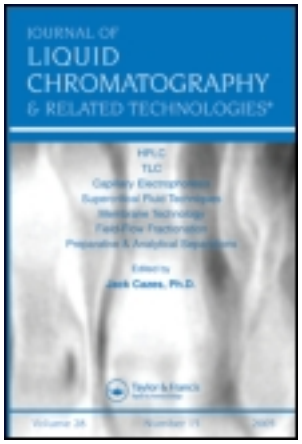

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STABILITY-INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF FLUCONAZOLE IN THE PRESENCE OF ITS OXIDATIVE DEGRADATION PRODUCT - KINETIC AND STRESS STUDY

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STABILITY-INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF FLUCONAZOLE IN THE PRESENCE OF ITS OXIDATIVE DEGRADATION PRODUCT - KINETIC AND STRESS STUDY

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□ *A simple, specific, accurate, and stability-indicating high performance liquid chromatographic method (HPLC) method has been established for analysis of fluconazole (FLZ) in the presence of its degradation products generated in the stress degradation study. FLZ was subjected to stress conditions of acid, alkali, and neutral hydrolysis, oxidation, photolysis, and thermal decomposition. Extensive degradation was found to occur in oxidative medium under thermal stress. Successful separation of drug from degradation products was achieved on a C-18 column using phosphoric acid 0.5% v/v: acetonitrile (80:20% v/v) as the mobile phase. The flow rate was 1.5 mL* min^{-1} *and the detector was set at 261 nm. The retention times of FLZ and its main oxidative degradation product were found to be 5.389 min and 2.729 min, respectively. Linearity was established for fluconazole in the range of 0.5–50 μg/ml. The percentage recovery of fluconazole was found to be* 99.91 ± 0.74*. Because the method effectively separates fluconazole from its oxidative degradation products, it can be used as stability-indicating method. The proposed method was also used to study the kinetics of fluconazole oxidative degradation that was found to follow a zero-order reaction. The* $t_{1/2}$ *was 21.66 min while k (reaction rate constant) was* 2.91×10^{-8} *mole/min.*

Keywords fluconazole, HPLC, kinetic study, oxidation, stability indicating method, stress study

INTRODUCTION

Fluconazole, (FLZ) [2-(2,4,-difluorophenyl)-1,3-bis(1H-1, 2,4,-triazol-1-yl)propan-2-ol], (Figure 1) is an orally active antifungal agent, which is used in the treatment of superficial and systemic candidiasis and in the treatment of cryptococcal infections in patients with the acquired immunodeficiency syndrome (AIDS). It acts by blocking the synthesis of ergosterol, an

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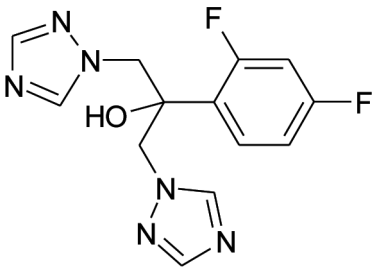

$C_{13}H_{12}F_2N_6O$ M.W. (306.27)

FIGURE 1 Structural formula of fluconazole.

essential component of the fungal cell membrane.[1] Mammalian cell is much less sensitive to FLZ inhibition.

Stability is considered as one of the most important criteria in pharmaceutical quality control. Only stable preparation would promise precise delivery of the drug to the patients. Expiration dating on any drug product is based upon scientific studies at normal and stressed conditions.[2,3]

The literature survey reveals several analytical methods used for quantitative determination of FLZ in body fluids and pharmaceutical preparations. These methods include high-performance liquid chromatography,[4–10] liquid chromatography-tandem mass spectrometry,[11–13] gas chromatography,[14–17] micellar electrokinetic capillary chromatography,[18] and bioassay.[19] Only one stability study was done to assess the stability of fluconazole in different stress conditions without separation and identification of these degradation products.[20]

The parent drug stability test guideline issued by the International Conference on Harmonization (ICH)[21] suggests that stress studies such as hydrolysis, oxidation, photolysis, and thermal stress should be carried out on a drug to establish its inherent stability characteristics, leading to identification of degradation products and, hence, supporting the suitability of the proposed analytical procedures. It also requires that analytical procedures for testing the stability of samples should be stability-indicating and should be fully validated.

The scientific novelty of the present work is that the developed stability-indicating RP-HPLC method was simple, rapid, selective, and less time- consuming (only about 5 min for a single run) compared with other published LC methods. This method was successfully validated according to the ICH guideline[22,23] which make it applicable for use in the quality control laboratories for the accurate quantification of FLZ in its pharmaceutical formulations

EXPERIMENTAL

Chemicals and Reagents

All chemicals used throughout this work were of analytical grade, and the solvents were of spectroscopic grade. O- phosphoric acid, 35% hydrogen peroxide solution, 33% aqueous ammonia solution, methanol, chloroform (El-Nasr Pharmaceutical Chemicals Co., Abu–Zaabal, Cairo, Egypt). Hydrochloric acid, sodium hydroxide (El-Nasr Pharmaceutical Chemicals Co., Abu–Zaabal, Cairo, Egypt). Methanol and acetonitrile of HPLC grade (Sigma-Aldrich, Germany). Deionized water was bidistilled from a "Aquatron" Automatic Water Still A4000 that was provided by Sterillin (Staffordshire, England).

