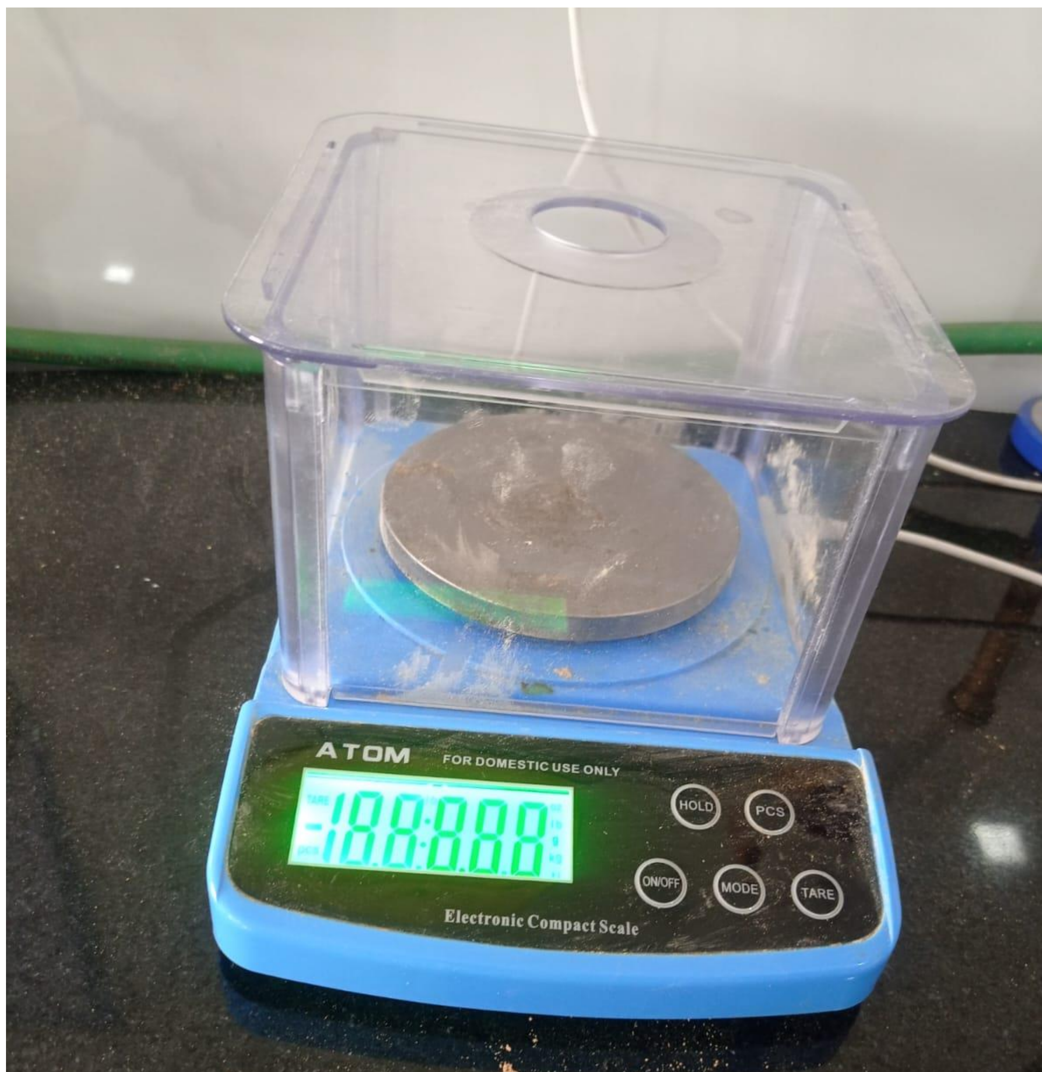


Fig 2 : Weighing Balance

EXPERIMENTAL INVESTIGATION

Preparation of standard stock solution and working solution: precisely weighed 100 milligrams of the medication ketoconazole and put into a volumetric flask that held 100 millilitres. To guarantee that the medication was completely dissolved, a tiny amount of methanol was added. Phosphate buffer was then added to get the volume up to the appropriate level. To totally dissolve and eliminate the air, the solution was sonicated.

