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REVIEW ARTICLE OPEN ACCESS

REVIEW ON VARIOUS ANALYTICAL AND BIOANALYTICAL METHODOLOGIES FOR FLUCONAZOLE

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ABSTRACT

Fluconazole is an azole antifungal drug, used to treat serious fungal or yeast infections, such as those affecting the vagina, mouth or throat, lungs, bladder, or blood. It comes in injectable, topical and oral dosageforms. The development and validation of analytical methods are ongoing, interrelated processes carried out during the drug development process. Establishing the suggested analytical method's accuracy, specificity, precision, and robustness for the pharmaceutical industry's analysis of a drug moiety is the main goal of its development and validation. The creation of a methodology is necessary for the pharmaceutical formulation's drug discovery, development, and assessment. Validation is required to demonstrate that an analytical method is appropriate and suitable for the intended use, which is frequently a crucial prerequisite for analytical purposes. This article focuses on the development of different analytical method development techniques and validation of fluconazole.

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INTRODUCTION

The triazole class of antifungals includes fluconazole, α-(2.4-diflurofenil)-α-(1H-triazol-1-methyl)-1H-1,2,4-triazol-1-ethanol.It is recommended for oropharyngeal candidiasis, esophageal, vaginal, and deep infections and exhibits activity against species of Candida. Ergosterol, a steroid that is specific to fungal cell membranes, is selectively inhibited by fluconazole. Developed in the 1980s, fluconazole is a triazole that exhibits activity against species of Candida and is available in oral tablet, capsule and suspension forms, topical gel, lotion and dusting powder,and injection for intravenous infusion (1).

Physicochemical Properties: Among the triazole class, fluconazole is a member of propan-2-ol that has a 2,4-difluorophenyl group at position 2 and 1H-1,2,4-triazol-1-yl groups at positions 1 and 3. It has a solid, white, crystalline powder appearance. It has molecular weight of 306.27 g/mol. The melting point isbetween 138-140 °C. ThepKa value is 1.76. Although it dissolves poorly in water, it dissolves in organic solvents including methanol, propylene glycol, chloroform, and others (2).

Pharmacokinetic: Fluconazole has an oral bioavailability of over 90%, according to measurements. Fluconazole's protein binding is thought to be between 11-12%. When given over an extended period of time, fluconazole's significant penetration in a variety of bodily fluids makes it an excellent treatment for systemic fungal infections.The liver metabolizes fluconazole minimally. Fluconazole is a CYP2C9, CYP3A4, and CYP2C19 inhibitor. About 80% of the administered dose of fluconazole is found in the urine as unmodified medication in healthy volunteers, where the drug is excreted through the kidneys. After oral treatment, the terminal elimination half-life in plasma is roughly 30 hours (range: 20–50 hours).(3)

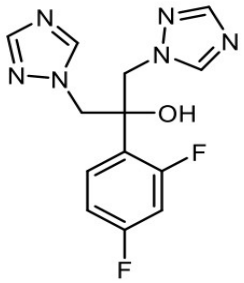

Figure 1. Chemical Structure of Fluconazole

Pharmacodynamic: Fluconazole exhibit fungistatic activity, against various microorganisms: Candida albicans, Candida glabrata, Candida Para psilosis, Candida tropicalis, and Cryptococcus neoformans. In order to treat fungal infections and their symptoms, steroidal inhibition in fungal cells is used to disrupt cell adhesion, proliferation, and synthesis of the cell wall. In normal and

immunocompromised animal models, fluconazole has also demonstrated fungistatic action against intracranial and systemic fungal infections.(4)

Mechanism of action: The fungal cytochrome P450-dependent enzyme lanosterol 14-α-demethylase is highly selectively inhibited by fluconazole. Normally, this enzyme converts lanosterol to ergosterol, which is required for the formation of fungal cell walls. One iron atom in the heme group of lanosterol 14-α-demethylase forms a bond with the free nitrogen atom on the azole ring of fluconazole. This stops the process of ergosterol manufacture by preventing oxygen activation, which in turn stops lanosterol from being demethylated. It is then discovered that methylated sterols build up in the fungal cellular membrane, which inhibits the growth of the fungus. The structure and functionality of the fungal cell plasma membrane are negatively impacted by these accumulating sterols (5).