Instruments

- Infrared spectrophotometer: FTIR Jasco 460 plus (Japan).
- Mass spectrophotometer: Shimadzu Qp-2010 Plus (Japan).
- Precoated TLC-plates, silica gel 60 F_{254} (20 cm × 20 cm, 0.25 nm), E. Merck (Darmstadt-Germany).
- Rotary evaporator: Normschliff Gerätebau Wertheim (Germany).
- Hot oven: Tecnomedica SRL Monte Bernina, Bareggio, MI, Italy; operated with a thermostat and a timer.
- Sonicator: Bandelin Sonorex RK 510S, Donation of Alexander von Humboldt–Foundation, Bonn-Bad, Godesberg, Federal Republic of Germany.
- Light-cabinet: containing UV-lamp operating to give overall illumination of $\geq$80 μw/cm^2 at 315–400 nm. Illuminance was 860 lux. The lamp was tested and calibrated in the National Institute for Standards (NIS).

HPLC Instrumentation and Conditions

- The HPLC system consisted of a two-pump (Shimadzu Model LC-20AT), an ultraviolet variable wavelength detector (Model SPD-20A, Shimadzu), and an auto-sampler (Model SIL-20A, Shimadzu)
- The chromatographic separation was performed using a Waters C 18 analytical column (250 mm × 4.6 mm i.d., 5 μm).
- Separation was achieved using a mobile phase consisting of (phosphoric acid 0.5% *v*/*v*: acetonitrile 80:20 (*v*/*v*); isocratically at 1.5 ml · min-1 and UV detection at 261 nm.
- The column was maintained at ambient temperature and an injection volume of 50 μL was used. The mobile phase was filtered through 0.45-μm membrane filter. The mobile phase was degassed for ~15 min in an ultrasonic bath prior to use.

Materials

Pure Standard

FLZ standard was kindly donated by Amoun Pharmaceutical Co. (El-Obour City, Cairo, Egypt). Its purity was found to be $99.13 \pm 0.95\%$ ($n = 6$) according to a reported method.[10]

Degradation Products Preparation

Pure FLZ powder of 100 mg was transferred into a 100-mL flask; 30 mL of 35% hydrogen peroxide solution were added and refluxed for 3 hr. Complete degradation was monitored by TLC using chloroform:methanol:33% aqueous ammonia solution (8.5:1.5:0.1 $v/v/v$) as a developing system. The solution was boiled on a small flame until free from hydrogen peroxide, evaporated on a rotary evaporator, and the residue was collected. The degradation product powder was elucidated using IR and mass spectrometry.

Standard Stock Solutions

All solutions were freshly prepared and stored in a refrigerator to be used within 72 hr: FLZ standard stock solution ($1\ mg \cdot mL^{-1}$) in methanol; and oxidative degradation products solution ($1\ mg \cdot mL^{-1}$) in methanol (prepared as mentioned and calculated with respect to pure FLZ).

Pharmaceutical Dosage Forms

- Diflucan syrup BN: 0305, labeled to contain 5 mg/mL; diflucan vial BN: 9742, labeled to contain 2 mg/mL; diflucan capsule BN: 0202, labeled to contain 50 mg/capsule; diflucan capsule BN: 0105, labeled to contain 150 mg/capsule (manufactured by Pfizer Egypt S.A.E., Cairo, Egypt, Under the authority of Pfizer Inc. U.S.A.).
- Fungican capsule BN: 101148, labeled to contain 150 mg/capsule (manufactured by Amoun Pharmaceutical Co. (El-Obour City, Cairo, Egypt)).
- Alkanazole capsule BN: 032, labeled to contain 150 mg/capsule (manufactured by Hikma Pharma S.A.E., 6th of October City - Egypt).
- Triflucan capsule BN: 1002159, labeled to contain 150 mg/capsule [manufactured by Egyptian International Pharmaceutical Industries Company (EIPICO)].

Procedures

Working standard solutions containing 0.5–50 μg/mL FLZ were prepared using its corresponding standard solution (1 mg/mL) in the mobile phase. The samples were filtered through a 0.45-membrane filter prior to

analysis. Triplicate 50-μL injections were made for each solution and chromatographed under the specified chromatographic conditions described before. To reach good equilibrium, the analysis was usually performed after passing ~50–60 mL of the mobile phase, for the purpose of conditioning and pre-washing of the stationary phase. The peak areas were recorded, the calibration curve was plotted representing the relative peak area (peak area of FLZ, to that of the external standard; 20 μg/mL) against the corresponding concentrations in μg/mL of FLZ and the regression equation was computed.

