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Determination of Ketoconazole in Pharmaceutical Formulations

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Abstract: Ketoconazole has been widely used as an antifungal drug that is formulated as tablets, cream and over-the-counter ketoconazole shampoo. The aim of this research was to study and to standardize an UV (ultraviolet spectrophotometric) method, potentiometry and a HPLC (high performance liquid chromatographic) method for the determination of ketoconazole in commercially available tablets. These three methods were compared and discussed with respect to their sensitivity, selectivity and ready-applicability in routine analytical work. Absorption spectra and spectrophotometric determinations were carried out on the UV spectrophotometer. Investigated concentrations that ranged from 0.003 mg·dm^{-3} to 0.02 mg·dm^{-3}. The absorbance was measured at 224 nm. In potentiometric titrations, glass and saturated (KCl) calomel electrodes were used to determine the end point of the titration. HPLC analyses of ketoconazole were carried in the presence of econazole as internal standard. It was concluded that the described methods are simple, fast and reliable for the identification of ketoconazole in pharmaceutical preparations. The preparation of the samples was easy, the excipients did not interfere with the active substance in the methods, so they can be used in routine quality control analysis.

Key words: Assay, ketoconazole, tablets.

1. Introduction

Ketoconazole is most often used to treat fungal infections that can spread to different parts of the body through the bloodstream such as yeast infections of the mouth, skin, urinary tract and blood, and certain fungal infections that begin on the skin or in the lungs and can spread through the body. Ketoconazole blocks the synthesis of ergosterol, a key component of the fungal cell membrane, through the inhibition of cytochrome P-450 dependent enzyme lanosterol 14α-demethylase responsible for the conversion of lanosterol to ergosterol in the fungal cell membrane. This results in an accumulation of methylated sterol precursors and a depletion of ergosterol within the cell membrane, thus weakening the structure and function of the fungal cell membrane [1].

Ketoconazole is used as a broad-spectrum antifungal agent for the treatment or prevention of fungal infections especially against thrush, gastrointestinal infections, and infections of the skin, nails, and scalp. It is also topically used in the formulation of cosmetic creams and in shampoos as an antidandruff agent [2].

Ketoconazole, cis-1-acetyl-4-[4-[[2-(2, 4-dichloroph -enyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl] methoxyl]phenyl] piperazine is in a class of antifungals called imidazole's and has the structural formula, as shown in Fig. 1.

Several analytical methods have been developed for quantitative determination of ketoconazole. These include visible spectrophotometry [3], UV (ultraviolet spectrophotometry) [4], spectrofluorimetry [5], thin-layer chromatography [6], supercritical fluid chromatography with UV detection [7], capillary electrophoresis with diode array detection [8] HPLC (highperformance liquid chromatography) using different detection modes such as UV [9], diode array [10], electrochemical detection [11] and stripping

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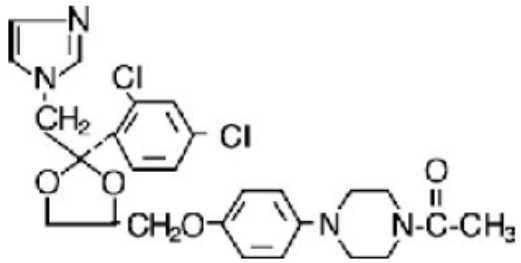

Fig. 1 Chemical structure of ketoconazole.

voltammetric and polarographic techniques [12].

The aim of this research was to use an UV (ultraviolet spectrophotometry), potentiometry and HPLC (high performance liquid chromatographic) for the determination of ketoconazole in pharmaceutical formulations and to point out the convenience of these methods for introduction as a routine procedure for quality control of commercially available tablets.

2. Materials and Methods

Absorption spectra and spectrophotometric determination were carried out on a "Gilford 250" and "LKB 4050" spectrophotometers in 1 cm cuvettes.

Potentiometric titrations were made using "radiometer pH meter" with a glass and saturated (KCl) calomel electrode which was used to determine the end point of the titration.

HPLC analyses were performed by "LKB system" using an Ultrapac LiChrosorb RP 18 (5 μm) column, with the mobile phase consisting of 0.2% diethyl amine in methanol/0.5% ammonium acetate solution (78:22); flow rate of 0.9 cm^3/min and UV detection at 224 nm.

