Extractive Spectrophotometric Determination of Fluconazole by Ion-pair Complex Formation with Bromocresol Green

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An extraction-spectrophotometric method for the determination of trace amounts of fluconazole was described. Fluconazole was effectively extracted as a 1 : 1 ion-pair complex with bromocresol green (BCG) at pH 3.0 into chloroform, followed by spectrophotometric determination at 420 nm. Beer’s law was obeyed over the range of 4—50 µg mL⁻¹ of fluconazole with a detection limit of 3.7 µg mL⁻¹. The method is simple, rapid and sensitive. The procedure was applied to the determination of fluconazole in pharmaceutical preparations as well as its recovery from a blood serum sample.

Keywords fluconazole, solvent extraction, spectrophotometry, capsule, blood serum

Introduction

Fluconazole, [2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol] (I), is an orally active antifungal agent, which is used in the treatment of superficial and systemic candidiasis and cryptococcal infections in patients with the acquired immunodeficiency syndromes (AIDS). It acts by blocking the synthesis of ergosterol, an essential component of the fungal cell membrane.¹

Due to the vital importance of fluconazole determination to biological fluids and pharmaceutical preparations, several chromatographic²-⁵ and spectroscopic⁶-⁸ methods for its quantitative determination have been reported. Simpler alternative methods that use inexpensive instruments are clearly needed for the determination of this pharmaceutically important antifungal drug. Following our interest in analysis of antifungal drugs⁹-¹¹ in this paper we report a new, simple, sensitive and inexpensive method for the extraction and determination of the fluconazole from pharmaceutical preparations and a blood serum sample. The method is based on the formation of a highly colored ion-pair complex between the drug and bromocresol green (BCG) in acidic solution. The resulting ion-pair complex was quantitatively extracted into chloroform, followed by spectrophotometric determination at 420 nm.

Experimental

Apparatus

All absorbance measurements were made on a Cecil CE 9050 UV-visible spectrophotometer with matched quartz cuvettes of 1 cm path length. pH measurements of the aqueous phase were performed using a JENWAY 3345 Ion meter. Shaking of the solutions was made using a JANKE & KUNKEL KS500 shaker.

Reagents

All chemicals were of analytical-reagent grade and used without further purification. Doubly distilled deionized water was used throughout. Reagent grade fluconazole was a gift from Dr Gholamreza Bahrami (School of Medical Science, Kermanshah, Iran) and capsules containing 50, 100, 150 mg of the drug were obtained from Pars Darou (Tehran Iran), Zahravi Pharm. Co. (Tabriz Iran), and Pars Darou (Tehran Iran), respectively. Analytical grade BCG and chloroform were purchased from Merck chemical company and used as received.

A stock standard solution of 1.0 mmol/L (306.0 µg mL⁻¹) fluconazole was prepared by dissolving 0.0153 g of the pure drug in 50.0 mL of water. Standard working solutions were prepared by dilution of the stock solution as required.

General procedure

1.8 mL of 0.010 mol/L BCG and 1.2 mL of phthalate buffer (pH=3.0) were mixed in a 10 ml calibrating volumetric flask. 1.0 mL of standard or sample solution of the fluconazole was pipetted into the flask and the volume of the solution was made to the mark using doubly distilled water. 4.0 mL of this solution were transferred to a 25 mL separatory funnel and after the
addition of 4.0 mL of chloroform the resulting mixture was shaken for 15 min, which was then allowed to stand for clear separation of the two phases. The absorbance of the chloroform layer was measured against a reagent blank at 420 nm.

**Capsule sample solutions**

Five capsules of fluconazole were weighed accurately and their contents were mixed and powdered thoroughly. An accurate amount of the powder equivalent to 1.53 mg of the drug (1.0 × 10⁻⁴ mol/L) was weighed and after addition of water the resulting mixture was shaken for a few minutes and then filtered. The volume was made to 50 mL in a calibrating volumetric flask. Appropriate dilution of the solution was made and the recommended procedure for the determination of the fluconazole was followed.

