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Communication

A sequential injection system for spectrophotometric determination of ketoconazole

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Abstract: Sequential injection analysis (SIA) with spectrophotometric detection is used for a fast determination of ketoconazole in pure and pharmaceutical preparations. The developed method is based on oxidation of ketoconazole with cerium ion in an acidic medium, which leads to the formation of a coloured product that absorbs at a wavelength of 496 nm. Then automation of the method is developed using the SIA technique. All variables that affect the reaction response are studied and optimised using univariate and simplex optimisation. The method is applicable for the determination of ketoconazole in the range of 20-120 μg mL^{-1}.

Keywords: ketoconazole, sequential injection analysis, spectrophotometric determination, oxidation

INTRODUCTION

Ketoconazole (KC) is chemically known as *cis*-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine. The drug is a highly effective broad-spectrum antifungal agent [1]. It is used to treat a wide variety of superficial and systemic mycoses [2] and has an advantage over other imidazole derivatives in producing adequate sustained blood levels following oral administration [3]. Different methods have been reported for its determination such as spectrophotometry [4-10], chromatography [11-16] and electrochemical method [17-22]. The oxidation of KC with Ce(IV) has not been extensively studied in the literature, thus warranting a comprehensive investigation of the reaction. Standard chromatographic methods of KC generic assay require elaborate and sophisticated instrumentation and have low sampling frequency. Some of the spectrophotometric methods require heating, extraction, a lengthy procedure and a long time for maximum colour development, but lack sensitivity, specificity and a wide dynamic range. Electrochemical methods sometimes suffer from interferences while chromatographic methods are time-consuming and require long procedures for filtration and

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extraction. Sequential injection analysis (SIA) has been adopted for the assay of many pharmaceutical and environmental compounds due to many advantages over classical methods. Specifically it is reproducible, robust, reagent-saving and adaptable to different detecting systems [23, 24]. In this paper SIA is utilised along with spectrophotometric measurement to determine KC in pharmaceutical forms. The newly adopted method is validated by its application to the real dosage form of the drug.

MATERIALS AND METHODS

Chemicals and Reagents

A standard stock solution (0.20 mg mL^{-1}) of KC (Sigma) was prepared daily and ten standard working solutions (5-200 mg mL^{-1}) were prepared daily by dilution. Cerium(IV) solution (0.05M) was prepared from ceric ammonium sulphate trihydrate (AR grade) dissolved in 0.5M sulphuric acid. Appropriate working solutions were prepared by dilution. Extra-pure-grade sulphuric acid (95-98 %) was used to prepare a solution of 1.84 g L^{-1} used in this study.

Apparatus

The manifold used consists of SIA apparatus of FIALab 3500 (Medina, USA) combined with a fibre-optic spectrometer, a syringe pump equipped with PVC pumping tube, an 8-port multi-position valve, a fibre-optic Z-flow cell equipped with an LS-1 tungsten halogen lamp and a USB2000 spectrometer (Ocean Optics, USA). A schematic diagram of components of the manifold is shown in Figure 1.

ESR spectra were recorded with a JEOL JES-RE1X spectrometer using an aqueous flat sample cell at 293 K, g = 1.9930, with a microwave frequency of 9.4371 GHz, microwave power of 4.00 mW, sweep width of 5.000 mT and sweep time of 1.0 min.

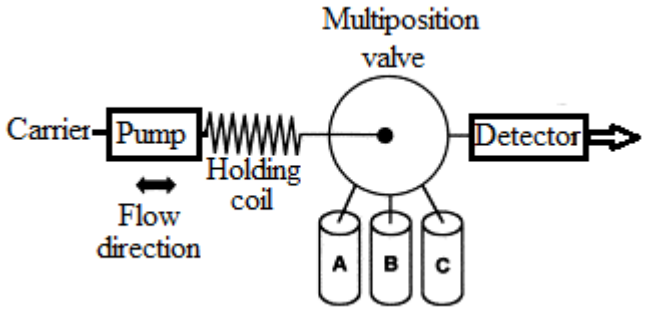

Figure 1. SIA manifold diagram: A = KC solution; B = Ce(IV) solution; C = Sulphuric acid solution

Method and Procedure

The analytical system control including control of the peristaltic pump and selection valve was achieved by FIALAB for Windows version 5.0 from FIAlab® (Medina, USA) using a compatible IBM personal computer.

SigmaPlot 2004 for Windows version 9.01 from Systat Software Inc. was employed for constructing kinetic curves and calculating linear regression equations.

The Chemometric Optimisation by Simplex programme was obtained from Elsevier Scientific Co. (Netherland) and utilised for the optimisation of variables using a compatible IBM personal computer.