Methanol was of HPLC grade was purchased from Merck (Darmstadt, Germany). Ketoconazole was purchased from Sigma (St. Louis, MO, USA). Econazole was also purchased from Sigma (St. Louis, MO, USA). Acetic acid and diethyl amine were of analytical grade and from Sigma. Deionized water was produced using a Millipore Milli-Q apparatus (Milford, MA, USA).

Tablets containing 200 mg ketoconazole per tablet, as declared, were taken from the market in the Republic of Macedonia.

The concentration of ketoconazole stock solutions was 10^{-4} mol dm^{-3} in 0.1 mol dm^{-3} HCl for spectrophotometric determinations. Solutions in range the investigated concentrations were obtained by diluting of stock in range from 0.003 mg dm^{-3} to 0.02 mg dm^{-3}. UV absorption absorbance was measured at a maximum wavelength of 224 nm.

For potentiometric determinations, samples are dissolved in acetic acid. A solution of $HClO_4$ (0.1 mol dm^{-3}) in acetic acid was used for titrations. Per cubic centimeter of $HClO_4$ is equivalent to 0.02657 g of ketoconazole.

For HPLC determinations of ketoconazole in the presence of econazole as internal standard a stock solution was prepared by dissolving of 50 mg ketoconazole (accurately weighed) and econazole (accurately weighed) in the 100 mL of mobile phase. The working standard solutions were prepared by diluting the stock solution with the mobile phase in a 10 mL volumetric flasks. The concentrations of ketoconazole working standard solutions were 25, 50, 100, 150 and 200 μg/mL. The liquid chromatograph is equipped with the UV-detector. The responses "major peaks", Fig. 2 were measured at the wavelength of maximum absorbance at 224 nm.

3. Results and Discussion

The results obtained by these three methods are given in Table1.

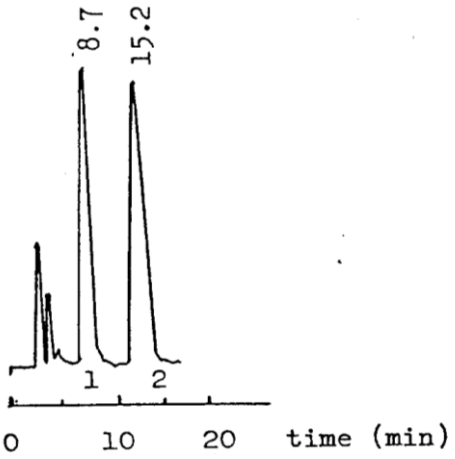

Fig. 2 Typical chromatogram of ketoconazole in tablets.

Table 1 Results for content of ketoconazole in tablets by spectrophotometric (I), potentiometric (II) and HPLC (III) methods.

Method	Statistic			
	$\overline{x}$	*SD*	*r*	*F*
I	196.86	1.28	I-II = 0.94	I–II = 1.54
II	198.32	1.59	II-III = 0.92	II-III = 1.71
III	197.28	2.08	I-III = 0.90	I-III = 2.64

Data for content of ketoconazole are presented in mg/tablet as the mean value on 10 samples and related standard deviations are given in Table 1. The results for content of ketoconazole in tablets driven from each method where compared (I-II, II-III and I-III) with regression analyses and corresponding results for *r* and *F*-value are given in Table1.

As it can be seen from the Table 1, the differences in assay values in all examined methods were not statistically significant.

Therefore, it can be considered that the described methods for quantitative determination of ketoconazole in tablets are simple and accurate. These methods can be performed directly without removing the excipients for identification of assay of ketoconazole in solid pharmaceutical formulations.

4. Conclusions

The described methods are simple, fast and reliable for determination of ketoconazole in pharmaceutical formulations. The preparation of the samples is easy, the excipients do not interfere with the active substance all the methods, so these methods therefore can be used in routine quality control analyses of pharmaceutical preparations.

The spectrophotometric method is recommended for qualitative determination in routine analysis, especially in routine dissolution tests. It is not only satisfactorily reproducible, but also a simple and selective method with respect to the excipients. It can be compared favorably, with respect to the sensitivity with the potentiometric and chromatographic methods. Although the potentiometric method cannot be considered as selective, it is rapid and reproducible enough to be used as an alternative routine method. The HPLC method is useful especially for determination of impurities and degradation products in stability studies of ketoconazole in pharmaceutical preparations.

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