A transparent solution with a strength of 1000 µg/ml (standard stock solution) was obtained. Ten millilitres of the stock solution were transferred into a 100 millilitre volumetric flask, and 100 millilitres of phosphate buffer 6.8 were added to create a working solution with a concentration of 100 µg/ml. The mixture was then filtered using Whatman filter paper.

Method Validation:

The International Conference on Harmonization (ICH) guidelines, section Q2, were followed in developing the process validation of the suggested technique(R1).

Linearity and calibration curve:

From the working solution, a serial dilution was made in the range of 10 to 60 µg/ml. The UV-Visible spectrophotometer was used to examine the samples, using phosphate buffer 6.8 as a reference. The drug

concentration level versus absorbance graph was plotted to create the calibration curve.
For statistical data processing, the ANOVA test and least-square regression analysis were utilized. The ketoconazole solution's UV spectrum at 208 nm in phosphate buffer 6.8 (10 µg/ml) is displayed.

Accuracy:
To verify the test sample's recovery, an accuracy study was carried out. It was executed by injecting the three distinct levels of the sample solution: 80, 100, and 120%. Based on estimated and actual concentrations, the accuracy was expressed as % recovery ± (% confidence interval) with % relative error.

Precision:
To assess the method's degree of repeatability, a precision test was to be carried out. In this instance, the sample measured three separate days for intra- and inter-day research, and at least three times on the same day at hourly intervals. We computed the relative standard deviation (RSD) and standard deviation (SD).

Limits of quantification (LOQ) and detection (LOD):
To assess the method's sensitivity, the LOD and LOQ were used. LOD stands for limit of detection, which is the method's ability to quantify an analyte's minimum concentration in a sample as precisely as possible . The limit of minimal detection capability of the method used to test the analyte in a sample that reliably quantitates it with the required degree of accuracy and precision is known as the limit of quantification, or LOQ.
The calibration curve's slopes were calculated as average values, and the estimation of LOD and LOQ is plotted on the response standard deviation . In order to verify LOD and LOQ experimentally, the known concentration was diluted. By dilution of the known drug concentration until the average responses were around three to ten times the LOD and LOQ, the parameters were experimentally confirmed. standard deviation of the replies for each of the six repeated calculations.

Robustness:
λ max of the analysis was changed by ± 3 nm to test the robustness of the suggested procedure. We calculated the percentage relative error and the mean recovery (±%) confidence interval. Analyzing the typical pure medication of ketoconazole solution in the experiment with phosphate buffer 6.8 was conducted at various wavelengths (± 3 nm).

Test of the commercial formulation:
In a pestle and mortar, ten tablets (NIZORAL 200) were weighed and smashed. A 10 milligram dose of drug powder was precisely weighed, transferred to a 10 milliliter volumetric flask, dissolved in around 4 milliliters of phosphate buffer 6.8, and sonicated for 10 minutes to produce a transparent solution . To create a sample solution, the volume was adjusted with phosphate buffer 6.8. A measurement was made of the sample solution's absorbance.

RESULTS AND DISCUSSION:

Method Validation: In accordance with ICH guidelines, the suggested method's robustness, linearity, accuracy, precision, limit of detection, and limit of robustness and quantitation. The validation parameters' total outcomes were combined and shown in

Table 1. Validation parameters for determination of Ketoconazole in Phosphate buffer 6.8.

Parameters	Values
Absorption Maxima (nm)	208 nm
Linearity range (µg/ml)	10 – 60
SRE	Y=0.040x + 0.528

CC (R^2)	0.999
Precision (%RSD)	Intraday assay = 0.10 to 0.56
	Intraday assay = 0.13 to 0.55
Accuracy (% recovery ± SD)	98.99 ± 0.28 to 99.32 ± 0.23
LOD (µg/ml)	1.37
LOQ (µg/ml)	4.07

