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ORIGINAL ARTICLE

Spectrophotometric method for the determination of ketoconazole based on amplification reactions

Shaligram S. Rane[a], P. Padmaja[b,*]

[a]*Department of Applied Chemistry, Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, Vadodara, India*
[b]*Department of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, India*

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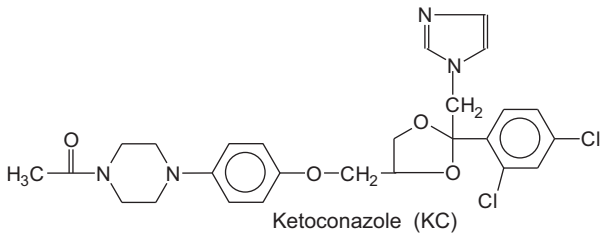
Abstract This paper describes a sensitive spectrophotometric method developed for determination of Ketoconazole (KC) in tablets based on amplification reactions. Ketoconazole was oxidized with periodate, resulting in formation of KC^{2+} and iodate ions. After masking the excess periodate with molybdate, the iodate was treated with iodide to release iodine. The liberated iodine was transformed to ICl_2^- 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 μg/mL and 1.7088 μg/mL of Ketoconazole with a molar absorptivity of 5×10^5 mol · L^{-1} cm^{-1}. The procedure was successfully applied for the determination of ketoconazole in tablet formulation.

1. Introduction

Ketoconazole (KC), Cis-1-acetyl-4-[4-[2-(2, 4-dichlorophenyl)-2-(1H-imidazole-1-ylmethyl)-1,3-dioxolon-4-yl] methoxy piperazine, is a highly effective broad spectrum antifungal agent. Ketoconazole has been determined in pharmaceutical preparations and in bio-logical fluids by spectroscopic [1–13], chromatographic [14–19] and electrochemical methods [20–24]. However, some of these methods need expensive equipment and/or are time consuming.



Ketoconazole (KC)

Amplification reactions have been the subject of extensive research in analytical chemistry for more than a century. Amplification reactions provide a chemical means of enhancing the sensitivity of an analytical measurement.

*Correspondence to: Department of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, India. Tel.: +91 9376214733; fax: +91 265 795552/331130.
E-mail addresses: Shaligram.rane@intasbiopharma.co.in (S.S. Rane), p_padmaja2001@yahoo.com (P. Padmaja)

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Amplification reaction is defined as a reaction, which replaces the conventional reaction used in a particular determination so that a more favorable measurement can be made. The sequence can be repeated to provide a further favorable increase in measurement. The liepert reaction for the determination of iodide is the most important amplification reaction, since many indirect and exchange reactions utilize same cycle of reaction

$$I^- \rightarrow IO_3^- \rightarrow 6I^- \quad \text{(a)}$$

where each iodide gives rise to six iodine atoms.

Recently a method was described for the determination of arsenic, after selective separation as AsI_3. In presence of chloride and acid, the reaction of arsenic (III) and the associated iodide with excess iodate caused the oxidation of the generated iodine and its stabilization as ICl_2 species. The anionic iodine complex was extracted into benzene as ion pair with Rh6G (Rhodamine 6G) for spectrophotometric determination at 535 nm [25]. Similarly amplification reactions were used for the determination of mercury [26]. The method involved selective separation of mercury (II) as tetra-iodo mercury (II) and oxidation of the associated iodide to iodate using bromine water. The iodate then formed when reacted with iodide in the presence of chloride in acid medium facilitated the formation of anionic iodine complex for extraction as ion pair with rhodomine 6G in to benzene.

Since periodate is known to react with mercury to form mercury (II) para periodate and also has a favorable potential for oxidations of organic compound, the formation of mercury (II) para periodate was put to advantage for the formation of anionic iodine complex for the determination of inorganic and organo-mercury (II) species present at trace levels [27].