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To carry out the simplex optimization method, a super-modified simplex programme was employed to find optimum concentrations of both H_2SO_4 and Ce(IV) solutions for sequential injection determination of KC with Ce(IV). The boundary conditions for the two variables lineated in Table 1, together with the start and step values, were fed into the programme.

Table 1. Simplex computer programme parameters and boundaries considered for the optimisation

Parameter	$[H_2SO_4]$	[Ce(IV)]
Step	0.01	0.001
Start-step	0.01	0.001
Lower	0.001	0.0001
Upper	0.1	0.01

RESULTS AND DISCUSSION

The method is based on the reaction of KC with Ce(IV) in sulphuric acid medium. A red-coloured product that is believed to be a radical cation $KC^{\cdot+}$ [25] formed as a result of the loss of an electron from the piperazine ring of the KC molecule (Figure 2). The reaction is instantaneous and the product is stable for several hours. Absorption spectra showed that the oxidised form of KC has λ_{max} at 496 nm.

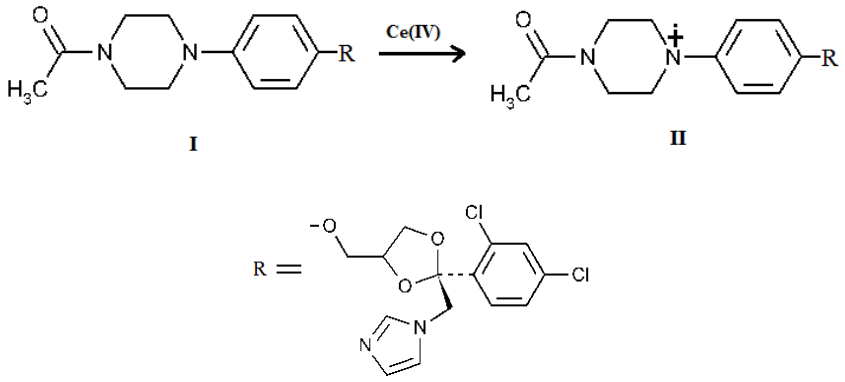

Figure 2. Proposed oxidation reaction of KC: I = KC; II = KC radical cation

It was reported earlier that KC reactions with metal ions proceed by either a complexation or an oxidation reaction [4, 19]. The KC oxidation reaction is accompanied by the formation of a radical cation during the oxidation process, which gradually decays via a chemical reaction to form some stable products. The presence of a radical accompanying the oxidation was confirmed by the ESR spectrum as shown in Figure 3, which illustrates the hyperfine structure of the resultant radical ion, reflecting the extent of delocalisation of the unpaired electron of $KC^{\cdot+}$.

At Ce(IV) concentration higher than 0.05M, the red colour of the product disappears, probably due to further oxidation of the radical formed in the first step of the reaction, producing a divalent cation in another step as shown below [20]:

$$KC \; -e \rightarrow \; KC^{\cdot+}$$
$$KC^{\cdot+} \; -e \rightarrow \; KC^{++}$$

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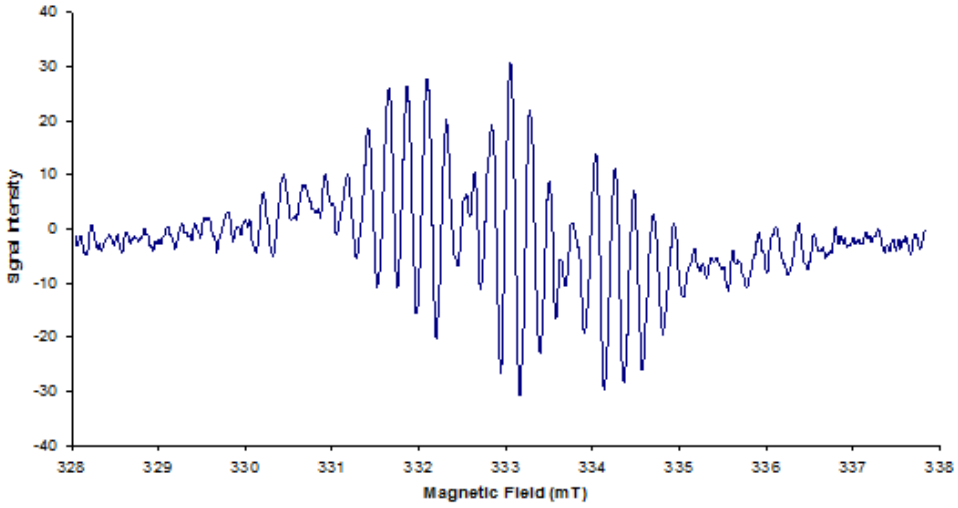

