THE SUPRAMICELLAR SOLUTIONS OF POLYMERIC SURFACTANT (PEG 400) FOR THE DETERMINATION OF POORLY SOLUBLE ANTIFUNGAL DRUG

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Abstract. The purpose of present study is to investigate the effect of Polyethylene glycol (PEG) upon the solubility of antifungal drug Ketoconazole (KTCZ). KTCZ is water insoluble drug. For water insoluble drugs difficulties are usually encountered in selecting dissolution medium with a good discriminating power. In the present study, a dissolution medium based on solubility data is developed for KTCZ. The solubility of KTCZ in various fluids such as purified water (pH 6.4), cetyl trimethyl ammonium bromide (CTAB), sodium lauryl sulphate (SLS), Polyethylene glycol (PEG) and also in 0.1 M sulphuric acid containing SLS, CTAB, PEG (at their critical micellar concentrations (CMC) values) was determined. The solubility of KTCZ is increased by 2.51 fold in PEG + purified water system. The surprising increase in solubility of same drug by 7.29 fold is found in PEG + 0.1 M H_2SO_4 system. Polymeric surfactants (PEG 400) form nanoscopic core-shell structures above the critical micellar concentrations. The hydrophobic drugs while the hydrophobic part serve as reservoirs for hydrophobic drugs while the hydrophilic part serves as interface between the bulk aqueous phase and the hydrophobic domain. This unique architecture enables polymeric micelles to serve as nanoscopic depots or stabilizers for poorly water-soluble compounds. A new spectrophotometric method was also proposed for the determination of KTCZ in pure form and pharmaceutical formulation. This method is based on the redox-complexation reactions, which proceed in the ketoconazole, ammonium metavanadate and PEG system and form a red-brown colored complex with absorption maxima of V(V) at 375 nm. A linear calibration graph was obtained between 1.06 µg/mL – 21.25 µg/mL of KTCZ. The results of analysis have been validated statistically and by recovery studies. The proposed method is simple, rapid, sensitive and economic.

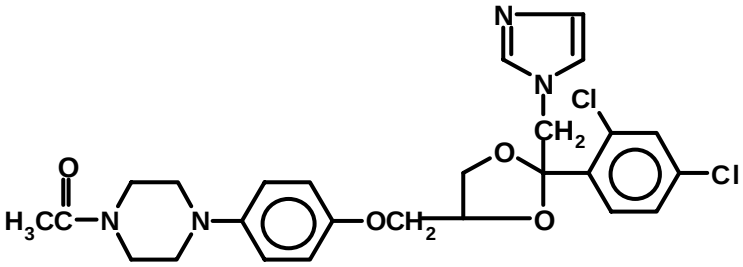
Keywords: spectrophotometry, ketoconazole, formulations, validation, solubility, polymeric surfactant

Introduction

Ketoconazole,cis-1-acetyl-4[4-(2-(2,4-dichlorophenyle]-2-(1H-imidazole-1ylmethyl)-1,3-dioxolon-4-yl]methoxy piperazin (KTCZ) is a highly effective broad spectrum antifungal agents. It is used to treat a wide variety of superficial and systematic mycoses [1,2] and has the advantages over other imidazole derivative of producing adequate sustained release blood level following oral administration [3]. KTCZ is poorly – water soluble drug. Oral administration of therapeutic agents represents by far the easiest and most convenient route of drug delivery, especially in case of chronic therapies [4]. In the case of poorly soluble drugs, the dissolution time in the gastrointestinal contents may be longer than the transmit time to the intended absorptive sites [5]. Therefore, dissolution of drugs is quit often the rate-limiting which, ultimately, controls the bioavailability of the drug [6].

Micelles as drug carriers provide a set of advantages – they physically entrap sparingly soluble pharmaceutics and deliver then to the desired site of action at concentration that can exceed their intrinsic water solubility and thus increase their bioavailability [7].

Due to the vital importance of KTCZ determination in pharmaceutical preparations and in biological fluids, several chromatographic [8], spectroscopic [9,10] and electrochemical methods [11-13] for its quantitative determination have been reported. However, some of these methods need expensive equipments or are time consuming. This method is accurate, reproducible and economical which can be useful for the routine analysis of drugs in laboratory.



In this article we also investigate the effect of polyethylene glycol (PEG) upon the solubility of KTCZ. Because KTCZ is slightly soluble in water.

Experimental: apparatus and reagent

An Elico model SL-164 UV-Vis-spectrophotometer with 1.0 cm matched quartz cell was used for all spectral and absorbance measurements. KTCZ was obtained as gift sample Glenmark pharmaceutical, Mumbai. Ammonium metavanadate purchased from Loba chemie, and polyethylene glycol obtained from Sigma as a gift sample.