SRE - Standard Regression Equation AND Correlation coefficient

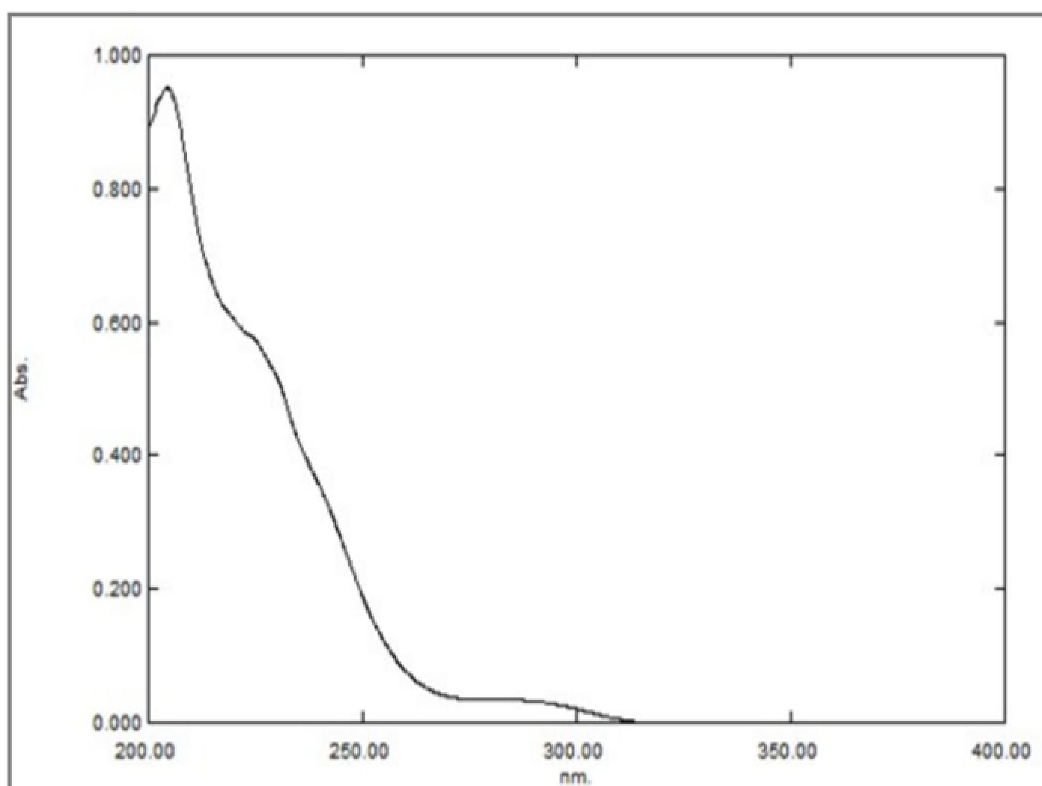


Fig 2. UV Spectrum of Ketoconazole at 208 nm in Phosphate buffer 6.8 (10 µg/ml).

Linearity:

Ketoconazole was discovered to have linearity in the range of 10 to 60 µg/ml and a 0.999 correlation value. Figures 3 and 4 display, respectively, the over-ray spectra and the calibration curve of ketoconazole in phosphate buffer 6.8 at 208 nm [20]. Table 2 displayed ketoconazole's linearity. The ANOVA test within the F value validates the significant linear regression. The result shows that the computed value of F is higher than the F critical value at 208.

