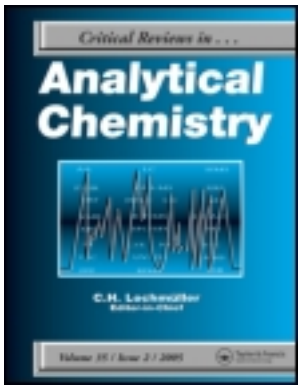

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/batc20

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Available online: 26 Jul 2011

To cite this article: Josilene Chaves Ruela Corrêa & Hérida Regina Nunes Salgado (2011): Review of Fluconazole Properties and Analytical Methods for Its Determination, Critical Reviews in Analytical Chemistry, 41:3, 270-279

To link to this article: http://dx.doi.org/10.1080/10408347.2011.588924

Critical Reviews in Analytical Chemistry, 41:270–279, 2011

ISSN: 1040-8347 print / 1547-6510 online
DOI: 10.1080/10408347.2011.588924

Review of Fluconazole Properties and Analytical Methods for Its Determination

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Fluconazole, α-(2.4-diflurofenil)-α-(1H-triazol-1-methyl)-1H-1,2,4-triazol-1-ethanol, is an antifungal of the triazoles class. It shows activity against species of *Candida* sp. and it is indicated in cases of oropharyngeal candidiasis, esophageal, vaginal, and deep infection. Fluconazole is a selective inhibitor of ergosterol, a steroid exclusive of the cell membrane of fungal cells. Fluconazole is highly absorbed by the gastrointestinal tract and spreads easily by body fluids. The main adverse reactions related to the use of fluconazole are nausea, vomiting, headache, rash, abdominal pain, diarrhea, and alopecia in patients undergoing prolonged treatment with a dose of 400 mg/day. In the form of raw material, pharmaceutical formulations, or biological material, fluconazole can be determined by methods such as titration, spectrophotometry, and thin-layer, gas, and liquid chromatography. This article discusses the pharmacological and physicochemical properties of fluconazole and also the methods of analysis applied to the determination of the drug.

Keywords Review, fluconazole, analytical methods

INTRODUCTION

Fungi are microorganisms found in soil and water and on plants, air, animals, and debris in general. Many fungi have pathogenic potential for humans. According to the tissues and organs affected, mycoses are classified into superficial mycoses (mycosis of the skin, nails, and hair) and mycoses subcutaneous, systemic or deep (Bergold and Georgiadis, 2004).

Invasive fungal infections remain the major cause of morbidity and mortality in severely ill patients and the immunocompromised, such as cancer patients, the polytraumatized, patients using antineoplastic therapy, and those with acquired immunodeficiency syndrome (AIDS), among others that have high risk of developing opportunistic infections.

The treatment of these infections is still limited by problems of drug safety, low efficiency. and microbial resistance (Carrillo-Muñoz et al., 2006).

The vast majority of fungal infections are due to yeast of the genus *Candida* and fungi of the genus *Aspergillus*. However, infections by other fungi, although rarer, are increasing in frequency. Recent advances in therapy and invasive techniques for diagnosis have led to the increase of immunocompromised patients due to their longer lives (Park et al., 2007a; Moretti, 2007). Risk factors have changed the spectrum of pathogens that cause systemic infection, leading to the emergence of fungal infections (Moretti, 2007).

Systemic infection by *Candida* sp. in these patients may proceed quickly and is often fatal. Yeasts of the genus *Candida* have been considered among the main causal agents of systemic infection of hospital origin and represent the main cause of fungal infection of the bloodstream. Patients infected by *Candida* show an overall mortality rate of around 50% to 60%, with long hospital stays of more than 30 days (Moretti, 2007).

The therapeutic arsenal of antifungal drugs is still limited, especially in the case of deep or systemic infections. There is a need for new antifungal drugs that are more effective and less toxic. In the recent years, amphotericin B and the azoles, especially ketoconazole, itraconazole, and fluconazole, have been the drugs of choice in therapy (Bergold and Georgiadis, 2004; Carrillo-Muñoz et al., 2006).