Ammonium metavanadate (0.1mol dm^{-3}): Oxidant solution of ammonium metavanadate (Loba chemie) is used as a primary standard [14]. The solution of oxidant was prepared by suspending weighed in a standard H_2SO_4 (AR, BDH) till a clear solution was obtained. The solution so prepared remains stable for 1 month, and hence used as such stock solution. All of the chemicals used in this study were of the highest purity available and used without further purification.

Ketoconazole: 0.01mol dm^{-3} stock solution of KTCZ (Glenmark Pharmacetical, Mumbai) was prepared by dissolving about 531 mg of drug in standard sulphuric acid and made up to the mark in 100 ml volumetric flask. The above stock solution was further diluted to get a working standard solution of 1 µg to 500 µg/mL.

Polyethylene glycol: 0.36 mL of PEG dissolve in 100 mL distilled water and diluted to get a 1.25 x 10^{-4} mol dm^{-3}(CMC of PEG).

Cetyl trimethyl ammonium bromide: 0.364 g of CTAB is dissolve in 100 ml distilled water and diluted the solution to get a 8.24 x 10^{-5}mol dm^{-3} (CMC of CTAB) solution.

Sodium lauryl sulphate: An aqueous solution of SLS (0.01mol dm^{-3}) was prepared by dissolving 288 mg of SLS in a 100 ml calibrated flask with water and diluted the solution to get a 2.78 x 10^{-3} mol dm^{-3}(CMC of SLS) solution.

Procedure

Solubility determination

Excess KTCZ (50mg) was added to 15 mL of each liquid such as PEG, CTAB, SLS(at their cmc value), 0.1 mol dm^{-3} H_2SO_4 + PEG, 0.1 mol dm^{-3} H_2SO_4 + CTAB, 0.1mol dm^{-3} H_2SO_4 + SLS taken in a 25 ml stopper flask and the mixtures were shaken 15 hrs at room temperature (28°C ± 1°C) on a rotary flask shaker. 5 ml aliquot were withdrawn at 1 hr interval and filter immediately using a 0.45 µ disc filter. The filtered sample were diluted suitably and assayed for KTCZ by UV-spectrophotometric method. Shaking continued until two consecutive estimation are the same.

Preparation of calibration curve ranging

Aliquot of standard solution of KTCZ ranging from 0.002 – 0.8 mL were transferred to a series of 10 mL volumetric flask. To that 0.1 mL of ammonium metavanadate (0.01mol dm^{-3}) and 0.125 mL of PEG (0.000125mol dm^{-3}) were successively added, and than added distilled water up to the mark. The absorbance of light pink colored species formed was measured at 375 nm of KTCZ present in the sample solution. The amount of KTCZ was obtained from its calibration curve.

Assay procedure

At least four tablets of the drug weighed in to a small dish, powdered and mixed well. A portion equivalent 200mg was weighed and dissolved in 1 ml of sulphuric acid

and made up to the mark using distilled water. Aliquot of the test solution was diluted in 10 ml in test tube. An aliquot of diluted drug solution was then treated as described above in pure drug.

Results and discussion

Solubility plays a prime role in the dissolution of a drug substance from a solid dosage form, correlation between solubility and dissolution rate of different drug substance in various media are well established [15-16]. In this study, solubility data will be used as basis for the development of dissolution medium for KTCZ in future. The solubility of KTCZ was determined at 37°C ± 1°C) in different fluids (shown in Table 1).

Table 1. Determination of solubility of ketoconazole in different fluids:

S. No.	Sample	Wt. of drug	Overall volume	Solubility in %	Solubility increase in fold
1.	KTCZ + Distilled Water	50	15	100.00	1.00
2.	KTCZ + SLS (at CMC)	50	15	143.78	1.43
3.	KTCZ + CTAB (at CMC)	50	15	155.72	1.55
4.	KTCZ + PEG (at CMC)	50	15	251.13	2.51
5.	KTCZ + 0.1 mol dm^{-3} H_2SO_4 + SLS(at CMC)	50	15	335.45	3.35
6.	KTCZ + 0.1 mol dm^{-3} H_2SO_4 + CTAB(at CMC)	50	15	450.74	4.507
7.	KTCZ + 0.1 mol dm^{-3} H_2SO_4 + PEG(at CMC)	50	15	729.00	7.29

Surfactant greatly increases the solubility of KTCZ. The solubility of KTCZ is increased by 2.51 fold in PEG + purified water system. The surprising increase in solubility of same drug by 7.29 fold is found in acidic PEG i.e. PEG + 0.1 mol dm^{-3} H_2SO_4 system.