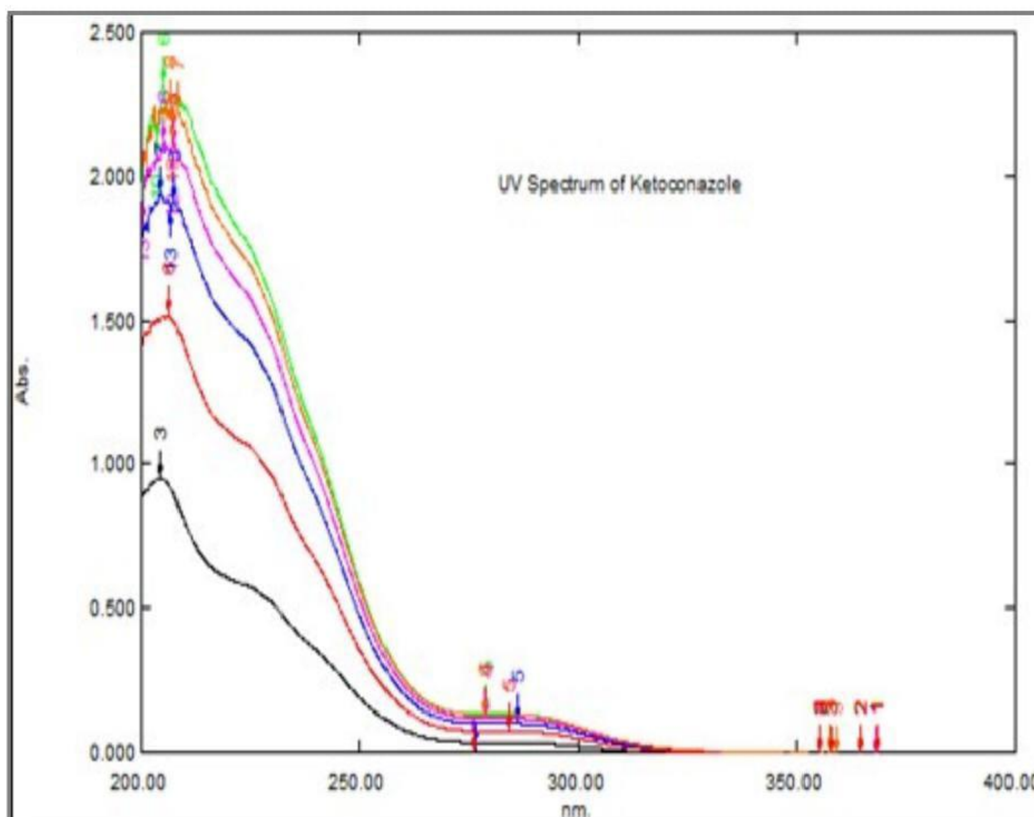


Fig 3. Overlay spectra of ketoconazole in Phosphate buffer 6.8 within the linearity range at 208 nm.

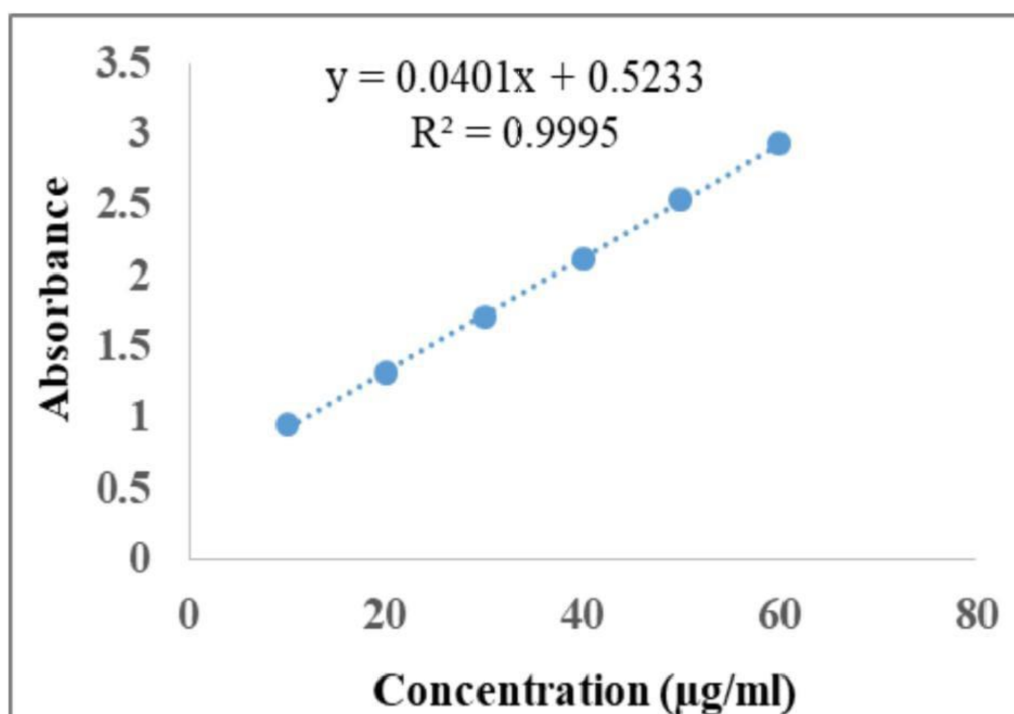


Fig 4. Calibration curve of Ketoconazole at 208 nm.

Table 2. Linearity of Ketoconazole at λ max of 208 nm.

Conc. (µg/ml)	Absorbance Value	Absorptivity
10	0.95	0.0955
20	1.31	0.0655
30	1.73	0.057667
40	2.12	0.053
50	2.54	0.0508
60	2.94	0.049
Avg.	1.93167	0.06183
SD	0.750	0.01729
%CV	0.388	0.2797
RE	Y=0.040x+0.528	
r^2	0.999	

SD - Standard Deviation, CV – Co-efficient of variance and RE - Regression equation.

