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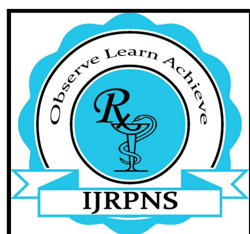

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VALIDATED STABILITY-INDICATING ULTRAVIOLET-SPECTROPHOTOMETRIC ASSAY OF KETACONAZOLE IN PHARMACEUTICALS

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ABSTRACT

A rapid, sensitive facile and stability-indicating UV-spectrophotometric method is described for the determination of ketoconazole, an antifungal drug, in bulk drug and formulations. The method is based on the measurement of absorbance of the drug solution in 0.1 M H_2SO_4 at 222 nm. Regression analysis of the Beer's law plot showed a good correlation in the concentration range 1.0-17.5 µg mL^{-1} with an apparent molar absorptivity value of 2.94×10^4 L mol^{-1} cm^{-1}. The limits of detection (LOD) and quantification (LOQ) were calculated to be 0.28 and 0.84 µg mL^{-1}, respectively. The method was validated for accuracy, precision, selectivity, robustness and ruggedness. The method was applied to formulations with a recovery of 97.10-100.5 % the standard deviation being 0.63-2.23. The drug was subjected to acid, base and hydrolytic, oxidative, thermal and photolytic stress conditions to determine its stability-indicating ability. Results showed that the drug underwent slight (~22%) degradation under base-hydrolysis stress conditions and remained intact under other stress conditions.

KEYWORDS

Ketoconazole, Assay, UV-spectrophotometry, Stability-indicating and Pharmaceuticals.

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INTRODUCTION

Ketoconazole (KTC), namely, 1-acetyl-4-[4-[[2-(2, 4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1, 3-dioxolan-4-yl]-methoxy] phenyl] piperazine (Figure No.1), is a synthetic imidazole derivative. Due to its advantages of oral administration, lower toxicity than most azole antimycotics, and effective against many fungal and gram positive microorganisms[1,2], KTC has been widely used as an antifungal drug. However, KTC can cause inhibition of cytochrome P-450 enzymes[3] and some phase II enzymes[4-7].

Some adverse reactions of KTC may lead to urticaria, angioedema, leukopenia, haemolytic anemia, nausea and thrombocytopenia[8,9]. Hence it is important to determine in body fluids and pharmaceutical formulations.

Several methods based on high performance liquid chromatography, HPLC[10-14], liquid chromatography-tandem mass spectrometry[15,16] spectrofluorimetry[17], and voltammetry[18,19]have been reported for the assay of drug in body fluids. The drug in pharmaceuticals has been assayed by method relying on HPLC[20-25], HPTLC[26],electrokinetic chromatography[27],capillary zone electrophoresis[25], electron paramagnetic resonance spectrometry[28], thermogravimetry[29], potentiometry[30-33], voltammetry[34-38], microbiological assay[39-41], visible spectrophotometry[42-48] and spectrofluorimetry[17,49-50]. Although these methods can yield satisfactory results, some of them usually require expensive instruments, time-consuming extraction or separation steps and critical pH adjustment.

UV-spectrophotometry offers the advantage of great speed and simplicity, low cost, sensitivity, wide linear dynamic range and above all short analysis time compared to other analytical techniques including voltammetry, visible-spectrophotometry and often chromatography. This technique has been applied to the assay of a variety of pharmaceuticals and includes isoniazid[51] terbinafine[52], repaglinide[53], dothipen[54], dexamethasome[55], resperidone[56], lamuvidine[57] and losartan[58] to mention a few. However, literature on the UV-spectrophotometric assay of KTC has been sparse with only a few methods reported sofar.

A direct method has been reported by Vojic*et al*[59] in which the absorbance of the drug solution or its tablet extract in 0.1 M-HCl was measured at 225 nm. Beer's law was obeyed over the concentration range, 3-30 µg mL^{-1}. With absorbance measurements being made at two wavelengths: 222 and 269 nm, in 0.01 M HCl, two direct methods for the determination of KTC in tablets and creams were developed by Kedor-Hackmann*et al*[60]. Beer's law was obeyed over 4-30 µg mL^{-1} (at 222 nm) and 100-280 µg mL^{-1} (at 269 nm). The drug in pharmaceutical emulsion was determined by Kedor-Hackmann[61] with first derivative mode using zero crossing method at 257 nm employing methanol as the diluent. Analytical curve was linear in the concentrationrange, 5-30 µg mL^{-1}. Two brands of emulsions containing KTC, when analysed by the method yielded percent of label claim of 101.5± 0.41 (brand-1) and 102.7±0.30 (brand-2). One more first derivative method, where the drug could be determined in the range, 2-20 µg mL^{-1}, has been described by Ragehy and Bagary[62].

