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Research Article

An Overview: Analytical Methods of Estimation of Azoles as Antifungal Agents

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ABSTRACT

One of the important aspects of the drug discovery process is analytical method development and validation. To ensure quality & safety of the drug development of analytical method is done which will provide accurate & precise data. Method can be developed by using various instruments but due to speed and accuracy generally reverse phase high performance liquid chromatography is preferred by researchers. In this review we discuss various kind analytical methods available for estimation of azoles but in literature survey but mainly RP-HPLC & UV method is used.

KEYWORDS: Azoles, RP-HPLC, Analytical methods.

INTRODUCTION

The fungal kingdom mainly involves yeast, moulds, rusts etc. The fungal infection caused by invasion of micro organism in epithelial tissue. Over all kind of fungal agents some are not harmful but some are pathogenic to humans. These pathogenic fungi after entry in human body may cause mild to severe infection. This antifungal agent selectively eliminates fungal micro organism from without or with minimal toxicity to host. They are classified on the basis of pharmacophore or according to mechanism. The classification which we have is according to pharmacophore[1, 2]

Classification[2]-

1) Polyenes - Nystatine
   - Amphotericin B
2) Imidazoles - Miconazole
   - Clotrimazole
   - Ketoconazole
3) Triazoles - Fluconazole
   - Itraconazole
   - Voriconazole
   - oxiconazole
4) Allylamines –Niftifine
   - Terbinafine
   - Butenafine
5) β-3-glucan inhibitor- Caspofungin
   - Micafungin
   - Anidulafungin
6) Other- Griseofulvin
   - flucytosine
   - Tolnaftate

Azoles primarily inhibit the fungal cytochrome P450 3A Enzyme lanosterol 14-alpha-demethylase which leads to prevention of conversion of lanosterol to ergosterol, these leads to depletion of ergosterol and and accumulation of sterol precursors. Due to ergosterol depletion the integrity and function of fungal cell membrane is disrupted, leads to cell lysis.[2, 3, 4]. Generally the analytical method development and validation is important for the newly introduced drug or drug combination to check and ensure the quality standards of the drugs and drug formulations, because such

drugs are not added in the pharmacopoeia due to which method for quantification is not available. Various analytical methods are available for estimation of drug or drug combination but most widely used analytical method for drug estimation is RP-HPLC.[5, 6, 7].in this review we see various methods of estimation but emphasis given to UV and RP-HPLC, Other are also included.

Most of the drugs in single or multi component dosage forms can be analyzed by HPLC method because of the associated advantages like speed, greater sensitivity, improved resolution, specificity, accuracy, precision, reusable columns and ease of automation in this method.[5, 6, 7] This review article briefly discusses the RP-HPLC methods available for the estimation of antifungal agents in bulk and in various formulations concentrating mainly on the mobile phase, stationary phase and detector type.

Table1- Conditions for various methods for estimation of Azole antifungal agent.