Accuracy:

The suggested method's accuracy was calculated using the percentage of recovery at the three-level of % increase. Table 3 displays the percentage recovery of ketoconazole, which was found to be between 98.99 and 99.32. Without a doubt, the recovery studies' findings show how accurate the suggested approach is.

Table 3. Estimation of Accuracy by percentage recovery method.

Conc.(µg/ml)	LOA (%)	Amount (µg) Mean ± SD*	%MR ± % RSD
10	80	7.89 ± 0.05	98.99 ± 0.28
	100	9.90 ± 0.02	
	120	11.91 ± 0.03	
20	80	15.82 ± 0.09	99.32 ± 0.23
	100	19.89 ± 0.08	
	120	23.9 ± 0.03	
30	80	23.74 ± 0.07	99.12 ± 0.05
	100	29.69 ± 0.09	
	120	35.81 ± 0.07	

LOA - Level of addition, MR - Mean Recovery and * (n=3) determinations.

Precision: At the three-level of Intraday and Interday Precision, the suggested method's precision was calculated. addition in percentage terms. The repeatability results show the accuracy both throughout the interday evaluation and over a brief period of time. The data were displayed in Table 4 and the percentage RSD of ketoconazole was determined to be 0.10 to 0.56 for intraday precision and 0.13 to 0.55 for interday precision. The suggested method yielded intraday and interday relative standard deviation (RSD) readings that are within two percent relative standard deviations.

Table 4. Intraday and Intraday Precision.

Conc.	Intraday precision		Intraday precision	
	X±SD	%RSD	Mean±SD	%RSD
10	09.84±0.05	0.56	09.88±0.05	0.55
20	19.89±0.03	0.15	19.89±0.17	0.17
30	29.91±0.03	0.10	29.21±0.13	0.13

X±SD – Mean ± Standard deviation (n=3). Concentration in µg/ml.

Limit of detection (LOD) and limit of quantification (LOQ):

The purpose of the LOD and LOQ study was to evaluate the suggested developed method's sensitivity. It was discovered that the LOD and LOQ were, respectively, 1.37 and 4.07 µg/ml.

Study of robustness:

The developed method's resilience demonstrates a non-significant influence of the absorption level through the analysis of the ketoconazole solution in Phosphate buffer 6.8 at different wavelengths (± 3 nm).

The data of the robustness study were shown in Table 5.

	At 206 nm			**At 210 nm**	
Abs	X ± SD	%R ±%RSD	Abs	X ± SD	%R ±%RSD
0.95			0.93		
0.96	0.94±0.015	99.64±1.613	0.94	0.93±0.015	99.29±1.619
0.93			0.96		

X±SD – Mean ± Standard deviation (n=3). R – Recovery and RSD – Relative Standard deviation.

Assay of marketed formulation:

The devised method was used to analyze the marketed tablet formulations. According to ICH recommendations.

The results showed that the values for each of these formulations ranged from 99.89 to 100%, with a maximum percentage confidence interval of ± 0.11. The claim made on the formulation label is supported by the performed accuracy, which displays a recovery percentage of 99.21%.

SUMMARY OF PROJECT

The validation of a novel UV spectrophotometric method for determining Ketoconazole in pharmaceutical formulations is a critical process ensuring the accuracy, reliability, and robustness of the analytical technique. The validation protocol typically involves several key steps:

1. **Specificity:** This step confirms that the UV method can accurately measure Ketoconazole in the presence of other components typically found in pharmaceutical formulations. It ensures that any interference from excipients or impurities does not affect the measurement of Ketoconazole.
2. **Linearity:** The linearity of the method establishes the relationship between the concentration of Ketoconazole and the corresponding absorbance values. This demonstrates that the UV method can reliably quantify Ketoconazole over a range of concentrations relevant to the pharmaceutical formulation.

3. **Accuracy:** Accuracy assesses how close the measured values are to the true values. It is typically evaluated by comparing the measured concentrations of Ketoconazole using the UV method against known concentrations in spiked samples.
4. **Precision:** Precision evaluates the repeatability (intra-day precision) and intermediate precision (inter-day precision) of th0e UV method. It ensures that the method produces consistent results when performed by the same operator, in the same laboratory, and under different conditions.
5. **Robustness:** Robustness examines the method's ability to remain unaffected by small variations in experimental conditions such as wavelength, pH, or temperature. It demonstrates the method's reliability under slightly altered conditions.
6. **Limit of Detection (LOD) and Limit of Quantitation (LOQ):** These parameters determine the lowest concentration of Ketoconazole that can be reliably detected and quantified by the UV method. They provide information about the method's sensitivity.