Forced Degradation Study (Stress Study)

In order to determine whether the analytical method and assay were stability-indicating, FLZ was stressed under various conditions to conduct forced degradation studies[21]. Regulatory guidance in ICH requires the development and validation of stability-indicating potency assays. Unfortunately, the current ICH guidance documents did not indicate detailed degradation conditions in stress testing. However, the used forced degradation conditions, stress agents concentrations, and time periods of stress were based on trial and error.

The stability of FLZ was studied under forced degradation studies (acid, alkali, oxidation, neutral, heat, and ultraviolet degradation) as follows:

Acid Degradation

FLZ (3 mL) stock solution ($1\,mg \cdot mL^{-1}$) was left with 12 mL of 1 M hydrochloric acid for 3 d protected from light at ambient temperature, and another 3 mL of FLZ stock solution was refluxed for 3 hr with 12 mL of 1 M hydrochloric acid. At end of the periods, each of the two solutions was neutralized with 12 mL 1 M NaOH.

Alkali Degradation

FLZ (3 mL) stock solution ($1\,mg \cdot mL^{-1}$) was left with 12 mL of 1 M sodium hydroxide for 3 d protected from light at ambient temperature, and another 3 mL of FLZ stock solution was refluxed for 3 hr with 12 mL of 1 M sodium hydroxide. At end of the periods, each of the two solutions was neutralized with 12 mL 1 M HCl.

Oxidation (Hydrogen Peroxide) Degradation

FLZ (3 mL) stock solution ($1\,mg \cdot mL^{-1}$) was left with 12 mL of 3% hydrogen peroxide for 3 d protected from light at ambient temperature. Another degradation with hydrogen peroxide was tested under drastic conditions and is presented in the Degradation Products Preparation section.

Neutral Medium Degradation

FLZ (3 mL) stock solution ($1\,mg \cdot mL^{-1}$) was left with 12 mL of distilled water for 3 d protected from light at ambient temperature.

Heat Degradation

FLZ powder was left in hot oven at 90°C for 3 d. Samples of the powder were taken after 3 hr and 72 hr (3 d) and analyzed for FLZ content. Another study was conducted at 40°C for 72 hr (3 d).

Ultraviolet Degradation

Four beakers were used; two containing 50 mg of FLZ powder spread on a glass dish in a layer less than 2-mm thickness [one was subjected to radiation (test) and the other was protected (blank)], and two other beakers containing 20 mL of FLZ stock solution (test and blank also as powders). All the beakers were placed in a light cabinet and exposed to light for 40 hr resulting in an overall illumination of $80\,\mu w/cm^2$ at ambient temperature with UV

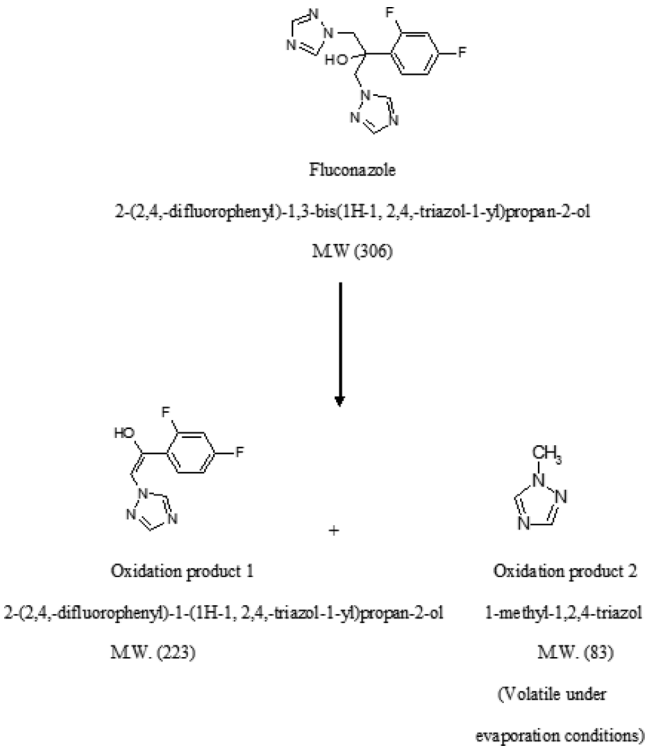

FIGURE 2 Scheme for oxidation of fluconazole.

radiation at 315–400 nm. The solution which was subjected to radiation was compensated with fresh methanol when needed in order not to be dry

N.B.: At the end of each degradation condition, the solution was transferred quantitatively and appropriate dilution was made using the mobile phase to get final FLZ concentration of $50\,\mu g \cdot mL^{-1}$. Analysis was accomplished by the adopted HPLC method and FLZ concentration was determined using its regression equation and compared with initial amount.