Need of analytical method development and validation: During the drug discovery, release to market, and development processes that lead to marketing approval, the development of sound analytical method or methods is crucial. In order to support routine, in-process, and stability analysis, methods for determining the selectivity, specificity, limit of detection, limit of quantitation, linearity, range accuracy, precision, recovery solution stability, ruggedness, and robustness of various analytical methods are being developed. These methods are intended for use in finished product or process tests, as well as in the preparation of drug products (6).

UV-visible spectroscopy is used to determine the absorbance spectrum of a chemical in solid or solution form. (7)

Analytical Method Development by HPLC: The most popular analytical method is High Performance Liquid Chromatography (HPLC). HPLC is a separation method that primarily consists of stationary and mobile phases with opposite polarities and uses high pressure pumps for separation. The interaction between the stationary and mobile phases allows for separation.Variables that are crucial to the creation of a method include the pH of the mobile phase, various types of buffer, column temperature, sample diluents, detection wavelength, and many more (15).

Analytical Method Development by HPTLC: Based on the full potential of thin layer chromatography, high performance thin layer chromatography (HPTLC) is an advanced instrumental technique. It is a potent analytical instrument for chromatographic information of complex mixtures of inorganic, organic, and biomolecules because of its benefits, which include automation, scanning, comprehensive optimization, the selective detection principle, minimal sample preparation, hyphenation, etc. HPTLC has great promise for estimating partitioning qualities to enable investigations on environmental destiny, combinatorial chemistry, and health effects. (21)

Analytical method development using bioanalytical methods: In the process of discovering and developing new drugs, bioanalysis is

Table 1. Analytical method development using UV-spectrophotometer

S. No.	Sample / Dosage form	Method / Instrument model	Solvent / Solution	Wavelength (nm)	References
1.	Tablet	UV-Visible Spectrophotometer (UV-1700 SHIMADZU)	Phosphate buffer pH 7.4	260	(8)
2.	Bulk	UV-visible spectrophotometer	0.1N HCl	239	(9)
3.	Bulk	UV-Visible Spectrophotometer, (Model UV1800)	Water	260.8	(10)
4.	Tablets	UV-visible spectrophotometer 1700 Ultra	Ethanol	252	(11)
5.	Tablets	Spectrophotometer UV-Vis Cary 100 Conc (Varian Analytical Instruments, Palo Alto, USA)	Methanol	261	(12)
6.	Tablets	Shimadzu UV 1700 double beam spectrophotometer	Phosphate buffer pH 6.8	260.57 & 264.23	(13)
7.	Capsules and IV Solutions	Shimadzu UV-160A recording double beam UV-visible spectrophotometer	Distilled water	261.6 & 274	(14)

Table 2. Analytical method development using HPLC method

S.No.	Sample	Stationary phase/column	Mobile phase	Wavelength (nm)	Flow rate (ml/min)	RT (min)	Reference
1.	Micro-emulsions & liquid crystals	C18-RP column (250 × 4.6 mm	Acetonitrile: water (50:50, v/v)	210	1.0	6.3	(16)
2.	Tablets and bulk	C18 column (4.6 x 250 mm, 5µm)	Acetonitrile and phosphate buffer pH 4.8 (50:50 % v/v)	210	1.0	5.25	(17)
3.	Capsules, IV infusions and bulk	C18 (10 mm, 25 cm, 4.6 mm)	Acetonitrile and 25 mMtris-hydroxymethyl aminomethane in phosphate butter pH 7 (55:45 v/v)	260	1.5	2.474	(18)
4.	Tablets and capsules	C-18 (150 mm x 4.6 mm, 5 µm)	Water and acetonitrile (65:35 v/v)	260	1.0	2.47	(19)
5.	Tablets	C18 column (4.6 ×250 mm, 5 µm)	0.05 M potassium dihydrogen phosphate buffer (pH 3.25 and acetonitrile (82:18, v/v).	210	1.5	5.38	(20)