Chemical stability of pharmaceutical substance is a matter of great concern as it affects the safety and efficacy of the drug product. The FDA and ICH guidance states the requirement of stability-indicating data to understand how the quality of drug substances and drug product changes with time under the influence of various environmental factors. Knowledge of the stability of the molecule helps in selecting proper formulation and package as well as providing proper storage conditions and shelf life which is essential for regulatory documentation[63-65]. Stability-testing of drug substance requires an accurate analytical method that quantitates active pharmaceutical ingredients(API) without interference from degradation products, process and other potential impurities[66]. With the advent of ICH guidelines, the requirement of establishing the stability-indicating assay method (SIAM) has become more clearly mandated. The guidelines explicitely require conduct of forced degradation studies under a variety of conditions like pH, oxidation, light, dry heat, etc[67]. But, none of the methods reported sofar, including the UV-spectrophotometric[59-62] is stability indicating.

Realising the importance of SIAM, and as an alternative to the existing sophisticated and complex methods, we propose to develop and validate a new direct UV-spectrophotometric method, to determine KTC in bulk drug and dosage forms, which is stability-indicating, by measuring the absorbance in 0.1M H_2SO_4.

Experimental

Instrument

Shimadzu Pharmaspec 1700 UV/visible spectrophotometer provided with 1-cm quartz cells was used for absorbance measurement.

Reagents and materials

All the chemicals used were of analytical reagent grade and double distilled water was used to prepare the solutions, whereever required. Hydrochloric acid, sulphuric acid, sodium hydroxide, and hydrogen peroxide were purchased from Merck, Mumbai, India. Pure Ketoconazole used as a reference material was received from Dr. Reddys Laboratories, Hyderabad, as a gift sample. Dosage forms containing KTC was purchased from local commercial sources. H_2SO_4 (0.1M) and HCl (1.0 M) were prepared by appropriate dilution of concentrated acids with water. NaOH (1.0 M) was prepared by dissolving the calculated quantity in water. H_2O_2 (5%) was prepared by appropriate dilution of the commercial sample (30%) with water.

Standard stock solution

A100 μg mL^{-1} KTC solution was prepared by dissolving 10 mg of pure drug in 0.1 M H_2SO_4, and diluting to 100 mL in a calibrated flask.

Assay procedures

Preparation of calibration graph

Varying aliquots (0.1, 0.25, 0.5 ...1.75 mL) of 100 μg mL^{-1} standard drug solution were measured accurately into 10 mL calibrated flasks and diluted to volume with 0.1M H_2SO_4. Absorbance of each solution was measured at 222 nm against 0.1 M H_2SO_4 blank.

A standard graph was prepared by plotting the absorbance values *vs* concentration of drug, and the concentration of the unknown was computed from the regression equation derived using the Beer's law data.

Procedure for dosage forms

Tablets

Finely grounded tablet powder equivalent to 10 mg KTC was quantitatively transferred into a 100 mL calibrated flask containing 60 mL 0.1 M H_2SO_4 and sonicated for 20 min, the content was diluted to the mark with 0.1 M H_2SO_4. The visible matter was filtered off using a quantitative filter paper. First 10 mL of the portion of the filtrate was discarded and subsequent portion was diluted to get 10 μg mL^{-1}. Five mL aliquot was then subjected to analysis in five replicates following the procedure described above.

Cream

A 0.5 g quantity of cream formulation which is equivalent to 10 mg of KTC was weighed into 100 mL of calibrated flask containing 60 mL of 0.1 M H_2SO_4. Sonicated the flask in ultrasonicator for 20 min to dissolve the drug. Finally diluted to the volume with 0.1 mL H_2SO_4. Then the solution was filtered using 0.22 μm nylon membrane filter. One mL aliquot was then subjected to analysis in five replicates following the procedure described above.

Topical solution

A 0.5 mL aliquot of topical solution which is equivalent to 10 mg of KTC was accurately measured and transferred into a 100 mL of calibrated flask containing 60 mL of 0.1 M H_2SO_4. Then, the steps described under cream were followed.

Procedure for placebo and synthetic mixture analysis

A placebo blank was prepared by mixing talk (20 mg), starch (30 mg), lactose (10 mg), sodium alginate (10 mg), magnesium stearate (20 mg), cellulose microcrystalline (20 mg) and acacia (50 mg) to obtain a homogeneous mixture. A 10 mg portion of the placebo was taken and its solution prepared by following the procedure described under "procedure for tablets". To 10 mg of the placebo prepared above 10 mg of pure KTC was added, homogenised and transferred to into 100 mL calibrated flask containing 60 mL 0.1M H_2SO_4 and its solution prepared as described under procedure for 'tablets'. One mL of the mixture solution (100 μg mL^{-1} in KTC) was analysed in five replicates following the general procedure.