Kinetic Calculations

The oxidation of FLZ showed a degradation of the intact compound (Figure 2). A set of identical test tubes containing 0.5 mL (equivalent to 400 µg FLZ) using FLZ stock solution (1 mg/mL) and 0.5 mL of 35% H_2O_2, were well-plugged and put in a hot oven at 150°C. At each 5-min interval, a test tube was removed from the oven and the contents of the test tube was quantitatively transferred into a 10-mL calibrated volumetric flask, and, then, volume was completed with the mobile phase to obtain a final FLZ concentration of $50\,\mu g \cdot mL^{-1}$. Analysis was accomplished by the adopted HPLC method and FLZ concentration was determined using its regression equation. The degradation kinetics was determined by plotting the concentration of remaining drug versus time.

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

A Waters C 18 analytical column (250 mm × 4.6 mm i.d., 5 µm) maintained at ambient temperature (25°C) was used for the separation and the method was validated for the determination of FLZ in pharmaceutical dosage forms. The mobile phase was chosen after several trials to reach the optimum stationary/mobile-phase matching. Eventually, a mobile phase consisting of phosphoric acid 0.5% *v/v*: acetonitrile 80:20 (*v/v*) with flow rate = 1.5 mL.min^{-1} provided the best chromatographic response and was used for further studies.

Method Validation

Method validation was performed according to USP guidelines[24] for all the proposed methods as follows:

Range and Linearity

The linearity of the method was evaluated by processing 6-point calibration curves on 3 different days. The calibration curves of which were

constructed within concentration ranges that were selected on the basis of the anticipated drug concentration during the assay of the dosage forms. A linear least-squares regression analysis was conducted to determine slope, intercept, and coefficient of determination to demonstrate linearity of the method. The goodness of fit in all cases was found to be >0.9966, indicating a functional linear relationship. The relevant slope values were statistically different from zero ($P < 0.05$) and, although intercepts of the calibration curves were significantly different from zero, they did not affect the accuracy of the method. The linear regression analysis data are summarized in Table 1.

Accuracy

To study the accuracy of the proposed methods, procedures under study of linearity were carried out for determination of six different concentrations of pure FLZ in triplicate. The accuracy expressed as percentage recoveries are shown in Table 2. Good accuracy of the developed methods was indicated by the results obtained.

Specificity

Specificity was ascertained by analyzing different mixtures containing FLZ and its degradation products in different ratios. The FLZ and the oxidation product could be eluted in the form of symmetrical peaks quite apart from each other. FLZ and its main degradation product were well-resolved in a reasonable time of 5.5 min. The average retention times under the conditions described are 5.389 min for FLZ and 2.729 min for the oxidation product (Figure 3). The chromatographic system described in this work allows complete separation of FLZ from its oxidation product. Other

TABLE 1 Assay Parameters and Evaluation Sheet for Determination of Fluconazole

Parameter	Value
Range	0.5–50 $\mu g \cdot mL^{-1}$
Slope	0.004
Intercept	0.0041
Correlation coefficient (r)	0.9998
L.O.D.($\mu g \cdot mL^{-1}$)	1.46
L.O.Q. ($\mu g \cdot mL^{-1}$)	0.46
RSD (%)[a]	0.98
RSD (%)[b]	0.93

[a]The interday ($n = 6$) relative standard deviations of (10 and 40 $\mu g \cdot mL^{-1}$) of FLZ by the proposed method.
[b]The intraday ($n = 5$) relative standard deviations of (10 and 40 $\mu g \cdot mL^{-1}$) of FLZ by the proposed methods.

TABLE 2 Statistical Comparison for the Results Obtained by the Prposed Methods and the Reported Method for the Analysis of Fluconazole

Parameter	HPLC	Reported Method[10]*
Mean	100.37	100.05
S.D.	0.66	0.67
Variance	0.43	0.44
Coefficient of variation	0.65	0.67
N	8	10
F-test	Calculated: 1.02 Tabulated: 3.68	
Student's t-test	Calculated: 1.89 Tabulated: 2.12	

*Reported method: HPLC on C8 column, mobile phase: water/acetonitrile 72:28 (*v*/*v*), UV detection at 260 nm.

parameters such as resolution, capacity factor, and selectivity for the separated peaks were then calculated. The retention time values of the separated peaks together with other chromatographic parameters are collected in Table 3

Precision

The precision of the proposed method, expressed as RSD, was determined by analysis of three different concentrations of pure FLZ within the linearity range for FLZ. The intra-day precision was assessed from the