Table 3. Analytical Method Development Using HPTLC Method

S.No.	Sample	Stationary Phase/ Column	Mobile phase	Wavelength (nm)	Reference
1.	Tablets and bulk	Silica gel 60 F254	Butanol: water: acetic acid (8:2:1 v/v)	254	(22)
2.	Tablets or capsules and bulk	Silica gel 60 GF254	Toluene: chloroform: methanol 1.2:3.0:0.4 (v/v)	210	(23)
3.	Tablets	Silica gel 60F254	Toluene: Ethyl acetate: Chloroform: Methanol (1.2:3:2:0.8 v/v)	205	(24)

Analytical Method Development by UV-Spectrophotometry: The pharmaceutical sector has been using UV spectroscopy as an important and efficient analytical tool. This pharmaceutical analysis includes all of the procedures needed to ascertain the identity, strength, quality, or purity of the substances. The study methodology uses colorless materials to test their absorption of monochromatic light in the 200–400 nm band of the near-ultraviolet spectrum.

crucial. The examination of analytes in biological samples is known as bioanalysis, and it entails a number of procedures, including sample collection, analysis, and data reporting. Samples from clinical or preclinical investigations are first collected, and they are subsequently sent to a lab for analysis. The second step is, sample cleanup or preparation. A steady and robust sample preparation technique should be used to get accurate results. The final step is the analysis and detection of the material.(25)

Table 4. Bioanalytical Method Development

S.No.	Method	Sample	Stationary phase/ column	Mobile phase	Wavelength (nm)	Flow rate (ml/min)	RT (min)	Reference
1.	LC-MS/MS	Human Plasma	C18 (100 x 2.1mm, 3.5 µm)	0.1% Formic acid water and acetonitrilewith 0.1% formic acid (70:30 v/v)	-	0.2	-	(26)
2.	HPLC	Biological Skin Matrices	RP 18 column (5 µm, 250 × 4 mm)	Methanol and 0.025 mol/L phosphate buffer (45:55, v/v), pH 7.0	260	1.0	5.1	(27)
3.	HPLC - UV	Human plasma and urine	ODS 100 A column (250 x 4.6 mm, 5 µm)	0.15M SDS, 0.3% TEA and 12% n-propanol in 0.02M orthophosphoric acid at pH 5.5	210	1.0	3.89	(28)
4.	HPLC	Human plasma	C18 column (150 × 4.6 mm)	10 mM acetate buffer at pH 5.0 and methanol (65:35 v/v)	210	1.0	7.00	(29)
5.	HPLC	GFS, VFS, topical simulatedmedia,PBS	C18 column (250 mm x 4.6 mm x 5 µm)	Waterand acetonitrile, pH 5.2 (80:20, v/v)	260	2.5	8.05 8.02 7.98 7.97	(30)
6.	HPLC	Human Whole Blood	C18 (5 µm, 4.6 x 250 mm)	Acetonitrile and water (36:64, v/v)	210	0.8	4.7	(31)
7.	HPLC-UV	Human plasma	C18 (250 x 4.6 mm, 5µm)	pH 5.0 with glacial acetic acid buffer-acetonitrile (80:20, v/v)	260	1.2	8.3	(32)
8.	HPLC	Human Urine	C18 (150 mm x 4.6 mm, 5 µm)	Methanol: water (70:30, v/v)	254	0.85	-	(33)
9.	HPLC	Intracellular concentrations	C18 (250 × 2 mm, 3 μm)	0.01 mol/L ammonium acetatein water (pH5) and acetonitrile	210	0.3	6.8	(34)
10.	HPLC	Human plasma	C18 (250 mm × 4.6 mm, 5 µm)	Acetonitrile and 10 mM sodium phosphate buffer (30:70, v/v)	210	1.0	4.6	(35)
11.	LC-MS	Human Plasma	50 x 4.6 mm, 4 µm	Methanol, 0.1%v/v formic acid	-	1.0	-	(36)

CONCLUSION

Drug development requires the development, validation, and transfer of analytical methods. For a biotech company, method development is essential for patient safety, regulatory, and commercial reasons. Approval of clinical trials and marketing authorizations are unattainable without the development and validation of high-quality methods. The many analytical techniques for estimating fluconazole in bulk and in its varied dosage forms were covered in this article. These techniques could be useful for fluconazole analysis in a variety of study fields.