Figure 3. ESR spectrum of 100 µg mL^{-1} KC prepared in 100 mL of 0.078M H_2SO_4 mixed with 0.0013M Ce(IV) solution in 0.5M H_2SO_4

Preliminary investigation of the reaction indicates that the oxidation reaction is dependent on the acid and Ce(IV) concentrations as well as the flow rate. At acid concentration lower than 1.0×10^{-4} mol L^{-1}, the drug is not soluble. When Ce(IV) is prepared lower than 1.0×10^{-4} mol L^{-1}, the molar equivalency of Ce(IV) is limited enough to cover a wider dynamic range of the adopted method. Ce(IV) was found to be insoluble when prepared at a concentration higher than 1.0 mol L^{-1}. The concentrations mentioned above delineate the boundaries of both parameters to be considered in further optimisation procedure.

Univariant Optimisation Method

The univariant optimisation method was applied to examine the effect of each parameter, i.e. flow rate, sulphuric acid concentration and cerium(IV) concentration, on the absorbance. In this method all chemical and instrument parameters were kept constant while varying the concentration of the one being investigated. The effects of H_2SO_4 and Ce(IV) concentrations on the absorbance measured at 496 nm are given in Figure 4 and Figure 5 respectively. It is clear that the absorbance has a maximum value at the acid concentration of around 0.03 mol L^{-1} and the Ce(IV) concentration of around 0.005 mol L^{-1}.

For the flow rate, a number of experiments were carried out keeping the concentrations of sulphuric acid and cerium(IV) constant at 0.01 mol L^{-1} and 0.005 mol L^{-1} respectively while varying the flow rate in the range of 10-50 µL s^{-1} as shown in Figure 6. It was found that the variation of the flow rate not only affects the peak height but it also influences the shape of the peak due to dispersion. The optimum value of flow rate was taken at 30 µL s^{-1} for the sharp and intense peaks in order to enhance sensitivity and sampling frequency.

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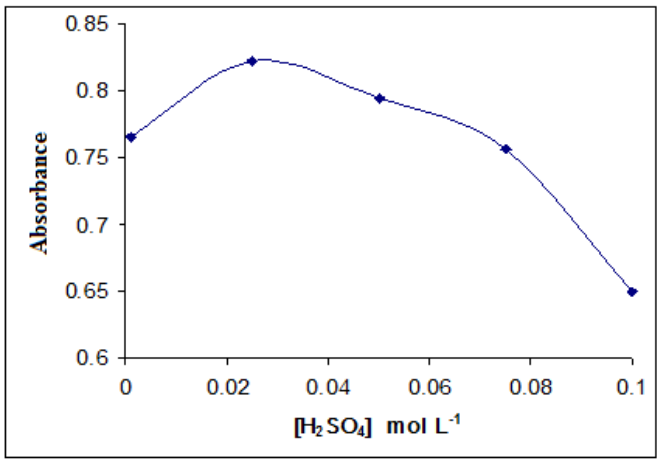

Figure 4. Absorbance as a function of $[H_2SO_4]$ ([Ce(IV)] = 0.005M, [KC] = 100 µg mL^{-1} and flow rate = 50 µL s^{-1})

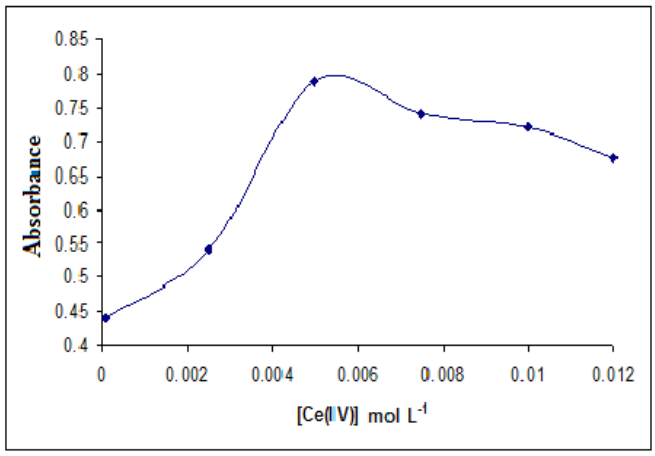

Figure 5. Absorbance as a function of [Ce(IV)] ($[H_2SO_4]$ = 0.003 M, [KC] = 100 µg mL-1 and flow rate =50 µL s^{-1})

Figure 7 shows the progress of the simplex, which indicates a gradual improvement in the response. Sixteen experiments were performed, enough to evaluate the proper conditions. Further investigations on the effects of other parameters such as injection volume and delay time on the absorbance indicated that the optimum operating conditions for KC assay were: $[H_2SO_4]$ = 0.00734 mol L^{-1} and [Ce(IV)] = 0.00176 mol L^{-1}, flow rate = 30 µL s^{-1} and injection volume = 180 µL.