Validation of the method

Optical characteristics such as Beer's law limits, molar absorbitivity and Sandell's sensitivity for KTCZ, are given in Table 1. Beer's law obeyed in the concentration range of 1.06µg/mL – 21.25µg/mL which is shown in Fig.1. Data of the regression analysis made for the calibration curve are also determined (Table 2). The accuracy

and precision of the method made for the calibration checked by analyzing the Beer's law range containing the same amount of each drug.

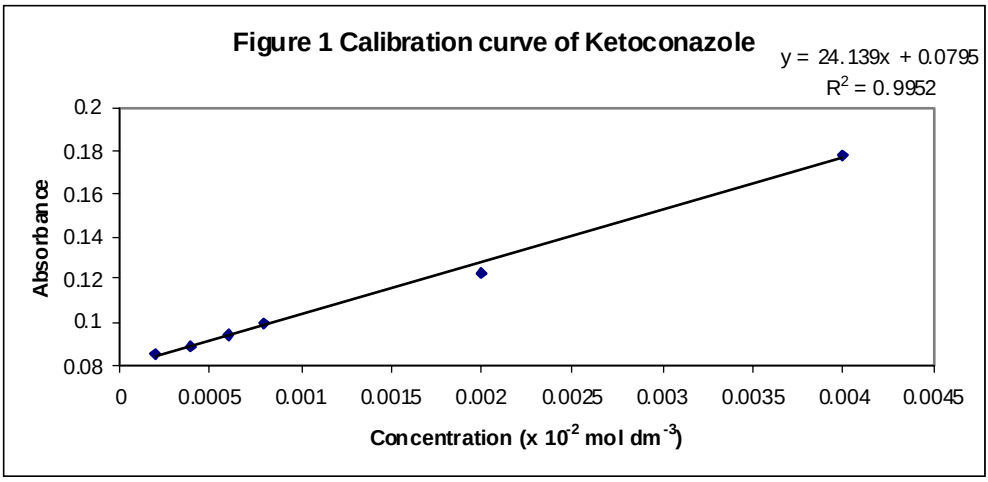


Fig. 1. Calibration curve of ketoconazole

Table 2. Results from assay of ketoconazole in tablets

S. No.	Parameters	Ketoconazole
1.	λ_{max}	375nm
2.	Beer's Range(µg/mL)	1.06 µg/mL - 21.25 µg/mL
3.	Molar Absorbtivity(L mol^{1} cm^{-1})	1.1898×10^{4}
4.	Sandell's Sensitivity(mol^{-1} L^{-2} cm)	0.0446
5.	Stability(h)	24
6.	Regression equation	0.9952
7.	Intercept	0.0795
8.	Slope	24.139×10^{2}
9.	Limit of Detection(mg/mL)	4.36×10^{-5}
11.	Standard Deviation of calibration line	0.03512

LOD and LOQ determination

The limit of detection (LOD) and the limit of quantization (LOQ) were determined by using the following equations

$$LOD = 3SD/m$$

$$LOQ = 10SD/m$$

Where SD is the standard deviation of the absorbance values of the second smallest concentration, m is the slope of the calibration curve [17]. The result obtained for pure drug was reproducible with low relative standard deviation (RSD).

The accuracy of the proposed method was checked by performing recovery experiments. For this, a known amount of the pure drug was added to pre-analyzed dosage forms and then determined by the recommended procedure. The result obtained in Table 3 showed that the mean recovery and relative standard deviation were in the range of 99.55±0.511 to 100.73±0.777 respectively. These results also suggested that there is no interference from the common excipients present in dosage forms.

Table 3. Results from recovery study

Commercial formulation analyzed	Drug content	Label claim in mg	% Recovery±SD
Phytorol	KTCZ	100 mg	99.55±0.51
Tocon	KTCZ	100 mg	99.36±0.66
Nizral	KTCZ	100 mg	101.73±0.77

Product identification of ketoconazole

Ketoconazole is a weak base, virtually insoluble in water, and are ionized only at low acidity. Consequently, Oxidation of these compounds and vanadium species are heavily dependent on acidity.

KTCZ is chiral drugs, and each is used clinically as stereo isomeric mixture. The drug is infact a mixture of the two race mates. i.e. four stereo isomers. In all four stereo isomers the hydrogen and the dichlorophenyl substituents at the two chiral centers of the dioxolane ring.

Main oxidation pathways were oxidative scission of the dioxolane ring, oxidative degradation of the piperazine ring and aliphatic oxidation and N-dealkylation at the 1-methyl propyl substituent. As a result of the various oxidation pathways, a very large number of products was formed, each representing less than 1-5%. The main oxidation product ND-Ketoconazole is characterized.