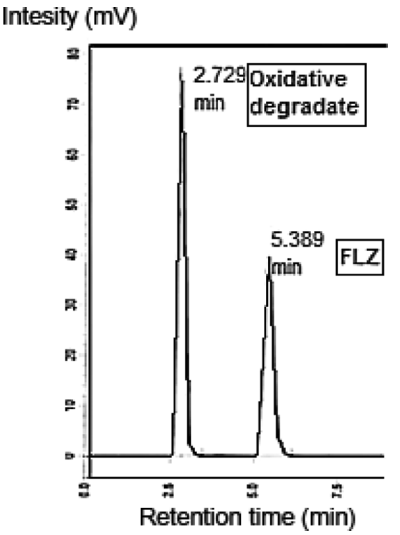

FIGURE 3 Liquid chromatographic separation of fluconazole (5.389 min) from its oxidation product (2.729 min).

TABLE 3 System Suitability Parameters of HPLC Method

Parameter	Obtained Value	Reference Value
Resolution (R)	4.75	$R > 0.8$
Tailing factor (T)	FLZ: 1 Ox.: 1	$T = 1$ for a typical symmetric peak
Selectivity (α)	2.54	>1
Column capacity (K')	FLZ: 4.39 Ox.: 1.73	1–10 acceptable
Column efficiency (N)	FLZ: 948.29 Ox.: 476.64	Increases with efficiency of the separation
Height equivalent to theoretical plate (HETP)	FLZ: 0.026 Ox.*: 0.052	The smaller the value, the higher the column efficiency

*Ox.: oxidative degradant.

results of three replicate analyses of pure FLZ on a single day. The inter-day precision was determined from the same samples analyzed on three consecutive days. The results of intra-day and inter-day precision are illustrated in Table 1.

Limits of Detection and Quantitation

The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the current ICH guidelines[22,23] as the ratio of 3.3 and 10 standard deviations of the blank, respectively, and the slope of the calibration line (Table 1).

Application to Commercial Preparations

The suggested method was successfully applied for determination FLZ in its commercial preparations. The results are shown in Table 4 were satisfactory and with good agreement with the labeled amounts. Applying the standard addition technique, no interference due to excipients was observed as shown from the results in Table 4.

System Suitability

System suitability parameters were tested by calculating the capacity factor, tailing factor, the sensitivity factor, and resolution to determine if the operating systems are performing properly. Good results were obtained as shown in Table 3.

Robustness

The robustness of the HPLC method was investigated by the analysis of samples under a variety of experimental conditions such as small changes in phosphoric acid/acetonitrile ratio (by up to $\pm 0.5\%$), flow rate, and changing the column using a Lichrosorb C18 (250 mm × 4.6 mm, i.d., 5 μm)

TABLE 4 Application of the Proposed and the Reported Methods for the Analysis of Fluconazole in Pharmaceutical Dosage Forms

Product	Proposed Method	Reported Method[10]*
Diflucan syrup 5 mg/mL, BN: 0305	102.64 ± 0.64	102.39 ± 0.55
Diflucan vial 2 mg/mL, BN: 9742	101.29 ± 0.56	101.04 ± 0.36
Diflucan capsule 50 mg/capsule, BN: 0202	98.87 ± 0.34	98.62 ± 0.78
Diflucan capsule 150 mg/capsule, BN: 0105	99.87 ± 0.45	99.62 ± 0.52
Fungican capsule 50 mg/capsule, BN: 101148	99.8 ± 0.33	99.55 ± 0.44
Alkanazole capsule 50 mg/capsule, BN: 032	98.7 ± 0.34	98.45 ± 0.36
Triflucan capsule 50 mg/capsule, BN: 1002159	100.9 ± 0.54	100.65 ± 0.41
Standard Addition Technique	99.8 ± 0.32	99.55 ± 0.53

*Reported method: HPLC on C_8 column, mobile phase: water/acetonitrile 72:28 (*v*/*v*), UV detection at 260 nm.

analytical column. The effect on retention time and peak parameters was studied. It was found that the method was robust when the column and mobile phase ratio and flow rate were varied. During these investigations, the retention times were modified, however the areas and peak symmetry were conserved.

Statistical Analysis

Table 2 shows statistical comparison of the results obtained by the proposed method and the reported HPLC method.[10] The calculated t and F values at two tails confidence intervals were found to be less than the tabulated ones.[25]

Forced Degradation Study (Stress Study)

The International Conference on Harmonization (ICH) guideline entitled "Stability Testing of New Drug Substances and Products" requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance.[21] An ideal stability-indicating method is the one that quantifies the standard drug alone and also resolves its degradation product.

All stressed samples in both solid and solution state remained colorless. All the results of the forced degradation studies are presented in Table 5 which describes the experimental conditions and percentage of FLZ remaining.