It has been reported that electro-oxidation of ketoconazole takes place in aqueous as well as in non-aqueous media. Ketoconazole was initially oxidized with the loss of one electron to form KC (+1) cation radical. Also it has been reported that KC (+1) can be further oxidized with the loss of second electron to give some stable products [28].

It thus seemed worthwhile to examine the possibility of oxidation of KC with periodate so as to develop a sensitive spectrophotometric method for determination of KC.

2. Experimental

2.1. Instrumentation

A UV 1601 spectrophotometer (Shimadzu, Tokyo) with a 10 mm matched silica cells was used for all spectral measurements. All pH values were measured on a Thermo Orion, pH meter (Model: 420 pH/mV) using a combined electrode.

2.2. Chemicals/standard

All chemicals, i.e. sodium periodate, ammonium molybdate, ammonia, potassium iodide, sulphuric acid, toluene, potassium iodate, sodium chloride, rhodamine 6G, sodium sulphate, were of the highest purity available (AR grade) and used without further purification. Water (HPLC grade) was used to prepare all solutions. Ketoconazole USP grade material and tablet containing ketoconazole (Phytoral) were used for preparation of standard and assay solutions, respectively.

2.3. Standard ketoconazole solution

A stock solution of standard KC was prepared by dissolving ~26.7 mg of KC (USP grade) in HPLC grade water containing few drops of 0.5 M sulphuric acid solution [28] and further diluted to 50 mL using HPLC grade water. Working standards were prepared by suitable dilution of an aliquot of the stock solution.

2.4. Procedure

The method reported elsewhere was followed for oxidation of KC by periodate and formation of ICl_2^- [26]. To the sample solution containing not more than 0.0054 mg of KC in a 50 mL beaker, 1.5 mL of 0.01 M of sodium periodate solution was added, pH was adjusted up to 3 by addition of dil. ammonia solution. The solution was stirred well, followed by the addition of 1 mL of 0.1% of ammonium molybdenum solution and again the pH was adjusted to 3. The solution was transferred to a separatory funnel and 2 mL of 0.1 M KI solution (freshly prepared) followed by 2 mL of 0.5 M sulphuric acid solution was added. The solution was diluted to about 25 mL with water and was made to stand for 2 min. The solution was then shaken with 10 mL of toluene for few seconds. The organic layer was separated and washed twice with 10 mL of water. The aqueous layer and washings were discarded.

The toluene layer was shaken with 25 mL of solution containing 2 mL each of 0.01% of potassium iodate solution and 2.5 M sulfuric acid solution, 4 mL of 15% of sodium chloride solution and 2 mL of 0.01% of Rh6G solution for 1 min. The toluene layer was separated into a dry test tube and about 1 g of anhydrous sodium sulfate was added. The absorbance of the extract was measured at 535 nm in 10 mm cells against the reagent blank run through the entire procedure.

3. Results and discussion

3.1. Effects of periodate concentration

To establish the optimum concentration of periodate required for complete oxidation of KC, reactions were carried out using 0.5 mL–2 mL of 0.01 M sodium periodate. In each instance, 10 mL of 2.136 μg/mL KC was present in a total volume of 25 mL maintained at pH 3.0. A reagent blank was prepared for each concentration of periodate. The results obtained for various volumes of periodate are shown in Fig. 1. From the graph it is evident that addition of 1.5 mL of 0.01 M periodate solution is sufficient for the quantitative oxidation of KC. It was decided to use 1.5 mL of 0.01 M solution of periodate in all subsequent work.

3.2. Effect of pH

The optimum pH for the oxidation of KC with periodate and for the liberation of iodine by reaction with iodide was evaluated. The variation of pH during oxidation of ketoconazole by periodate is shown in Fig. 2. It is observed that the oxidation of KC by periodate was quantitative in the pH range 3.0–9.0. On the basis of these experiments it was decided to maintain the pH 3 for the oxidation of KC in all subsequent studies.