Conflict of interests: The author reported no conflict of interests.

REFERENCE

Janowski M, Demchuk OM, Wujec M. Fluconazole Analogs and Derivatives: An Overview of Synthesis, Chemical Transformations, and Biological Activity. Molecules. 2024; 29(12).

Corrêa JCR, Salgado HRN. Review of fluconazole properties and analytical methods for its determination. Crit Rev Anal Chem. 2011;41(2):124–32.

Carrasco-Portugal MDC, Flores-Murrieta FJ. Gender differences in the oral pharmacokinetics of fluconazole. Clin Drug Investig. 2007;27(12):851–5.

Lee W, Lee DG. A novel mechanism of fluconazole: Fungicidal activity through dose-dependent apoptotic responses in Candida albicans. Microbiol (United Kingdom). 2018;164(2):194–204.

Ali OT, Elgendy KM, Saad MZ, Hassan WS, Sebaiy MM. Analytical Techniques for Determination of Albendazole , Fenbendazole , Omeprazole and Fluconazole in Pharmaceutical and Biological Samples. 2021;2(1).

Lavanya G, Sunil M, Eswarudu M., Eswaraiah MC, Harisudha K, Spandana BN. ANALYTICAL METHOD VALIDATION: AN UPDATED REVIEW Department of Pharmaceutical Analysis, Anurag Pharmacy College, Ananthagiri (V), Kodad (M)- 508206, Nalgonda (Dt),. Int J Pharm Sci Res. 2013;4(4):1280–6.

Khokrale S, Lulla J, Borse L. A Review Of The UV-Visible Spectroscopy ' s Method Development And Validation. Int J Pharm Sci. 2024;2(6):527–38.

Shoaeb Mohammad Syed, Marathe RP. Development and Validation of UV Spectrophotometric Method for the Estimation of Fluconazole in the Marketed Dosage Formulations. *Pharm Biosci J.* 2020;8(5):22–6.

THAKUR B, KUMAR I. New Developed and Validated Spectroscopic Method for the Simultaneous Estimation of Terbinafine Hydrochloride and Fluconazole. *Int J Pharm Pharm Sci.* 2020; (November):19–25.

Anandkumar Y, Kapse-mistry S, Kadu P. U . V . Spectrophotometric Method Development and. 2015;4(9):1472–80.

Chanchala M, Nimita M, Archana D, Shweta C. Development and Validation of UV Spectroscopic Method for Estimation of Lamivudine in Tablet Dosage Form. *Int J Pharm Sci Med.* 2023;8(6):73–9.

Ekiert RJ, Krzek J. Determination of azole antifungal medicines using zero-order and derivative UV spectrophotometry. *Acta Pol Pharm - Drug Res.* 2009;66(1):19–24.

Pandey S, Pandey P, Dubey S, Chaturvedi U, Rai AK. Facile derivative UV spectroscopy method: Simultaneous estimation of tinidazole and fluconazole in combined tablet dosage form. *Thai J Pharm Sci.* 2012;36(2):55–62.

Göğer NG, Aboul-Enein HY. Quantitative determination of fluconazole in capsules and IV solutions by UV spectrophotometric methods. Anal Lett. 2001;34(12):2089–98.

P. Ravisankar*1, 2, S. Gowthami1 GDR. A Review on Analytical Method Devlopment. *Indian J Res Pharm Biotechnol.* 2014;2(3):1183–95.

e Silva HR, dos Santos FK, da Luz GM, Chorilli M, Gremião MPD. Validation of high-performance liquid chromatographic method for analysis of fluconazole in microemulsions and liquid crystals. *Brazilian J Pharm Sci.* 2014;50(2):381–9.

MALOTHU N, KONA SB, MUTHYALA B, KATAMANENI P. a Simple Liquid Chromatographic Method for Simultaneous Estimation of Azithromycin, Fluconazole and Ornidazole in Bulk and Pharmaceutical Dosage Forms. *Int J Pharm Pharm Sci.* 2019;11(8):26–34.

Abdel-Moety EM, Khattab FI, Kelani KM, AbouAl-Alamein AM. Chromatographic determination of clotrimazole, ketoconazole and fluconazole in pharmaceutical formulations. *Farmaco.* 2002;57(11):931–8.