Mechanism proposed for ketoconazole

In preliminary experiments, it was observed that by oxidation of V(V) in presence of PEG to acidic solution of KTCZ, at first a pink acidic solution was obtained which become brownish during a few minutes. This is due to oxidation of KTCZ to unstable pink-red keto cation radical KTCZ. On the other hand, it found that the color of chemically produced cation radical was immediately disappeared in the presence of V(V) i.e. completely oxidized to products. Based on preliminary tests and mass spectra the oxidation product was identified as N-desalkyl-KTCZ and corresponding ketone.

$$VO_2^+ + H_3O^+ + \overset{K_1}{\rightleftharpoons} V(OH)_3^{2+} \quad \text{.....(1)}$$

$$V(OH)_3^{2+} + HSO_4^- \overset{K_2}{\rightleftharpoons} [V(OH)_3HSO_4]^+ \text{.....(2)}$$

[A]

[A] + Ktcz $\rightleftharpoons$ Ktcz - V(V) .....(3)

(B) Intermediate complex

[B] + D_n $\rightleftharpoons$ Ktcz - V(V) with D_n .....(4)

[C] Adduct

[C] $\xrightarrow[\text{Proton transfer}]{\text{Fast}}$ OH — Ktcz (D_n) + V(IV) .....(5)

[D] Hydroxy-Ktcz

[D] + V(V) $\xrightarrow[\text{Dealkylation}]{\text{slow}}$ ND — Ktcz + V(IV) + D_n .....(6)

Applying the condition for steady state approximation the above scheme leads to following rate equation

$$\frac{[V(OH)_3HSO_4]}{[V(OH)_3^{2+}][HSO_4]} = K_2$$

$[V(OH)_3HSO_4^+] = K2\ [V(OH)_3^{2+}]$ .....(7)

$$\frac{[V(OH)_3^{2+}]}{[VO_2^{2+}]\ [H^+]} = K_1$$

$[V\ (OH)_3^{2+}] = K_1\ [VO_3^{2+}]\ [H^+]$ ..... (8)

Putting Eq (2) in Eq (1), we get,

$[V(OH)_3HSO_4^+] = K_1\ K_2\ [VO_2^+]\ [H^+]\ [HSO_4^-]$

Rate = k [D]

Rate = $K_1\ K_2\ K_3\ [V(OH)_3HSO_4^+]\ [HSO_4^-]$ .....(9)

Rate = $K_1\ K_2\ K_3\ [VO_2^+][H^+][HSO_4^-]$ .....(10)

$[V(V)]T = [VO_2^+] + [V(OH)_2HSO_4^+] + [D]$

$[V(V)]T = [VO_2+]+K_1K_2[VO_2^+][H^+][HSO_4^-]+K_1K_2K_3[C][VO_2^+]\ [H^+][HSO_4^-]$

$[V(V)]T = ([VO_2^+])+(1+K_1K_2[H^+][HSO_4^-]+K_1K_2K_3[C][H^+][HSO_4^-]$ .....(11)

$$[VO_2^+] = \frac{[V(V)]_T}{1+K_1K_2[H^+][HSO_4^-]+K_1K_2K_3[C][H^+][HSO_4^-]} \quad \text{.....(12)}$$

Putting $[VO_2^+]$ in Eq. (13)

$$\text{Rate} = \frac{k\,K_1K_2K_3[C][H^+][HSO_4^-][V(V)]_T}{1+ K_1K_2[H^+][HSO_4^-]+K_1K_2K_3[C][H^+][HSO_4^-]} \quad \text{.....(14)}$$

$K_2 << 1$

$$k_{obs} = \frac{Rate}{[V(V)]_T}$$

$k_{obs} = k'[C][H+][HSO_4^-]$

Where $k' = k\ K_1K_2K_3$

$k_{obs}\ \alpha\ [C][H^+]$

Above rate is agreement of experimental finding i.e. rate of oxidation (as well as solubility) is governed by physiochemical properties of drug and pharmacokinetics.

Conclusions

The proposed method is found to be more sensitive and it does not require any pretreatment of the drug and tedious extraction procedure prior to its determination. The other ingredients and excipients usually present in pharmaceutical dosage form did not interfere in the estimation when some commercial dosage forms were analyzed by this method. The accuracy of this method was confirmed by the recoveries studies by adding a known amount of the pure drug to the formulations already analyzed by this method. The method was successfully applied to enable estimation of drug in pharmaceutical dosage forms at a lower concentration level (each tablet claiming 100 mg) and complete with other existing assay methods for routine quality control analysis of KTCZ in pharmaceutical formulations.

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