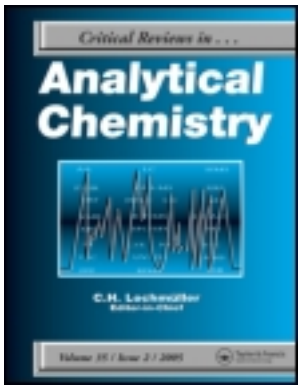

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Review of Fluconazole Properties and Analytical Methods for Its Determination

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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 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 it 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 physical-chemical 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 water, and air, on plants, 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 a 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 a 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).

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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, rarer, are increasing in frequency. Recent advances in therapy and invasive techniques for diagnosis have led to the increase of immunocompromised patients due to longer their life expectancies (Park et al., 2007a; Moretti, 2007). The risk factors have changed the spectrum of pathogens that cause systemic infection, to the emergence of fungal infections (Moretti, 2007).

Systemic infection by *Candida* sp. in these patients may go quickly and is often fatal. The yeast of the genus *Candida* has been considered among the main causal agents of systemic infection of hospital origin and represents 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 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. Much effort has been undertaken towards the introduction of new antifungal agents; however, progress in this area is slow in comparison with antibacterial agents (Carrillo-Muñoz et al., 2006).

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

This document is structured as follows: the next section discusses fluconazole and its therapeutic class, followed by sections on pharmacological and physical-chemical properties and analytical methods, and ends with some conclusions and indications of future studies.

FLUCONAZOLE

Among the classes of drugs available, the azoles are used extensively, 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. There are also many generic pharmaceuticals or those similar to Zoltec®available in the Brazilian market (Brasil, 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), and HPLC and by microbiological methods.

Currently there is great effort to ensure the quality, effectiveness, and stability of pharmaceutical products up to the moment of use. Thus, several research endeavors 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 and Moreno, 2009).

We have studied drugs still poorly researched 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 serum half-life sufficiently long to allow the 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 fungal cell membrane. Its essential function is to be a bioregulator of the membrane fluidity, asymmetry, and integrity. This is the target of azole derivatives (Carrillo-Muñoz et al., 2006).

However, new triazole agents have a more specific target. 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 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, strains resistant to fluconazole have emerged after prophylactic use for a long time. This prophylaxis decreases the occurrence of infections by *Candida* but increases the 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 11–12% (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 in patients using fluconazole have been reported, 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 (Fig. 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 & Elmquist, 2001; O'Neil, 2006).

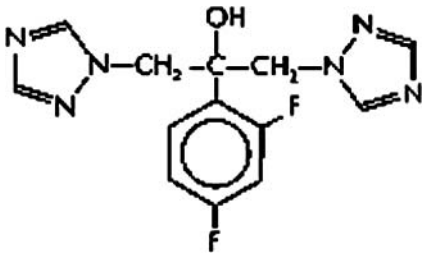

FIG. 1. Chemical structure of fluconazole (CAS 86386-73-4).

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: the 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 metabolics led to the development of the series of bis-triazoles compounds (Pereira, 2007).

Fluconazole differs from the 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 a high metabolic stability. This has been 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; Dentinger and Swenson, 2009). The stability of injectable formulations for 1 to 2 years when stored in glass or plastic jars at the temperature of 5 to 30°C has also been reported (Dash and Elmquist, 2001).

Polymorphism

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

1995); the study showed two different crystalline forms for the drug. Currently another polymorphic form is also known (Dash and Elmquist, 2001).

Alkhamis and collaborators (2002) showed 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 happened when the product is compressed, which was observed in a 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 others. The monohydrate form has the lowest solubility and dissolution rate.

Still, according to the report by Alkhamis and collaborators (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 collaborators (2007b) showed that some characteristics of fluconazole in the solid state can be modified by recrystallization using a 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 collaborators (2002), the transformation of the anhydrous form II to a more stable form (anhydrous I).

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

METHODS OF ANALYSIS

The official monograph for fluconazole raw material, described in the *United States Pharmacopoeia* (USP) (*United States Pharmacopoeia*, 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 Pharmacopoeia*, 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 (*United States Pharmacopoeia*, 2008).

The Brazilian Pharmacopoeia (BF) (*Farmacopéia Brasileira*, 2005) has fluconazole monographs for raw material and capsules. Titration is recommended for the determination of the raw material in a nonaqueous medium. For 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 and becoming 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 (*Farmacopéia Brasileira*, 2005) and USP (*United States Pharmacopoeia*, 2008) 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 collaborators (2004) developed a method for dissolution testing of fluconazole in capsules. They used paddles and 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. For fluconazole dissolution Porta and collaborators (2002) used USP apparatus 1 (basket), 0.1 M HCl, kept at 37.0°C, as the medium, and medium stirring speed equal to 100 rpm.

The Food and Drug Administration (FDA) recommends the use of deionized water as the dissolution medium for fluconazole tablets and suspension also marketed in the United States. It also recommends paddles at agitation equal to 50 rpm. For tablets the FDA recommends the use of 900 or 500 mL of medium dissolution depending on the dosage. In the achievement of dissolution profile, the sampling should occur after 10, 20, 30, 45, and 60 minutes of testing (U.S. Food and Drug Administration, 2006).

For the assay of fluconazole are several published methods to determine it in pharmaceutical dose forms: raw material or biological material.

Porta and collaborators (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, and limit of quantification and is linearity suitable and does not suffer interference from excipients of the formulations tested.

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
Bharathi et al., 2008	Plasma	HyPurity C18 (50 × 4.6 mm; 5.0μm)	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	School of Pharmacy, University of Pittsburgh. 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, U.K.
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 mM 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

(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
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 sodium phosphate of sodium, dehydrogenate, 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) e (17 : 83, v/v) / 0.3 mL/min / isocratic	UV, 210 nm	Faculty of Pharmacy, The University of Sydney, Australia
Aboul-Moety 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, 1 mM, pH 3.0 (30:70, v/v) / 1 mL/min / isocratic	UV, 210 nm	Institute of Pharmacology, National Yang-Ming Uni ersity, Taipei, Taiwan
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 Na_2HPO_4, 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

(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
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.

Coelho and collaborators (2004) proposed a method similar to that developed by Porta and collaborators (2002). The method 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 showed 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 collaborators (2007) proposed some methods for the determination of fluconazole raw materials and 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 a concentration of 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 different mobile phase.

Hurtado and collaborators (2008) developed an analytical method by HPLC for the quantification of injectable fluconazole. 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 to determine fluconazole was proposed by Abdel-Moety and collaborators (2002). This method used 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 a proportion of 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 column chromatographic 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 a 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 a microbiological method (Hurtado et al., 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 did not find 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 treatment. These characteristics lead to the high number of prescriptions for these drugs and their associates.

The technological advances of formulations containing fluconazole suggest 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. It must be the object 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 a rise 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.

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