Once each of these parameters is evaluated and meets the acceptance criteria established by regulatory guidelines (such as ICH guidelines), the UV spectrophotometric method can be considered validated for the determination of Ketoconazole in pharmaceutical formulations. This validated method can then be used for routine quality control analysis to ensure the potency and stability of Ketoconazole-containing products on the market.

CONCLUSION:

This UV spectrophotometer method that was created was thought to be straightforward, dependable, and selective offering acceptable levels of sensitivity, specific quantification, precision with lower detection limits, and accuracy. This approach is more rapid, less expensive, has adequate precision, and has acceptable specificity for determining the analyte's existence in components.

All instances had satisfactory recoveries, and the consistent agreement with the documented protocol demonstrated that the suggested method could be effectively used to determine ketoconazole. The technique can be easily used for routine quality control analysis of ketoconazole in bulk medicine, marketed tablets, and other formulations. It was successfully verified in accordance with ICH criteria.

BIBLOGRAPHY

1. Kaur IP, Kakkar S. Expert Opin Drug Deliv, 2010; 7(11): 1303-1327.
2. Perez BSH. Med Hypotheses, 2004; 62(1): 112-115.
3. Wang L, Tang X. Int J Pharm, 2008; 350(1-2): 181-187.
4. Alizadeh N, Rezakhani Z. J Chil Chem Soc, 2012; 57(2): 1104-1108.
5. Farhadi K, Maleki R. J Pharm Biomed Anal, 2002; 30(4): 1023-1033.
6. El-Ragehy NA, El-Saharty YS. J AOAC Int, 2001; 84(2): 563-568.
7. Abou-Attia FM, Issa YM, Abdel-Gawad FM, Abdel-Hamid SM. Farmaco, 2003; 58(8): 573-579.
8. Kedor-Hackmann ERM, Santoro MIRM, Singh AK, Peraro AC. Braz J Pharm Sci, 2006; 42(1): 91-98.
9. Vojić MP, Popović GV, Sladić DM, Pfendt LB. J Serb Chem Soc, 2005; 70(1): 67-78.
10. Khashaba PY, El-Shabouri SR, Emara KM, Mohamed AM. J Pharm Biomed Anal, 2000; 22(2): 363-376.
11. Saysin S, Liawruangrath B, Liawruangrath S. J Cosmet Sci, 2010; 61(5): 367-376.
12. De Bruijn P, Kehrer DFS, Verweij J, Sparreboom A. J Chromatogr B, 2001; 753(2): 395-400.

13. Abdel-Moety EM, Khattab FI, Kelani KM, AbouAl-Alamein AM. II Farmaco, 2003; 57(11): 931-938.
14. Dayyih WA, Al saadi N, Hamad M, Mallah E, Matalka K, Arafat T. Int J Pharm Sci Res, 2012; 3(10): 3686-3692.
15. Chou WL, Chang CY, Liu HM, Yang KC, Wu CC. J Food Drug Anal, 2007; 15(1): 25-32.
16. Jat RK, Sharma S, Chhipa RC, Singh R, Alam I. Pharmacophore, 2012; 3(2): 123-129.
17. Staub I, Bergold AM. Acta Farm Bonaerense, 2004; 23(3): 387-390.
18. Velikinac I, Cudina O, Jankovič I, Agbaba D, Vladimirov S. Farmaco, 2004; 59(5): 419-424.
19. Arranz P, Arranz A, Moreda JM, Cid A, Arranz JF. J Pharm Biomed Anal, 2003; 33(4): 589-596.
20. Rane SS, Padmaja P. J Pharm Anal, 2012; 2(1): 43-47.
21. Chan CC. Potency method validation. In: Chan CC, Lam H, Lee YC and Zhang XM (eds.). Analytical Method Validation and Instrument Performance Verification, New Jersey; John Wiley and Sons: 2004, pp. 11-26.
22. Aher KB, Bhavar GB, Joshi HP. J Curr Pharm Res, 2012; 9(1): 49-54.