Regression Data

To obtain the best and widest linear calibration curve for KC analysis, different KC standard solutions were prepared keeping concentrations of the other reactants constant at 0.00734 mol L^{-1} H_2SO_4 and 0.00176 mol L^{-1} Ce(IV). The volumes of Ce and KC solutions were 30 µL and 180 µL respectively and the flow rate was 30 µL s^{-1}. The calibration equation was obtained from the plot of absorbance of the coloured product at 496 nm and the absorbance of Ce(IV) solution at that wavelength. The regression data in Table 2 show that the linear dynamic range is 20-120 µg mL^{-1} with high sensitivity, low detection limit and a sampling rate of 120 h^{-1}.

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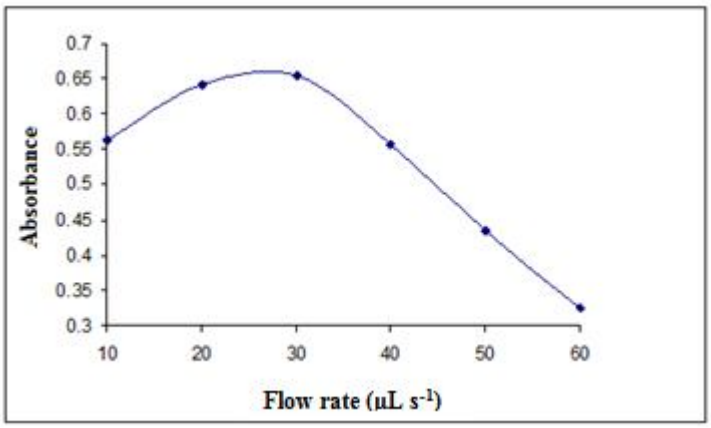

Figure 6. Absorbance as a function of flow rate ($[H_2SO_4]$ = 0.01mol L^{-1}, [Ce(IV)] = 0.005 mol L^{-1})

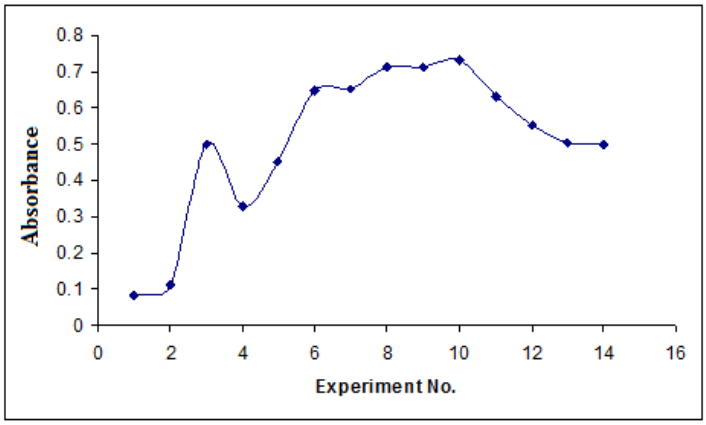

Figure 7. Absorbance progress of the simplex

Table 2. Regression data for SIA method for determination of KC

Wavelength (nm)	496
Concentration range (µg mL^{-1})	20-120
Correlation coefficient	0.997
Slope	0.00471
Intercept	0.0433
Sandell's sensitivity (µg cm^{-2})	0.0047
Detection limit (µg mL^{-1})	13.0
Relative standard deviation (RSD) %	11

Note: Detection limit = 3 Sb/slope; Sb = Standard deviation for blank using five replicates

Application

The effect of excipients in the dosage forms that may interfere with KC determination was studied by applying the proposed SIA method to some drug tablets from the market. The British Pharmacopoeia (BP) method [26] for the assay of KC tablets was also performed for the same batch samples.

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Statistical comparison was performed by calculating the t-test of recovery data for two commercial forms of KC tablets. The results showed high accuracy and repeatability of the proposed method compared with the standard method as shown in Table 3.

Table 3. Results of KC determination in proprietary drugs

Drug	Supplier	KC content	Mean recovery ± RSD (%)* SIA method	BP method	t**
Nizoral tablet	JANSSEN	200 mg	95 ± 3.5%	96 ± 0.4%	0.26
	Samf, Sudan	100 mg	94 ± 3.5%	95 ± 0.4%	0.31

* Relative standard deviation for five replicates

** Student t-test value

CONCLUSIONS

The developed method for KC determination based on the oxidation reaction with cerium ion was successfully applied using the SIA system for analysis of commercial samples and the results were comparable with standard method.

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