The ideal antifungal agent must have a broad spectrum of activity without toxicity to the patient. Many efforts have been made towards the introduction of new antifungal agents;

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however, progress in this area is slow in comparison with that for antibacterial agents (Carrillo-Muñoz et al., 2006).

In this work, fluconazole will be addressed; its properties and analytical methods will be described with the emphasis on using high-performance liquid chromatography (HPLC). An extensive review was carried out using the databases Scopus and SciFinder and the keywords: fluconazole AND HPLC AND assay, fluconazole AND dissolution, fluconazole AND polymorphos, fluconazole AND stability.

This article is structured as follows: the next topic discusses fluconazole and its therapeutic class, followed by the topics of pharmacological and physicochemical properties and analytical methods, and the article ends with some conclusions and indications of future studies.

FLUCONAZOLE

Among the classes of drugs available, the azoles are used a great deal, especially the triazoles. They are metabolized slowly and have less effect on the synthesis of human steroids than imidazole. Therefore, the azoles in development are primarily triazoles. Fluconazole, a triazole developed in the 1980s, has activity against species of *Candida*, and it is indicated in cases of oropharyngeal, esophageal, vaginal, and deep candidiasis. It is also used in cases of cryptococcal meningitis, and it is the drug of choice for the treatment of *Coccidioides* meningitis (Park et al., 2007a; Bennett, 2003). The use of fluconazole as prophylaxis in neutropenic patients has an impact on the reduction of fungal infections by *Candida* sp. in this population of patients (Moretti, 2007).

In Brazil, fluconazole is marketed in capsules in doses of 50, 100, and 150 mg. The drug of reference is Zoltec, produced by Pfizer. In the Brazilian market there are many generics of fluconazole or pharmaceuticals similar to Zoltec (Brazil, 2009).

In the form of raw material or in pharmaceutical formulations, fluconazole can be determined by methods such as titration, spectrophotometry, thin-layer chromatography (TLC), gas chromatography (GC), HPLC, and by microbiological methods.

Currently, there is extensive effort to ensure the quality, effectiveness, and stability of pharmaceutical products up to the moment of use. Thus, several investigations have been carried out by our study group. These studies describe methods able to determine the level of antimicrobial agents in various products, such as gatifloxacin (Salgado et al., 2006), lomefloxacin (Tozo and Salgado, 2006), azithromycin (Salgado and Roncari, 2005), ceftazidime (Moreno and Salgado, 2008), cefoxitin (Tozo and Salgado, 2007), linezolid (Lopes and Salgado, 2009), and fleroxacino (Salgado et al., 2009).

We have studied drugs still poorly studied and used in Brazil, thus many analytical methods are developed or optimized. Since fluconazole has no dissolution method or indicative of stability analytical methods published in official compendia, this drug is currently the object of study in our laboratory.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamics

The azole antifungal compounds are fully synthetic (Bergold and Georgiadis, 2004). The use of these drugs in antifungal therapy has great impact because of the broad spectrum of action and a serum half-life sufficiently long to allow therapy with one or two doses per day (Park et al., 2007a; Martinez, 2006).

The action of azole derivatives is based on the inhibition of biosynthesis of ergosterol in different stages. Ergosterol is the main component of the fungal cell membrane. Its essential function is to be a bioregulator of membrane fluidity, asymmetry, and integrity. This is the target of azole derivatives (Carrillo-Muñoz et al., 2006).

However, new triazole agents have more specific targets. The cytochrome P-450 lanosterol 14-alpha-demethylase, encoded by the ERG11 gene for Erg11p, is the point of action of fluconazole, voriconazole, itraconazole, and posaconazole. Some mutations in this gene may confer resistance to fluconazole in yeasts (Carrillo-Muñoz et al., 2006).