Procedure for forced degradation study

Four 10mgportions of pure KTC were transferred to four separate 100 mL calibrated flasks. The drug was mixed with5mL of 1.0 M HCl, 5 mL of 1.0 M NaOH, 5 mL of 5% H_2O_2 or 5 mL of water. The

flasks were kept in a water bath maintained at 80°C for 3 h. The flasks were cooled, neutralised with 5 mL of 1.0 M NaOH (for acid hydrolysis) and 5 mL of 1.0 M HCl (for base hydrolysis) and contents of all the flasks were diluted to mark with 0.1 M H_2SO_4. For thermal degradation, solid sample was kept in a petridish in an oven at 105°C for 24 h. After cooling, 100 µg mL^{-1} KTC solutions were prepared in 0.1 M H_2SO_4. For photo degradation, the solid sample was exposed to 1.2 million lux hours of visible radiation and 200 wh m^{-2} of UV radiation and its solution (100 µg mL^{-1}) in 0.1 M H_2SO_4 solution prepared. One mL aliquot of each of the above solutions was taken and absorbance measured at 222 nm against blank prepared in the same way as sample but without taking drug.

RESULTS AND DISCUSSION

The absorption of 10 µg mL^{-1} KTC solution in 0.1 M H_2SO_4 recorded between 200 and 400 nm showed an absorption maximum at 222 nm, and at this wavelength 0.1 M H_2SO_4 has insignificant absorbance (Figure No.2), and, hence 222 nm was chosen as the analytical wavelength for the assay.

Method validation

The method was validated according to ICH Q2B guidelines for linearity, sensitivity, LOD, LOQ, accuracy and precision, selectivity, robustness and ruggedness.

Linearity, sensitivity, LOD and LOQ

Calibration graph for KTC was obtained throughout the concentration range studied (Figure No.3) 1-17.5 µg mL^{-1}. Regression analysis was performed for the Beer's law data on the results are presented in Table No.1. Sensitivity parameters such as molar absorptivity and send ell sensitivity values were also calculated from the Beer's law data and are compiled in Table No.1.

LOD and LOQ values were calculated using the equations

$$LOD = \frac{3.3s}{m} \qquad LOQ = \frac{10s}{m}$$

Where "s" is the standard deviation of replicate absorbances of blanks and "m" is the slope of the calibration curve. The calculated values are given in Table No.1.

Precision and Accuracy

To ascertain the precision and accuracy of the method, standard drug solution at three concentration levels was assayed on the same day (intraday) in seven replicates and on five consecutive days (inter-day) by preparing the solutions after each day. The results of precision expressed as % relative standard deviation (%RSD) and accuracy expressed as percent relative error (%RE) are summarised in Table No.2 and speak of excellent precision and accuracy of the proposed method.

Selectivity

The absorbance of the placebo blank solution was the same as that of 0.1M H_2SO_4. The synthetic mixture solution at 10 µg mL^{-1} level when analysed yielded a percent recovery of 98.56 ± 1.26 indicating non-interference from the usual tablet excipients. The specimen UV-spectra of placebo, synthetic mixture and tablet extract shown in Figure No.4a to 4c supplement the above conclusion since no peaks were observed for the placebo blank and no additional peaks were observed in respect of tablet extract.

Robustness and ruggedness

The robustness of the method was assessed by making small alterations in the wavelength of measurement (222 and 222±1nm). Ruggedness was determined by analysis of standard solution at three concentration levels by three analysts and also by a single analyst using three different cuvettes with the same instruments. The results, expressed as intermediate precision (%RSD) which are ≤ 0.98% indicate that the method's performance is unaffected by small alterations in the experimental variable and instruments as well as personnel performing the analysis Table No.3.

Application to dosage forms:

The results of analysis of formulations containing KTC by the proposed method are shown in Table No.4. The same batch formulations were also assayed by the reference method[68] in which sample in a mixture of anhydrous acetic acid and methyl

ethyl ketone was titrated with perchloric acid, and the end point being determined potentiometrically for comparison. The statical evaluation of the results as shown by Student's t-value and F-value at the 95% confidence level did not indicate any difference between the proposed method and the reference method with respect to accuracy and precision.

Accuracy by recovery study

Pre-analysed tablet powder was spiked with pure KTC at three levels and the total was found by the proposed methods. The added KTC recovery ranged from 98.23 to 100.60% with a standard deviation ≤ 0.95% indicating the absence of matrix effect in the assay as shown by the results in Table No.5.

Results of degradation study

The drug was subjected to aqueous, acid and base hydrolysis and oxidative (H_2O_2) stress conditions in solution state and thermal and photo degradation in solid state. The effect was studied by comparing the UV-spectra of the solution before and after forced degradation. The results of this study, shown in Table No.6 revealed that the drug undergoes slight degradation (upto ~22%) under base-induced stress condition and remained stable to other stress conditions (Figure No.5a to 5f).