**Blood serum sample**

0.5 mL of a 1.0 mol/L fluconazole solution was added into 1.0 mL of a blood serum sample. The volume of the mixture was made to 10.0 mL in a calibrating volumetric flask and the general procedure of the proposed method was followed. The recovery of the fluconazole was determined using a standard addition technique.¹²

**Results and discussion**

**Fluconazole-BCG complexation**

Figure 1 shows the electronic absorption spectra of 1.0 × 10⁻³ molL⁻¹ BCG in the presence of increased amounts of the fluconazole (1.0 × 10⁻² molL⁻¹). As shown obviously, upon addition of the fluconazole the intensity of the absorption peak was greatly increased in addition to a gradual red shift of the absorption peak. These observations are indicative of formation of an adduct between the fluconazole and BCG. A mole-ratio plot was obtained at 420 nm, which shows an inflectional point at [fluconazole] : [BCG] = 1 : 1. This stoichiometry was further confirmed by a continuous variation plot (Figure 2).

**Fluconazole determination**

An ion-pair complex extraction spectrophotometry has been frequently used for the quantitative determination of many pharmaceutical compounds.¹⁴-¹⁹ The method has simplicity and selectivity, which are two requirements for pharmaceutical analysis of biological fluids such as blood and urine, and does not need any sophisticated instrument or construction of selective electrodes. To the best of our knowledge, no work about ion-pair complex extraction of fluconazole was reported in the literature.

Among different dyes tested for extraction of the fluconazole, BCG had the least background absorption when using chloroform as the extracting medium. The optimum pH value for the quantitative extraction of fluconazole with BCG in chloroform was studied over the pH range of 2.5—7.0. The results are shown in Figure 3. According to the results a pH below 4.5 was
recommended. At higher pH values, the fluconazole existed mainly in its neutral form (pKₐ=2.03) and the extent of ion pairing between anionic BCG and cationic fluconazole decreased, thus decreasing the color developed in chloroform phase. A pH value of 3.0 was used for further studies.

The influence of BCG concentration on the absorbance of the extracted ion-pair complex was studied by using a constant concentration of fluconazole (1.0×10⁻⁴ molL⁻¹) and different concentrations of BCG. The results are plotted in Figure 4, which shows that the absorbance remains approximately constant at the molar ratios of BCG/fluconazole greater than 16. A molar ratio of 18 that corresponds to 1.8×10⁻³ molL⁻¹ BCG was selected as the optimum concentration of BCG and used in further studies.

The optimum volume of chloroform and the times of extractions required were also studied (Figure 4). A ratio of chloroform : water (1 : 1) during a single extraction stage yielded the maximum extraction of the drug. A volume of 4.0 mL of chloroform was used throughout the procedure. In addition, the extraction of fluconazole with BCG under the optimum conditions was found to be rapid and thus a shaking time of 15 min was sufficient for complete extraction of the resulting ion-pair complex.

**Beer’s law study**

A calibration graph for the fluconazole was obtained under the optimum conditions (Figure 5). The results are summarized in Table 1.

**Interference study**

The effect of some commonly present species in pharmaceutical preparations and biological fluids on the ion-pair extraction of the fluconazole was investigated. As shown in Table 2, none of these species interfered in the determination of fluconazole.

**Determination of fluconazole in capsules**

Assay of the fluconazole in its capsules using the proposed method was carried out and the results are shown in Table 3. As is clear a very good agreement between the fluconazole contents determined by the proposed method and the declared amounts of the drug in the preparations is observed.

**Recovery of fluconazole from blood serum**

In order to investigate the applicability of the pro-
posed method to the determination of the fluconazole in biological fluids, it was applied to the recovery of the fluconazole from a blood serum sample. The recovery of the fluconazole using the proposed method and the standard addition technique \(^{12}\) was found to be 99.56 %. In fasted normal volunteers, administration of a single oral 400 mg dose of fluconazole led to a mean concentration of 6.72 \(\mu\)g/mL (4.12—8.08 \(\mu\)g/mL) in blood serum,\(^{22}\) which is above the detection limit of the proposed method.

**Conclusion**

The method described provided a simple, fast and reliable means of determining the fluconazole in pharmaceutical preparations as well as biological fluids. The proposed method made use of simple reagents, which a common analytical laboratory could afford. The commonly used additives such as starch, lactose, glucose did not interfere with the procedure.

**References**


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