As shown in Table 5, it was found that no decomposition was noted after exposure of FLZ methanolic solution to acid, alkali, water, and ultraviolet radiation. Only 2.47% degradation occurred after exposure of methanolic solution of FLZ to 1 M HCl for 72 hr at 25°C, while refluxing with 1 M HCl

TABLE 5 Results of Forced Degradation Study

Sample Condition	Procedure	Percentage of FLZ Remaining	Percentage of Degradation	Remarks
Acid degradation	1 M HCl – 72 hr –25°C	97.53%	2.47%	No degradation
Acid degradation	1 M HCl – 3 hr–reflux	95.13%	4.87%	No degradation
Alkali degradation	1 M NaOH – 72 hr –25°C	97.26%	2.74%	No degradation
Alkali degradation	1 M NaOH – 3 hr.–reflux	94.68%	5.32%	No degradation
Hydrogen Peroxide degradation	3% H_2O_2 – 72 hr –25°C	100%	0%	No degradation
	35% H_2O_2 – 30 min. –150°C	31.79%	68.21%	Degradation observed
Water degradation	Distilled water – 72 hr –25°C	100%	0%	No degradation
Thermal degradation	40°C – 72 hr	99.11%	0.89%	No degradation
	90°C – 3 hr	97.87%	2.13%	No degradation
	90°C – 72 hr	42.83%	57.17%	Degradation observed
UV degradation for solution	80 μw/cm^2 –25°C with UV radiation at 315 – 400 nm – 40 hr	98.42%	1.58%	No degradation
UV degradation for solid		96.43%	3.57%	No degradation

for 72 hr resulted in 4.87% degradation. Degree of degradation of FLZ in presence of 1 M NaOH was 2.74 and 5.32%; for 72 hr exposure at 25°C and 3 hr reflux, respectively. FLZ methanolic solution showed no degradation in water (0%). After 40 hr of exposure to ultraviolet radiation, FLZ methanolic solution showed only 1.58% degradation.

No decomposition was seen on exposure of solid drug powder to ultraviolet light (only 3.57%), and on exposure to high temperature (90°C) for a 3 hr only 2.13% degradation was observed. After prolonged exposure to heat at 40°C for 72 hr, degradation was only 0.89% but in case of very drastic condition as high temperature (90°C) for a long time (72 hr), a remarked degradation was observed (57.17%).

In the case of hydrogen peroxide exposure, it was noted that using a low concentration of hydrogen peroxide (3%) at low temperature (25°C) did not show any degradation of FLZ (0% degradation), even for long period (3 d). A remarked degradation was observed (68.21%) with a concentrated hydrogen peroxide solution (35%) on high temperature (150°C) after a short period (Table 5). Hence, it can be called a thermo-oxidative degradation.

Oxidation Pathway of FLZ

Fluconazole was subjected to oxidation by refluxing with 35% hydrogen peroxide for 3 hr. On the other hand, oxidative degradation product was isolated and characterized by MS- and IR-spectrometry. Also

TLC-monitoring of the drug degradation was done on thin layer plates of silica gel F_{254} using chloroform:methanol:33% aqueous ammonia solution (8.5:1.5:0.1, *v/v/v*) as a developing solvent. The developed plates were visualized under short UV-lamp. The oxidative degradation product (R_f value = 0.02) could be separated elegantly from the intact drug (R_f value = 0.58).

It is noted that only one oxidation product is detected as one peak using the proposed HPLC method and as one spot in TLC using chloroform: methanol:33% aqueous ammonia solution (8.5:1.5:0.1, *v/v/v*) as a mobile phase. This main degradation product has a molecular weight of 223 while the second oxidation product that is expected from the scheme (Figure 2) has a molecular weight of 83. The latter is reported to be volatile[26] under high temperature during evaporation and removal of hydrogen peroxide. This scheme agrees with the fundamental basis of organic chemistry in which tertiary alcohols undergo to oxidation through the cleavage of C-C bond.[27]

Elucidation of the Main Degradation Product of FLZ

Mass Spectrometry

The structure of main oxidation product is evident and verified by structure elucidation and investigations made by MS and IR. In the MS chart of FLZ (Figure 4), there is a peak at 307 *m/z* (molecular ion, $[M+1]^+$); while an evident peak is present at 224 *m/z* in the MS chart of the oxidation product (Figure 5).

Infrared Spectrometry

IR spectra of FLZ and its main oxidative degradation product are shown in Figure 6 and Figure 7, respectively. The broad peak for OH stretch is present at 3132 cm^{-1} in oxidative degradation product spectrum, which confirms presence of hydroxyl group in it. Presence of a peak at 1620 cm^{-1} (C=N) in both spectra demonstrates the presence of a triazole ring in the oxidative degradation product as well as intact FLZ; this confirms that only one ring is detached from the parent compound during oxidation process.