Fluconazole, at concentrations achieved during systemic use, has its main mechanism of action on the inhibition of fungal sterol 14-alpha-desmetilase, a microsomal enzyme system dependent on cytochrome P450. Therefore, it compromises the biosynthesis of ergosterol in the cytoplasmic membrane, which leads to the accumulation of 14-alpha-methilesteroes. These methilesteroes can break the compact arrangement of acyclic chains of phospholipids, compromising the function of certain enzyme systems linked to the membrane, such as ATPase and enzymes of the electron transport system, thus inhibiting the growth of fungi. The damage to the cell membrane confirms the fungicide or fungistatic action of the drug (Park et al., 2007a; Bennett, 2003; Telles Filho, 2007).

As already described, fluconazole is also used as prophylaxis in immunocompromised patients. However, there has been emergence of strains resistant to fluconazole after prophylactic use for a long time. This prophylaxis decreases the occurrence of infections by *Candida* but increases infections caused by *Candida* resistant to fluconazole, such as *C. glabrata* and *C. krusei*, and it also increases the occurrence of infections by filamentous fungi (Moretti, 2007; Bennett, 2003; Sasongko et al., 2003).

Pharmacokinetics and Drug Interactions

Fluconazole is not freely soluble in water but has higher solubility than imidazoles. Its relative aqueous solubility allows it to be administered orally or intravenously. Fluconazole shows excellent bioavailability, and it is highly absorbed by the gastrointestinal tract. It also spreads easily by body fluids, including sputum, saliva, breast milk, and cerebrospinal fluid. Its bioavailability is not altered by the presence of food or gastric acidity (Bennett, 2003; Marciniec et al., 2007).

Plasma concentrations of fluconazole are essentially the same whether the drug is administered orally or intravenously and

reaches maximum plasma concentrations of 4 to 8 g/mL after repeated doses of 100 mg. The concentrations in cerebrospinal fluid correspond to 50–90% of plasma levels. Fluconazole binds to plasma proteins at the rate of 11 to 12% (Bennett, 2003). The drug displays elimination half-life of between 25 and 30 hours, and renal excretion is responsible for more than 90% of the disposal (Bennett, 2003; Martinez, 2006).

Fluconazole shows few drug interactions. The combined use of rifampicin and fluconazole may lead to decreased plasma concentration of the antifungal (Bennett, 2003; Dash and Elmquist, 2001).

Fluconazole may interfere with metabolism of some drugs, mainly through the inhibition of cytochrome P450 and the CYP3A4 and CYP2C9 isoenzymes. This can lead to increased plasma of some agents such as nevirapine, phenytoin, and midazolam. It may reduce clearance of theophylline. Cisapride and amitriptyline may have their plasma concentrations increased if used concurrently with fluconazole. It is not recommended to use fluconazole with astemizole, cisapride, or terfenadine due to the risk of cardiac arrhythmias (Bennett, 2003).

The increase and decrease of contraceptive steroids have been reported in patients using fluconazole, and the effectiveness of oral contraceptives is affected (Bennett, 2003).

Toxicology

The main adverse reactions related to the use of fluconazole are nausea, vomiting, headache, rash, abdominal pain, diarrhea, and alopecia in patients undergoing prolonged treatment with a dose of 400 mg/day. Rare cases of deaths due to liver failure or Stevens-Johnson syndrome were reported (Bennett, 2003).

Fluconazole is teratogenic in rodents, and it has been associated with cardiac and skeletal deformities in three babies born of women who used high doses of fluconazole during pregnancy. Therefore, it should be avoided during pregnancy and lactation (Bennett, 2003; Dash and Elmquist, 2001).

PHYSICOCHEMICAL PROPERTIES

Fluconazole is chemically known as α-(2.4-diflurofenil)-α-(1H-triazol-1-methyl)-1H-1,2,4-triazole-1-ethanol (Figure 1). It is the first of the triazoles class, and its appearance is like crystalline powder, white or almost white. Fluconazole is slightly soluble in water, soluble in alcohol and acetone, readily soluble in methanol, and very slightly soluble in toluene. Its molecular shape is $C_{13}H_{12}F_2N_{6O}$ (C = 50.98%, H = 3.95%, F = 12.41%, N = 27.44%, O = 5.22%), and its molecular weight is 306.27 g/mol. Fluconazole's melting point is 223–224°C. It is a weak base and its ionization constant (pKa) measured in 1.1 M NaOH is 1.76 ± 0.10, with the predominant nitrogen protonation (Dash and Elmquist, 2001; O'Neil, 2006).