It was observed that the absorbance of the blank was low only when freshly prepared aqueous solution of periodate was

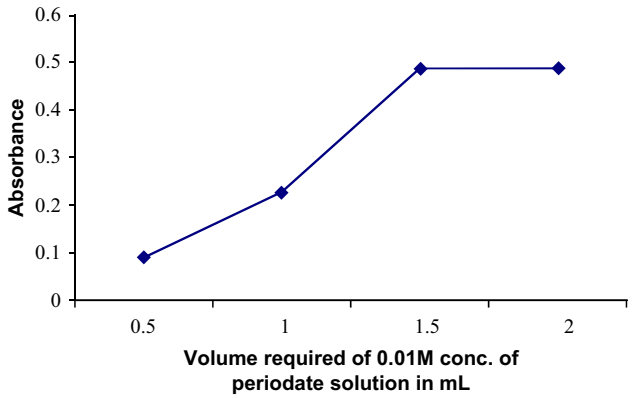

Figure 1 Effect of periodate concentration for oxidation of ketoconazole.
2.136 μg/mL KC—10 mL, periodate—*x* mL of 0.01 M, pH—3, 0.1% of ammonium molybdenum solution—1 mL, 0.1 M KI—1 mL, aqueous volume—25 mL, toluene for extraction—10 mL.

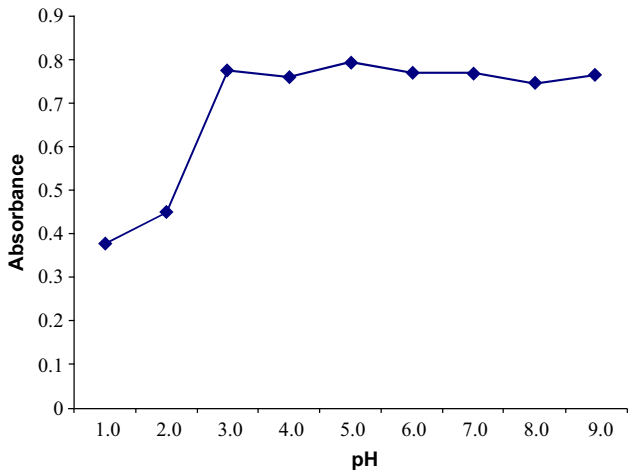

Figure 2 Effect of pH for oxidation of ketoconazole.
2.136 μg/mL KC—10 mL, 0.01 M periodate—1.5 mL, pH—varied, 0.1% of ammonium molybdenum solution—1mL, 0.1 M KI—1 mL, aqueous volume—25 mL, toluene for extraction—10 mL.

used. This was possibly due to photo decomposition of periodate to iodate.

3.3. *Calibration graph and molar absorptivity*

The adherence of oxidation of KC with periodate to Beer's law was next examined under the optimum conditions. Beer's law was obeyed over the concentration range of 0.2136 μg/mL–1.7088 μg/mL with molar absorption coefficient of 5×10^5 mol · L^{-1} cm^{-1} and a regression coefficient of 0.9963, indicating good linearity. It compares favorably in sensitivity with the spectroscopic methods described in literature for the determination of ketoconazole as shown in Table 1. The detection limit of the method was found to be 0.127 μg/mL. The measurement precision was determined by performing six replicate measurements. The RSD was found to be 0.89%. The validation parameters are summarized in Table 2, which shows good repeatability and reproducibility of the proposed method. Precision was verified with six measurements on same day and on different days. RSD was found to be less than 2.0%.

3.4. *Reaction sequence for the observed enhancement*

In the method proposed the oxidation of KC with periodate would liberate 2 atoms of iodate. The reduction of iodate in

Table 2 Spectral data for the amplification reaction of ketoconazole.