Drug	Formulation	Method	column	Mobile phase/ Diluent	Detector	Lamda max/ Wavelength
Clotrimazole[8]	Tablet	UV-VIS Spectroscopy		NH_2So_4	-	263nm
Clotrimazole & Clindamycin phosphate[9]	Soft Gelatine Passeries	RP-HPLC	Hypersil BDS C8 (250x4.6mm) 5µm	Orthophosphoric acid(2.5PH):Acetonitrile 70:30 v/v	UV-Vis Dual Absorbance Detector	210nm
Clotrimazole[10]	Lozenges	HPLC	Gracemart C18, (250x4.6mm) 5µm	0.1%tri-ethanol amine(PH3 adjusted by OPA):methanol 25:75 v/v	SPD m-10avp Photodiode Array Detector (PDA)	215nm
Clotrimazole &Betamethasone [11]	Ceame	HPLC	Merck C18(250x4.6mm) 5µm	Methanol: Acetate buffer: Acetonotrile 33:27:40 v/v	Photo Diode Array Detector	254nm
Clotrimazole and Clindamycin Phosphate[12]	P'ceutical dosage form, Bulk Drug	RP-HPLC	Hypersil BDS C8 (250x4.6mm) 5µm	Phosphate Buffer: Acetonitrile 48:52v/v	Photo Diode Array Detector	220nm
Clotrimazole[13]	Bulk drug, Tablet	HPTLC	Silica Gel $60f_{254}$ TLC plates (20×20 cm, layer thickness 0.2 mm	Cyclohexane:Toluene:Methanol:Triethyleamine 8:2:0.5:0.2 v/v/v/v	Hamilton syringe (100 µl), Camag TLC Scanner-3	262nm
Clotrimazole and Beclomethasone Dipropionate [14]	Lotion & Cream	HPLC	Kromasil C18 (150 mm × 4.6 mm, 5 µm)	Acetonitrile: water 70:30, v/v	Photo diode array detector	254 nm.
Clotrimazolze & Tinidazole[15]	Pharmaceutical formulation	HPTLC	Precoated silica gel 60F254 TLC Aluminum plate, (20×10 cm 2).	Toluene: Methanol: Ethyl Acetate: Tri Ethylamine (5:1:1:0.1v/v/v/v)	Camag tlc Scanner "Scanner_171010" s/n 171010 (2.01.02),	254nm
Ketoconazole[16]	Pharmaceutical dosage form	HPLC	Lichrospher®100 c-18 Column (150 mm length x 4.6 mm, 5 µm	Methanol: Water (90:10 v/v)	PDA	
Ketoconzol[17]	Bulk& p'ceutical formulatiom	U.V. Spectrophotometric method	-	Methanol: phosphate buffer ph 7.4 (10:90)	UV Detector	287 nm
Ketoconazole[18]	Emulsion	RP-HPLC	Lichrospher® 100 rp-18 (5µm)	Mixture of Triethylamine in Methanol (1:500 v/v) and 0.5% Ammonium Acetate Solution 75:25 v/v	UV detector	225nm

Ketoconazole[19]	Emulsion	U.V. Spectrophotometric method		Methanol	UV Detector	257nm
Prednicarbate, Mupirocin and Ketoconazole [19]	Topical dosage form	RP-HPLC	Hypersil Gold C18, 5 µm, 250 mm × 4.6 mm	Methanol: Water (80: 20, v/v) adjusted to PH 5.0 with OPA	UV Detector	243nm
Hamycin Ketoconazole [20]	Cream	RP-HPLC	Thermosil c-18, (250 mm, 4.6 mm, 5µm)	0.4 % (v/v) Di isopropylamine in Methanol (v/v): 0.5% (w/v) Ammonium acetate in distilled water (90:10 % v/v) PH 6.5 adjusted with glacial acetic acid	UV Detector	263nm
Ketoconazole[21]	Bulk drug	RP-HPLC	Promosil C18, (250 mm, 4.6 mm, 5µm)	Water : acetonitrile : Buffer PH 6.8 (51:45:4 v/v)	Photodiode Array	238nm
Miconazole[22]	Bulk drug	RP-HPLC	Thermo Scientific Hypersil gold C18 column (50x 4.6) mm i.d., 1.9 mm	Acetonitrile–methanol–ammonium acetate (1.5 w/v) 30:32:38 v/v	UV Detector	235nm
Miconazole Nitrate & Metronidazole[23]	Pharmaceutical dosage form	Gas Chromatography &Flame Ionization detector (GC-FID)	Capillary Column ae.se-54 (15 m × 0.53 mm)	Nitrogen (carrier gas)	Flame Ionization Detector	
Miconazole & Clindamycin[24]	Pharmaceutical dosage form	RP-HPLC	Inertsil ODS C18 (250x4.6 mm, 5 mm)	Buffer (PH 3.5) and acetonitrile 65:35 v/v	PDA	220nm
Mometasone Furoate & Miconazole nitrate[25]	Cream	HPTLC	Silica Gel 60F254	Hexane:Chloroform:Methanol:Ammonia 6:3:1:0.1 v/v/v/v	Densitometric desaga TLC Scanner	230nm
Mometasone furoate & Miconazole nitrate[25]	Cream	HPLC	Ace column C18 (150 mm x 4.6 mm) 5 µm	Acetonitrile:water (80:20 v/v)	Deuterium lamp	229nm
Miconazole nitrate, Metronidazole[26]	Bulk Powder Form	Spectrometric method		Phosphate Buffer		318.8nm,279 nm
Miconazole,ornidazole[27]	Cream Formulation	RP-HPLC	Phenomenex luna C8 (250 x 4.6mm)5µ Column.	Acetonitrile: distilled water: o-phosphoric acid: triethyl amine 50:50:0.2:0.2 v/v	UV Detector	237nm
Miconazole[28]	Oral Sustained Release Mucoadhesive Tablets	RP-HPLC	C18 column (100 mm x 4.6 mm)	0.6% Ammonium Acetate in a mixture of 370 parts acetonitrile, 290 parts Methanol and 340 parts water.	UV Detector	235nm
Oxiconazol[29]	Bulk,Lotion, Cream	RP-LC	LiChrocart C8 column (125 x 4.0) mm, i.d., 5 µm	Methanol-0.02 M Ammonium Acetate Buffer 85:15 v/v	UV Detector	254nm
Oxiconazole[30]	Pharmaceutical Dosage Form	UPLC	BEH(50 x 2.1mm) 1.7 µm	Acetonitrile : Sodium Dihydrogen Ortho Phosphate (50:50)	UV Detector	296nm