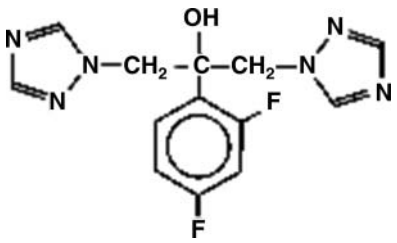

FIG. 1. Chemical structure of fluconazole (CAS 86386-73-4).

It should be stored in hermetically sealed vials and the storage temperature must be kept below 30°C (O'Neil, 2006).

Fluconazole was developed at Pfizer Central Research, in Sandwich, Kent, UK, and the first publication on this new agent was made by Kenneth Richardson in 1985 (O'Neil, 2006; Richardson et al., 1985).

Fluconazole development was the result of a research program for design of a broad-spectrum antifungal agent, active by oral and intravenous via, for treatment of superficial and systemic infections. The starting materials were imidazole derivatives, which were generally well tolerated and also because they offered the advantage of a selective mode of action and the inhibition of a crucial enzyme in the membrane of ergosterol biosynthesis of fungi: C-14 desmetilase. When administered orally, however, these compounds suffered extensive first-pass metabolism in the liver, which consequently resulted in a low bioavailability. Moreover, the high lipophilicity of many of them led to a high degree of plasma protein binding (often > 99%) and consequently to low levels of drug in the site of infection. The search for antifungal agents with acceptable pharmacokinetic metabolism led to the development of the series of bis-triazoles compounds (Pereira, 2007).

Fluconazole differs from imidazoles in its pharmacokinetic properties being less lipophilic. The presence of two triazole rings in its structure is responsible for lower lipophilicity. Its log P octanol is equal to 0.5 and its solubility in water is 8 mg/mL at 37°C, which assures sufficient conditions to be formulated for intravenous use. The presence of a halogenated phenyl ring increases the activity of the drug against fungi. Fluconazole also has high metabolic stability achieved by the combination of three structural elements: the strength of the triazole ring to oxidative attack, the blockage of aromatic hydroxylation by the presence of two fluorine atoms, and the steric hindrance of the hydroxyl, a possible site to conjugation (Dash and Elmquist, 2001; Pereira, 2007).

Fluconazole shows good stability in aqueous solution; some studies show extemporaneous or injectable preparations of the drug whose content has remained stable for more than 15 days (Yamreudeewong et al., 1993; Dentingerand Swenson, 2009). The stability of injectable formulations for 1 to 2 years when stored in glass or plastic jars at a temperature of 5 to 30°C has been reported (Dash and Elmquist, 2001).

Polymorphism

Fluconazole has polymorphism. The first time its polymorphism was reported was by Gu and Jiang (1995), which showed

two different crystalline forms for the drug. Currently another polymorphic form is also known (Dash and Elmquist, 2001).

Alkhamis and coworkers (2002) discovered two crystalline forms (I and II), two solvates, and a monohydrate form. The X-ray analyzed forms showed different patterns of diffraction. When the forms were analyzed by infrared radiation differences in spectra were observed. Through the technique of differential calorimetry, thermograms were obtained indicating fusion of each form at different temperatures and that the polymorphic form II becomes form I, the more stable form. This transformation also happens when the product is compressed, which was observed in the test of intrinsic dissolution. The solubility in water and the intrinsic dissolution of these various forms of fluconazole are different. The amorphous form has higher solubility and dissolution rate than the others. The monohydrate form has the lowest solubility and dissolution rate.

Still, according to the report by Alkhamis and coworkers (2002), the transformation of the less stable polymorphic form into the most stable form, under normal conditions of temperature and pressure, is a matter of time. Therefore, in agreement with these authors, the polymorphic form II is not stable enough to be used in the production of medicines containing fluconazole.