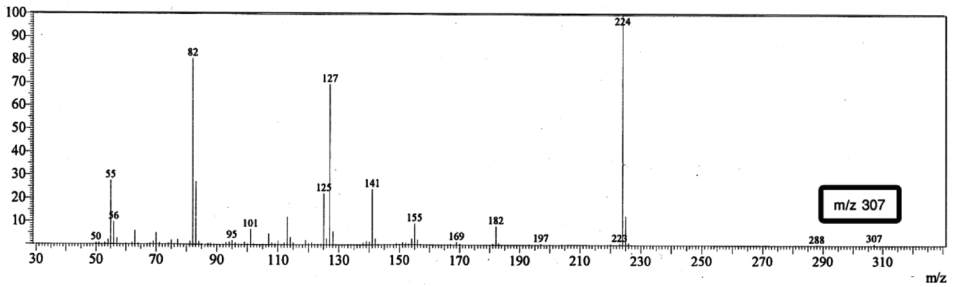

FIGURE 4 MS chart of fluconazole.

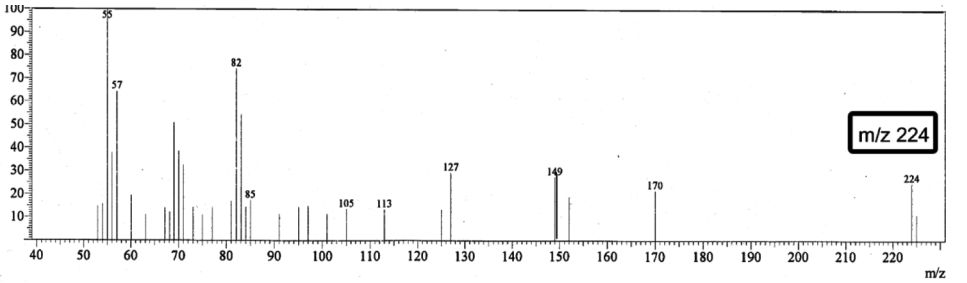

FIGURE 5 MS chart of the main oxidative degradation product.

N.B.: The products of thermal decomposition (90°c for 72 hr) were isolated, characterized as in case of hydrogen peroxide (35% for 3 hours). Structure elucidation using IR and MS showed no difference between thermal and thermo-oxidative degradation products.

Kinetic Order of Oxidative Degradation

To determine the kinetics of FLZ oxidative degradation with hydrogen peroxide, the remaining FLZ was plotted against time during the oxidation process (Figure 8). Using least square regression linear relationships with correlation coefficient ($r = 0.98$) was obtained indicating that FLZ under the oxidation process follows zero order kinetics. The degradation

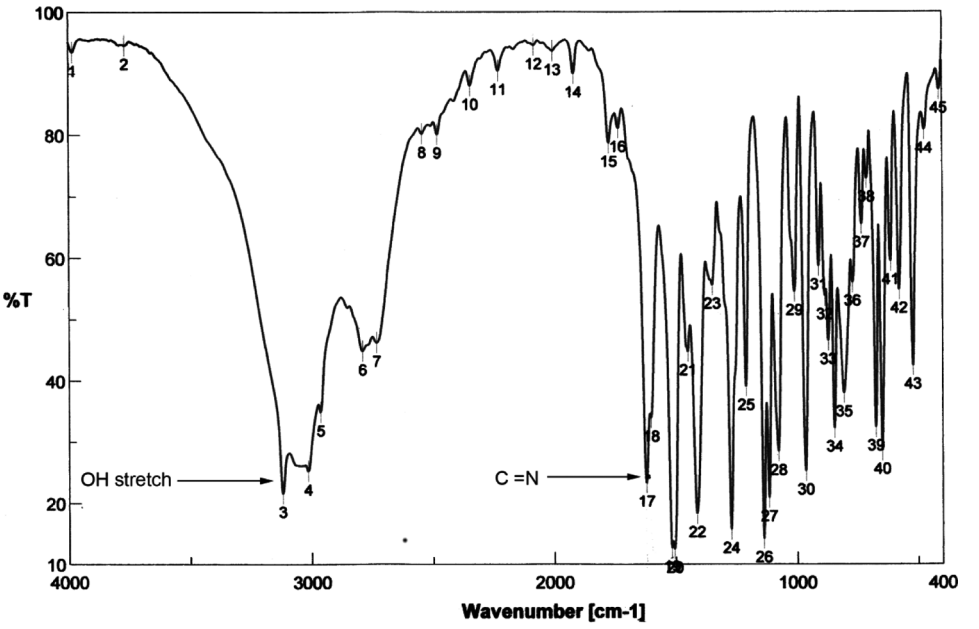

FIGURE 6 IR spectrum of fluconazole.