Table No.1: Sensitivity and regression parameters

S.No	Parameter	Value
1	λ_{max}, nm	222
2	Linear range, µg mL^{-1}	1.0 - 17.5
3	Molar absorptivity (ε), L mol^{-1} cm^{-1}	2.94 X 10^4
4	Sand ell sensitivity*, µg cm^{-2}	0.0181
5	Regression (Y^{**} = a + bX)	
6	Slope (b)	0.0554
7	Intercept (a)	-0.0005
8	Standard deviation of intercept (S_a)	0.0029
9	Standard deviation of Slope (S_b)	0.00027
10	Standard deviation about regression (S_r)	0.0043
11	Regression co-efficient (r^2)	0.9999
12	Limit of detection (LOD, µg mL^{-1})	0.28
13	Limit of quantification (LOQ, µg mL^{-1})	0.84

* Limit of determination as the weight in µg per mL of solution, which corresponds to an absorbance of A=0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1cm. **Y=a+bX, where Y is the absorbance, X is concentration in µg mL^{-1}, a is intercept, b is slope.

Table No.2: Evaluation of intra-day and inter day accuracy and precision

S.No	KTC taken µg mL^{-1}	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=6)		
		KTC found, µg mL^{-1}	% RE	%RSD	KTC found, µg mL^{-1}	% RE	%RSD
1	5	5.04	0.8	1.51	4.99	-0.2	0.92
2	10	9.85	-1.5	0.51	10.01	0.1	0.79
3	15	14.88	-0.8	0.34	14.88	-0.8	0.24

% RE. Percent relative error, %RSD. Relative standard deviation.

Table No.3: Method robustness and ruggedness expressed as intermediate precision (%RSD)

S.No	KTC taken µg mL^{-1}	Robustness# (%RSD)	Ruggedness	
			Inter-analysts (%RSD), (n=3)	Inter-cuvettes (%RSD), (n=3)
1	5	0.92	1.1	0.98
2	10	0.85	0.95	0.85
3	15	0.76	0.68	0.80
#Wavelengths used were 221, 222 and 223.				

Table No.4: Results of determination of KTC in tablets, cream, topical solution and statistical comparison with the reference method

S.No	Formulation brand name	Nominal amount	Found* (Percent of label claim ± SD)	
			Reference method	Proposed method
1	Ketozole tablets*	200 mg/tablet	99.1±0.82	97.72±1.84
				t=1.53
				F=5.05
2	Fungicide tablets**	200 mg/tablet	98.5±1.10	98.42±2.23
				t=0.07
				F=4.12
3	Funginoc cream**	2%	99.4±1.40	100.5±0.63
				t=1.61
				F=4.95
4	Danket topical solution$	2%	98.5±1.70	97.10±1.08
				t=1.56
				F=2.47
* Mean value of 5 determinations.				
Tabulated t-value at the 95% confidence level and for four degrees of freedom is 2.77. Tabulated F-value at the 95% confidence level and for four degrees of freedom is 6.39.				
Marketed by: *Ranbaxy, **Torrent Pharmaceuticals, $ Dermacare Laboratories				

Table No.5: Results of recovery study by standard addition method

S.No	Tablet	KTC in tablet, µg mL^{-1}	Pure KTC added, µg mL^{-1}	Total found, µg mL^{-1}	Pure KTC recovered*, Percent ± SD
1	Ketozole	4.93	2.5	7.39	99.42±0.61
		4.93	5	9.99	100.60±0.53
		4.93	10	14.9	99.80±0.34
2	Fungicide	4.96	2.5	7.33	98.30±0.95
		4.96	5	9.78	98.23±0.59
		4.96	10	14.73	98.44±0.47
*Mean value of three determinations					

Table No.6: Percentage degradation of KTC in various stress conditions

S.No	Condition	% Degradation
1	Acidic degradation	-2.2
2	Base degradation	21.9
3	Water hydrolysis	-0.5
4	Thermal degradation	0.0
5	Photolytic degradation	-1.5
6	Oxidative degradation	1.1

CONCLUSION

The proposed method is simple, rapid, selective and sensitive for the determination of KTC in bulk drug and formulations. It can be easily adopted to routine quality control in quality control laboratories of pharmaceutical industries since it is free from matrix effect.

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COMPETING INTERESTS

Here by we declare that we don't have any competing interests (*Financial or Non-financial).*

AUTHORS' CONTRIBUTIONS

BK participated in conception and design of the study. Coordinated and helped to draft the manuscript and revising it critically for important intellectual content. VKP involved in literature collection and carried out the computational experiments. Acquired the data, and analysis and interpretation of data. Both the authors read and approved the final manuscript.

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