Park and coworkers (2007b) showed that some characteristics of fluconazole in the solid state can be modified by recrystallization using supercritical antisolvent. The polymorphic forms are affected by experimental conditions such as temperature and organic solvent used, and the variation of pressure changes the orientation of the formation of crystals. These authors confirm what was suggested by Alkhamis and coworkers (2002), that the transformation of the anhydrous form II to a more stable form (anhydrous I) occurs.

In a thermo analytic study of polymorphic transformations of fluconazole, Desai and coworkers (2003) showed that cycles of heating and cooling of fluconazole lead to changes between the polymorphic forms of the drug.

METHODS OF ANALYSIS

The official monograph on fluconazole raw material, in the *United States Pharmacopeia* (USP) (*United States Pharmacopeia*, 2008), advocates the identification of the drug through the equivalence of the infrared spectrum or the spectrum of absorption of ultraviolet radiation with the standard spectrum. For the assay using nonaqueous titration with perchloric acid as titrant is recommended. Fluconazole has some related compounds from its synthesis route: compounds A, B, and C (*United States Pharmacopeia*, 2008). The USP describes three different tests and recommends test 1 or tests 2 and 3 for the determination of related compounds. Tests 1 and 2 are methods using HPLC and test 3 uses TLC. In test 1 the mobile phase consists of acetonitrile and water in isocratic mode and test 2 uses two acetate buffers, acetonitrile and methanol, in gradient mode (USP, 2008).

The Brazilian Pharmacopoeia (BF) (*Farmacopéia Brasileira*, 2005) includes fluconazole monographs for the raw material and capsules. It recommends titration for the determination of the raw material in a nonaqueous medium. For the capsules it recommends spectrophotometry in the ultraviolet region at 261 nm. There is no recommendation for the dissolution test for fluconazole capsules.

The dissolution process is the release of the drug from its pharmaceutical form to become available to be absorbed by the body. Dissolution testing is an important tool in quality control of medicines throughout their life. The dissolution characteristics of medicines should remain constant through the period of validity. This test becomes more important for drugs of relatively low aqueous solubility, such as fluconazole.

The method of dissolution must be discriminatory and able to evaluate the performance of the product and detect possible changes occurring during the stability study. The BF and USP state that the dosage forms should not remain on the surface of the liquid during the dissolution test. When the USP apparatus 2 (paddles) is used, sinkers can be employed to help sink the capsules. Sinkers are made of inert material and they have a shape and size compatible with the capsules.

Coelho and coworkers (2004) developed a method for dissolution testing of fluconazole in capsules. They used paddles, 900 mL of medium consisting of 0.2 M phosphate buffer, pH 7.0. It was maintained at $37.0 \pm 0.5^{\circ}C$ and 100 rpm stirring speed. Sinkers were not used. Porta and coworkers (2002) used for fluconazole dissolution USP apparatus 1 (basket), 0.1 M HCl kept at $37.0^{\circ}C$ as the medium, and medium stirring speed equal to 100 rpm.

The U.S. Food and Drug Administration (FDA) recommends the use of deionized water as the dissolution medium for fluconazole tablets and suspension marketed in the United States. It also recommends paddles at agitation equal to 50 rpm. The FDA recommends for tablets the use of 900 or 500 mL of dissolution medium depending on the dosage. In the achievement of the dissolution profile, sampling should occur after 10, 20, 30, 45, and 60 minutes of testing (U.S. FDA, 2010).

There are several published methods to determine fluconazole in pharmaceutical dose forms: raw material or biological material.

Porta and coworkers (2002) developed a method using spectrophotometry in the ultraviolet region for the determination of fluconazole in dissolution tests. It uses hydrochloric acid 0.1 M as solvent. Beer's law was observed in the range of 0.0080 mg/mL and 0.5601 mg/mL. The spectrum of absorption with the best resolution was obtained in the concentration of 200 mg/mL of fluconazole. The maximum absorption was observed at 261 nm and 267 nm. The readings were taken at 261 nm. The method shows accuracy, precision, specificity, limit of detection, limit of quantification, and suitable linearity and does not suffer interference from excipients of the formulations tested.