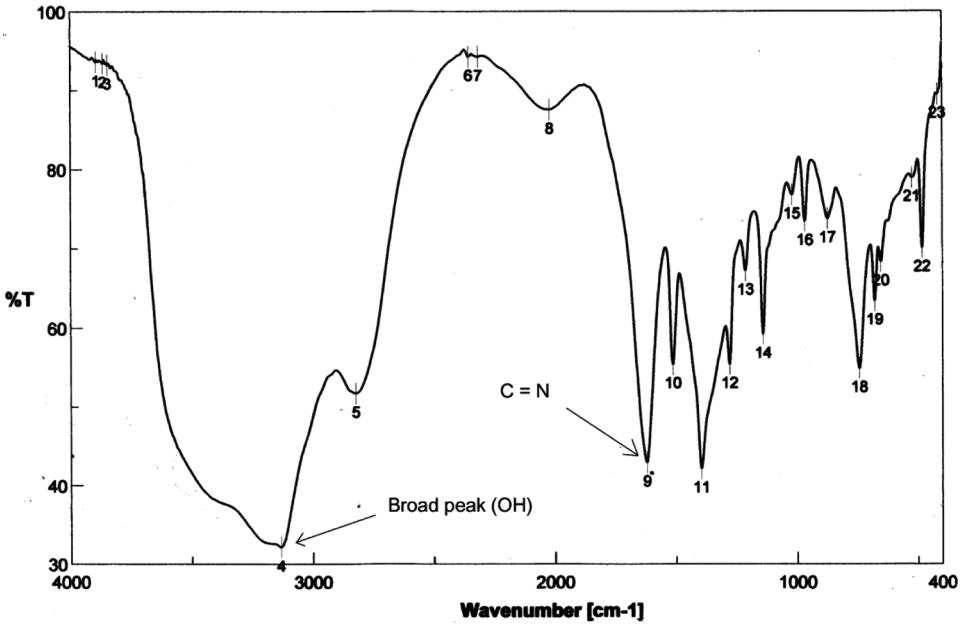

FIGURE 7 IR spectrum of main oxidative degradation product of fluconazole.

rate constant (k), half-life ($t_{1/2}$) for oxidative degradation of FLZ were -2.91×10^{-8} mole/min and 21.66 min, respectively.

Comparing the results of this study with other reported stability study for FLZ[10,20], it was found that the proposed HPLC method is more

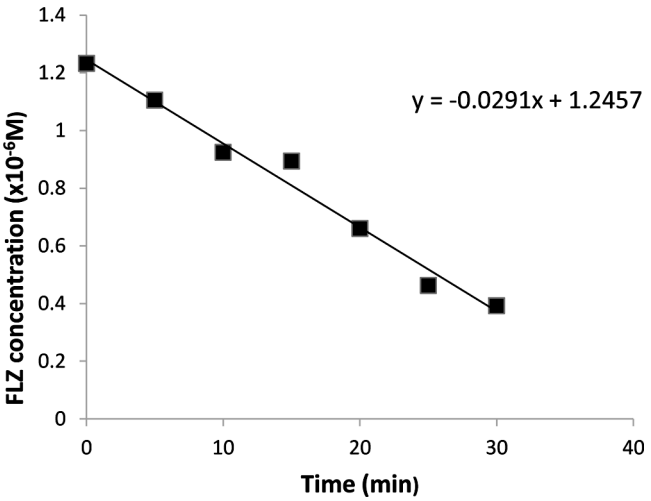

FIGURE 8 Zero order plot of fluconazole oxidative degradation with 35% hydrogen peroxide. (Color figure available online.)

sensitive than the reported ones; this is clear from its range ($0.5–50\,\mu g \cdot mL^{-1}$). The proposed stress study was carried out at more drastic conditions as higher concentrations (1 M) of hydrochloric acid and sodium hydroxide and higher temperature for a shorter time than the other stress study.[20] This gives more stress and assures better stability. Shorter experimental time gives more convenience to the method. In the proposed study, the main degradation product was separated; an oxidation scheme was suggested and proved by comparison to the supplied data of infrared and mass spectra of FLZ and that of main oxidative degradation product.

CONCLUSION

FLZ is a widely used drug and present in many antifungal formulations. It is important to find a simple, rapid, and specific method of its analysis especially in quality control laboratories. The suggested chromatographic method provides a simple, accurate, and reproducible stability-indicating means for its quantitative analysis in the presence of its oxidative degradation products. The developed HPLC method provides a good resolution between the two proposed components within suitable analysis time (5.5 min). It is highly specific and less expensive than UPLC. The proposed methods have advantage over other published methods of analyzing FLZ in presence of its degradation products. Therefore, the applied method could be useful for stability investigation of the active drug and checking the extent of degradation in pharmaceutical formulations. The proposed HPLC method is highly sensitive, and it may be used for analysis of the suggested drug in biological fluids.

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