Econazole[31]	Topical Dosage Form	HPTLC	60F254Silica Gel plates	N Butyl Acetate, Carbon Tetrachloride:Methanol: Diethylamine (3+6+2.5+0.5)		
Econazole[31]	Topical dosage form	HPLC	C18(250 x 4.6mm, 5µ)	Acetonitrile:Water (30:70)	UV Detector	230nm
Econazole[32]	Cream	Capillary Zone Electrophoresis	31.5 cm x 50 microm I.D. capillary	PH 2.5 Phosphate Buffer	UV Detector	200nm
Econazole[33]	Human plasma	RP-HPLC	Phenomenex Luna C18 Column (250mm x 4.6mm i.d, 5øm)	0.5% Triethylamine at pH 6.5 and Acetonitrile at pH 3.5	UV Detector	260nm
Itraconazol[34]	Plasma	Fluorometric detection	ShimadzuC-18 column(3.9mmx150mm	Methanol : Water 75:25v/v	UV Detector	250nm
Itraconazol[35]	P'ceutical Formulation	UV-Spectrophotometer		Chloroform	UV Detector	267nm
Itraconazol[36]	Capsule, Bulk powder	UV-Spectrophotometer		Methanol	UV Detector	262nm
Itraconazol[37]	Pharmaceutical dosage form	RP-HPLC	Dionex C18 4.6 X 250mm, 5µm	Methanol & pH 7.5 Potassium Dihydrogen Phosphate 40:60v/v	UV Detector	306nm
Itraconazol[38]	Bulk& Capsule	RP-HPLC	C18G Column (250 × 4.6 mm), 5µm	Acetonitrile and Glacial Acetic Acid 0.1% w/v,50:50 v/v	UV Detector	264 nm
Itraconazol[39]	Capsule	RP-HPLC Method	Inertsil C-18, 5µm, (250 x 4.6mm)	Tetrabutyl Ammonium Hydrogen Sulphate Buffer Solution :Acetonitrile 40:60v/v	UV Detector	225nm

CONCLUSION

In this review we cover the various analytical methods for estimation of azoles as antifungal agent. For this various pharmaceutical dosage forms, Biological samples and active pharmaceutical ingredients single or in combination are taken for estimation. For estimation of all above mentioned form of drug are analysed by various methods such as RP-HPLC, UV-Spectroscopy etc.

But during literature survey it was observed that majority of methods are estimated by RP-HPLC & UV-Spectroscopy.

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