Coelho and coworkers (2004) proposed a method similar to that developed by Porta and coworkers (2002). The method

TABLE 1
Parameters Described in the Literature to Determine Fluconazole using HPLC

Reference	Sample	Column	Mobile phase/flow/gradient	Detector	Origin
Hurtado et al., 2008	Injectable	Phenomenex Synergi Fusion RP-80 C18 (150 × 4.60 mm, 4 μm)	Water : MEOH, (55:45, v/v)/isocratic	UV, 260 nm	UFSM, Santa Maria, Brazil
Barathi et al., 2008	Plasma	HyPurity C18 (50 × 4.6 mm; 5.0 μm)	CAN : ammonia 0,2% (v/v), (80:20, v/v)/ 0.5 mL/min/isocratic	Mass	Hyderabad, India
Zhang et al., 2008	Blood	Waters C18 (250 × 4.6 mm, 5 μm)	CAN : water (36:64, v/v)/ 0.8 mL/min/isocratic	UV, 210 nm	University of Pittsburgh School of Pharmacy, Pittsburgh, USA
Youdim et al., 2008	Human fluids	Phenomenex Synergi Fusion high pressure (20 × 2 mm, 2.5μm)	Solution A: water : CAN, with 0.1% formic acid (95:5 v/v). Solution B: CAN, with 0.1% formic acid/1 mL/min/gradient	Mass	Pfizer Global Research and Development, Sandwich, Kent, UK
Marciniec et al., 2007	Raw material	Purosphere STAR C18 (55 × 4 mm, 3 μm)	$NaH_2PO_4.H_2O$–MEOH–CAN (82.7:7.1:10.2, v/v/v)/1.5 mL/min/isocratic	UV, 254 nm	Poznan University of Medical Sciences, Poland
Ayub et al., 2007	Human fluids	C 18 (250 × 4 mm, 5 μm), 30°C	MEOH : B phosphate, 0.025 mol/L, pH 7,0, (45:55)/1.0 mL/min/isocratic	UV, 260 nm	UFMG, Belo Horizonte, Brazil
Carrasco-Portugal and Flores-Murrieta, 2007	Plasma	C18 (150 × 3.9 mm, 5 μm)	Sodium acetate 0.01 mol/L, pH 5.0 (with NaOH) : MeOH : CAN (75:20:5 v/v/v)/1.2 mL/min/isocratic	UV, 260 nm	Health Department, Mexico
Conrado et al., 2007	Rat plasma	C18 (150 × 4.6 mm, 5 μm)	CAN : water (80:20, v/v), with 0.4 mM of ammonium hydroxide, and 0.2 mM of acetic acid, pH: 8.0/1.0 mL/min/isocratic	Mass	UFRGS, Porto Alegre, Brazil
Holler and Valenta, 2007	*Candida albicans*	Nucleosil 100-5, C18 (240 × 4 mm)	B phosphate, 0.012 *M*, pH 7.4 : MEOH, (55:45 v/v), with addition of 1 m*M* of octanossulfonic acid/1.0 mL/min/isocratic	UV, 260 nm	Faculty of Life Sciences, Vienna, Austria