Parameters	Values
λ_{max} (nm)	535
Beer's law limits (μg/mL)	0.2–1.7
Molar absorptivity (mol · L^{-1} cm^{-1})	5×10^5
Limit of detection (μg/mL)	0.127
Slope	0.1157
Limit of quantitation (μg/mL)	0.17
Intercept	0.0294
Correlation coefficient	0.9963
R.S.D of 6 determinations (%)	0.89

Table 1 Comparative data of proposed method with literature survey of the spectrophotometric determination of ketoconazole.

Reagents used	λ_{max} (nm)	Beer's law range in μg/mL or molar absorptivity	Experimental conditions involved	Reference
Picric acid	410	1–58 μg/mL	Involves extraction into chloroform	[1]
Cu(II) and Co(II) complexes	720 and 612.5, respectively	35.36 ± 1.95 mol · L^{-1} cm^{-1} and 59.62 ± 1.87 mol · L^{-1} cm^{-1}, respectively	Involves extraction into dichloromethane	[2]
Iodine	290	1–40 μg/mL		[3]
First-derivative ultraviolet	257	5.0 to 30.0 μg/mL	Zero crossing method	[4]
Iron(III) chloride		1–15 μg/mL		[5]
Iron (III) chloride and 1.10-phenanthroline	512	1.6–16.0 μg/mL	Redox complexation reaction	[6]
Tri-iodide ion and alizarin red	425	10^{-5}–10^{-2} M	Ion pairs	[29]
Amplification method	535	0.2–1.7 μg/mL	Ion pair	Present method

Table 3 Results of determination of ketoconazole in its formulations.

Sr. No.	Sample	Amount of drug in extract (μg/mL)	Amount of pure KC added (μg/mL)	Total found (μg/mL)	Recovery (%)
1	Tablet 500 mg	0.534	–	0.540	98.88
2	Tablet 500 mg	0.534	0.534	1.048	97.77
3	Tablet 500 mg	0.534	0.400	0.935	99.89

acid medium with iodide ion would produce 12 atoms of iodine:

$$KC + IO_4^- \rightarrow KC^+ + IO_3^- \quad (1)$$

$$KC^+ + IO_4^- \rightarrow KC^{+2} + IO_3^- \quad (2)$$

$$IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O \quad (3)$$

As both un-reacted periodate and iodate formed due to reduction of periodate would react with iodide to liberate iodine, it was decided to mask the excess periodate using ammonium molybdate.

When 1.2 μg of ketoconazole was determined by the developed method it gave absorbance of 0.382. In accordance with Eqs. (1)–(3), as 1.2 μg of ketoconazole would yield 3.45 μg of iodine and since the absorbance was found identical to that obtained when 3.45 μg of iodine in toluene was directly subjected to determination, it was concluded that under the reaction conditions there was stoichiometric oxidation of ketoconazole in accordance with Eqs. (1)–(3).

3.5. *Application*

The method developed for the determination of KC was applied for establishing ketoconazole concentration levels in tablets. Samples to which known amount of KC were added were analyzed to ascertain whether the recovery was quantitative. Four tablets, each containing 200 mg of KC were crushed and powdered. A suitable amount of the powder (~5.78 mg) was weighed and dissolved in 50 mL of HPLC grade water containing a few drops of 0.5 M H_2SO_4 [28]. The excipients were separated by filtration and the filter paper was washed three times with water. The filtrate and washing solutions of the tablet were transferred quantitatively into a 50 mL calibrated flask and diluted to the mark with HPLC grade water, and the developed method was followed. The recovery was calculated by comparing the concentration obtained from the spiked mixtures with that of the standard KC (USP grade material) added to the sample. The results of analysis of commercial dosage forms and the recovery study (standard addition method) as shown in Table 3, indicate that the developed method is suitable for the assay of ketoconazole in commercial dosage forms.

4. Conclusions

The method described provides a simple, fast and reliable means of determining ketoconazole in pharmaceutical preparations. The method developed has very high sensitivity. The method has been applied to establish the ketoconazole content in commercial tablet dosage forms.

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