Sadasivudu P, Shastri N, Sadanandam M. Development and validation of RP-HPLC and UV methods of analysis for fluconazole in pharmaceutical solid dosage forms. *Int J ChemTech Res.* 2009;1(4):1131–6.

Meshram DB, Bagade SB, Tajne MR. Simple HPLC method for simultaneous estimation of fluconazole and tinidazole in combined dose tablet. *J Chromatogr Sci.* 2009;47(10):885–8.

Srivastava M. High-performance thin-layer chromatography (HPTLC). *High-Performance Thin-Layer Chromatogr. 2*011;1–397.

Narayana PS, Reddy AS, Ramesh B, Devi PS. Simultaneous determination and method validation of fluconazole and its impurities by high performance thin layer chromatography using reflectance scanning densitometry. *Eurasian J Anal Chem.* 2013;8(1):39–49.

Meshram DB, Bagade SB, Tajne MR. A simple TLC method for analysis of fluconazole in pharmaceutical dosage forms. J Planar Chromatogr - Mod TLC. 2008;21(3):191–5.

Meshram DB, Mishra P, Desai SD, Tajne MR. Simultaneous Determination of Fluconazole and Tinidazole in Combined Dose Tablet using High Performance Thin Layer Chromatography. Der Chem Sin. 2017;8(1):133–7.

Moein MM, El Beqqali A, Abdel-Rehim M. Bioanalytical method development and validation: Critical concepts and strategies. J Chromatogr B Anal Technol Biomed Life Sci [Internet]. 2017;1043:3–11. Available from: http://dx.doi.org/10.1016/j.jchromb.2016.09.028

Wu D, Wade KC, Paul DJ, Barrett JS. A rapid and sensitive LC-MS/MS method for determination of fluconazole in human plasma and its application in infants with candida infections. Ther Drug Monit. 2009;31(6):703–9.

Ayub AC, Vianna-Soares CD, Ferreira LAM. Fluconazol method validation by RP-HPLC for determination in biological skin matrices. J Chromatogr Sci. 2007;45(5):286–90.

Belal F, Sharaf El-Din MK, Eid MI, El-Gamal RM. Micellar HPLC and derivative spectrophotometric methods for the simultaneous determination of fluconazole and tinidazole in pharmaceuticals and biological fluids. J Chromatogr Sci. 2014;52(4):298–309.

Wattananat T, Akarawtu W. Validated HPLC method for the determination of fluconazole in human plasma. *Biomed Chromatogr.* 2006;20(1):1–3.

Singh A, Sharma PK, Majumdar DK. Development and validation of new HPLC-methods for estimation of fluconazole in different simulated biological fluids: A comparative study. *J Liq Chromatogr Relat Technol.* 2014;37(4):594–607.

Zhang S, Mada SR, Torch M, Goyal RK, Venkataramanan R. Development and validation of a high-performance liquid chromatographic assay for the determination of fluconazole in human whole blood using solid phase extraction. Ther Drug Monit. 2008;30(3):314–9.

Liew K Bin, Loh GOK, Tan YTF, Peh KK. Development and application of simple HPLC-UV method for fluconazole quantification in human plasma. *Int J Pharm Pharm Sci.* 2012;4(SUPPL. 4):107–11.

Hermawan D, Md Ali NA, Wan Ibrahim WA, Sanagi MM. Analysis of fluconazole in human urine sample by high performance liquid chromatography method. J Phys Conf Ser. 2013;423(1).

International Journal of Analytical Chemistry - 2015 - Davtyan - Development and Validation of Simple RP-HPLC Method for.pdf.

Kim SS, Im HT, Kang IM, Lee HS, Lee HW, Cho SH, et al. An optimized analytical method of fluconazole in human plasma by high-performance liquid chromatography with ultraviolet detection and its application to a bioequivalence study. *J Chromatogr B Anal Technol Biomed Life Sci.* 2007;852(1–2):174–9.

Hillis J, Morelli I, Neville D, Fox J, Leary AC. The Validation of a Bioanalytical Method for the Determination of Fluconazole in Human Plasma. Chromatographia. 2004;59(S2):203–7.

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