Kim et al., 2007	Plasma	C18 (250 × 4.6 mm, 5 μm), 30°C	CAN : ST sodium phosphate, 10 mM (30:70, v/v), pH 5.7/1.0 mL/min/isocratic	UV, 210 nm	Seoul, Korea
Wattananat and Akarawut, 2006	Plasma	C18 (150 × 4.6 mm), 35°C	B sodium acetate, 10 mM, pH 5.0 : MEOH, (65:35, v/v)/1.0 mL/min/isocratic	UV, 210 nm	Ministry of Public Health, Nonthaburi, Thailand
Porta et al., 2005	Plasma	C18 (150 × 4.6 mm, 5 μm)	Water : CAN, (70:30)/1.0 mL/min/isocratic	UV, 210 nm	USP, São Paulo, Brazil
Egle et al., 2004	Serum	Nucleosil 100-5, C18 (250 × 4.6 mm, 5 μm)	ACN : B dehydrogenate sodium phosphate, pH 5.0, 50 mM, (26.8:73.2, v/v)	UV, 210 nm	University Hospital Freiburg, Freiburg, Germany
Sun et al., 2004	Fluconazole in gel	C18 (125 × 3 mm, 3 μm)	MEOH : water : ammonium hydroxide, (80:20:0.001, v/v/v)/0.3 mL/min/isocratic	Mass	Shandong University, Jinan, China
Mathy et al., 2003	Rat fluids	Microbore Nucleosil C18 (150 × 1 mm, 3 μm)	B phosphate (20 mM) : CAN (75:25, v/ v, pH 7.0)/40 μL/min/isocratic	UV, 210 nm	Unité de Pharmacie Galénique, Université Catholique de Louvain, Brussels, Belgium
Sasongko et al., 2003	Human fluids	C18 (150 × 2.1 mm, 5 μm)	CAN : B sodium orthophosphate dehydrogenate (0.05 M), pH 4, (20 : 80, v/v) and (17 : 83, v/v)/0.3 mL/min/isocratic	UV, 210 nm	Faculty of Pharmacy, The University of Sydney, Australia
Aboul-Eneim et al., 2002	Capsule and injectable	C18 (25 × 4.6 mm, 10 μm)	CAN : B phosphate (pH 7) with trihydroximetil aminomethane (25 mM) (55:45, v/v)/1.5 mL/min/isocratic	UV, 260 nm	Faculty of Pharmacy, Cairo University, Egypt
Lee et al., 2002	Rat fluids	C18 (150 × 4.6 mm, 5 μm)	MEOH : octanossulfonic acid, 1mM, pH 3.0 (30:70, v/v)/1 mL/min/isocratic	UV, 210 nm	Institute of Pharmacology, National Yang-Ming Uni ersity, Taipei, Taiwan

(*Continued on next page*)

TABLE 1
Parameters Described in the Literature to Determine Fluconazole using HPLC (*Continued*)

Reference	Sample	Column	Mobile phase/flow/gradient	Detector	Origin
Majcherczyk et al., 2002	Plasma	C18 (250 × 4 mm, 5 μm), 30°C	B sodium acetate, 0.1 M, pH 5.0 : MEOH (70:30, v/v)/1 mL/min	UV, 210 nm	Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland
McLachlan et al., 2001	Human fluids	C8 (250 × 4.6 mm, 5 μm)	B Na2HPO4, 0.05 mol/L, pH 4.0 : CAN (80:20, v/v)/1.3 mL/min/isocratic	UV, 260 nm	The University of Sydney, Australia
Moraes et al., 1999	Plasma	C18 (150 × 4.6 mm, 4 μm), 40°C	Acetic acid, 5 mM : CAN, (60:40), pH 3.7/0.9 mL/min/isocratic	Mass	Institute of Biomedical Sciences, USP, Brazil
Vaden et al., 1997	Cat fluids	C18 (220 × 4.6 mm, 5 μm)	Water : CAN (84:16, v/v), pH 3.0/1.0 mL/min/isocratic	UV, 210 nm	North Carolina State University, USA
Coclgho et al., 1996	Plasma	C 8 (125 × 4 mm, 5 μm)	Water : CAN (72:28, v/v)/1.0 mL/min/isocratic	UV, 260 nm	Institut de Biologie, Montpellier, France
Koks et al., 1995	Human fluids	C18 (125 × 4 mm, 5 μm)	B sodium acetate, 0.01 M, pH 5.0 : MEOH, (70:30, v/v)/1.0 mL/min/isocratic	UV, 261 nm	Slotervaart Hospital, Amsterdam, Netherlands
Wallace et al., 1992	Human fluids	Mixed phase to liquid chromatography Varian PTHAA-5 (150 × 4 mm)	B monobasic phosphate, 0.051 M, pH 3.0 : ACN, (15:85, v/v)/0.9 mL/min/isocratic	UV, 210 nm	University of Texas Health Science, Texas, USA

ACN = acetonitrile; MEOH = methanol; B = buffer; UV = ultraviolet.

used for determination of fluconazole raw material and product used sodium hydroxide solution 0.1 M as solvent. The method also does not suffer interference from excipients of the formulations tested and was shown to be accurate and with good repeatability.

Due to low solubility in water and the possible protonation of the triazole ring nitrogens, the same researchers (Coelho et al., 2004) also proposed two other methods for determination of fluconazole raw material by titration in nonaqueous medium using 0.1 M perchloric acid as titrant and methilrosanilinic chloride and p-naftolbenzeina as indicators. The two methods' results do not differ by more than 1%.

Marciniec and coworkers (2007) proposed some methods for the determination of fluconazole raw materials and in stability study after sterilization by ionizing radiation. One of these methods was developed with spectrophotometry in the ultraviolet region. They used a methanol-water mixture (1:4) as solvent and fluconazole at concentration equal to 0.02% w/v.

Another method proposed uses HPLC. It employs a reversed-phase C18 column (Purosphere STAR) and solution of $NaH_2PO_4.H_2O$-methanol-acetonitrile (82.7:7.1:10.2, v/v/v) like mobile phase. Flow of 1.5 mL/min and a wavelength of 254 nm were used (Marciniec et al., 2007). Five methods using TLC were also proposed, each with a different mobile phase.

Hurtado and coworkers (2008) developed an analytical method by HPLC for the quantification of fluconazole in injectable form. They used a reversed-phase C18 column (150 × 4.60 mm, 4 nm; Phenomenex), isocratic elution of mobile phase composed of water and methanol (55:45, v/v), and detection by ultraviolet at 260 nm. The retention time of fluconazole was 4.9 minutes.

Another HPLC method developed for determination of fluconazole was proposed by Abdel-Moety and coworkers (2002). This method uses a reversed-phase C18 column and the mobile phase consisted of a mixture of acetonitrile and triidroximetil aminomethane (25 mM) dissolved in phosphate buffer (pH 7) at the proportion 55:45 (v/v). It used a flow rate of 1.5 mL/min and 260 nm. The retention time of fluconazole was 2.4 minutes, approximately.

Many published studies provide methods for the determination of fluconazole in body fluids, tissues, and cells permeated by HPLC, as seen in Table 1. Some of these published methods were developed without the use of buffer solutions like mobile phase, which increases the useful life of the chromatographic column and the equipment (Hurtado et al., 2008; Zhang et al., 2008; Porta et al., 2005; Vaden et al., 1997; Cociglio et al., 1996). Many studies also employ mass detector for the detection of small concentrations of fluconazole in biological samples (Bharathi et al., 2008; Youdim et al., 2008; Conrado et al., 2007; Sun et al., 2004; Moraes et al., 1999).

Different methods for determination of fluconazole include microbiological method (Hurtadoetal., 2008), gas chromatography (Debruyne et al., 1988), and micellar electrokinetic capillary chromatography (MECC) (Heeren et al., 1996).

Recently some articles have been published on the identification and characterization of fluconazole impurities in raw materials (Dongre et al., 2006, 2007). However, this review has not found data on degradation products of fluconazole.

CONCLUSION

It is true that the new triazoles are distinguished by their broad spectrum of activity and lower toxicity to patients, which brings benefits to treatments. These characteristics lead to the high number of prescriptions for these drugs and their associates.

The technological advance of formulations containing fluconazole suggests the need for development and optimization of analytical methods capable of ensuring the quality of such pharmaceuticals. Some issues are still not addressed in relation to fluconazole, such as study of its stability and degradation; these topics must be objects of study to improve knowledge about the drug and lead to the correct and safe handling of fluconazole.

Pharmaceutical products have to obey the law and ensure their efficacy without an increase in risk of the life of the consumer. It is necessary for the routine quality control of pharmaceutical products to employ well-characterized and fully validated analytical methods to yield reliable results that can be satisfactorily interpreted. This review is important because it presents several methods to analyze fluconazole and their advantages.

ACKNOWLEDGMENTS

The authors acknowledge CNPq (Brasília, Brasil) and FAPESP (São Paulo, Brasil).

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