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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SERTACONAZOLE NITRATE AND ZINC PYRITHIONE IN COMBINED DOSAGE FORM.

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Abstract: Drugs like Sertraconazole nitrate and Zinc Pyrithion were analyzed by many methods individually viz HPLC (High performance liquid chromatography) etc but there is no single method reported for the simultaneous determination of Sertraconazole nitrate and Zinc Pyrithion in combination. Although Sertraconazole nitrate is official in British Pharmacopoeia but Zinc Pyrithion is not official in any Pharmacopoeia. Hence a proper research work is well aimed to develop and validate a new analytical method for simultaneous estimation of Sertraconazole nitrate and zinc Pyrithion in Pharmaceutical Dosage form.

Keywords: HPLC, Method Development, Sertraconazole nitrate, Zinc Pyrithion, Pharmaceutical Dosage Form

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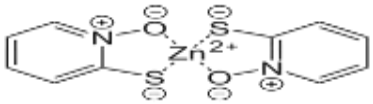
INTRODUCTION

DRUG PROFILE.

ZINC PYRITHION:-



SYNONYMS: 2-pyridinethiol 1-oxide, Omadine, Pyridinethiol N-Oxide.

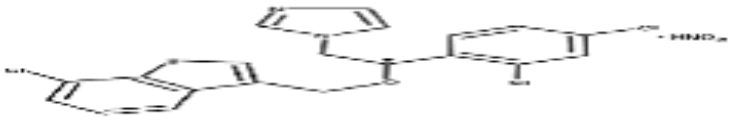
IUPAC name: bis (2-pyridylthio) zinc 1, 1'-dioxide

Molecular Formula: $C_{10}H_8N_2O_2S_2Zn$

Zinc Pyrithion being cordinated complex of pyrithione ligands chelated to Zinc (2+)ions via oxygen and sulfur centres. Dynamic fungistatic and bacteriostatic properties Zinc Prrithion is used to treat dandruff and seborrheic dermatitis. Available for topical treatments such as Shampoos, it medates its action by increasing the cellular levels of copper and damaging iron-sulfur cluster of proteins essential for fungal metabolism and growth.

Low solubility of Zinc Pyrithion helps to get well onto the target skin surface. Well approved by FDA for its safety, effectiveness and no estrogenic activity have been reported both *in vivo* and *in vitro* assays. Further the molecular basis for the antifungal activity of Zinc Pyrithion has been well elucidated, as utilizing a copper toxicity mechanisms that targets critical iron sulfur proteins.

SERTACONAZOLE NITRATE:-



SYNONYMS: Demofix, Sertaconazol.

IUPAC name: 1-{2-[(7-Chloro-1-benzothiophen-3-yl) methoxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole.

Molecular Formula: $C_{20}H_{15}Cl_3N_2OS.HNO_3$.

Being an Imidazole, Sertaconazole is highly selective inhibitor of fungal cytochrome P-450 sterol C-14 α demethylation via the inhabitation of the enzyme cytochrome P-450 14α-demethylase. The enzyme converts lanosterol to ergosterol, and is required in fungal wall synthesis. The

subsequent loss of normal sterols correlates with the accumulation of 14 α methyl sterols in fungi and may be responsible for the fungistatic activity of fluconazole.

An ergosterol an essential component of the fungal cell membrane,inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents. Sertaconazole also 1) inhibit endogeneous respiration,2) interact with membrane phospholipids,3) inhibit the transformation of yeasts to mycelia forms,4) inhibit purine uptake, and 5) Impair triglyceride and or phospholipid biosynthesis.

REVIEW OF LITERATURE [24-36]

ZINC PYRITHIONE [24-27]

Official Method available for Zinc Pyrithione

Zinc Pyrithione is not official in any Pharmacopoeia

Reported method available for Zinc Pyrithione. [24-27]

Sr.no	Drug	Method	Description	Ref.no
1	Zinc Pyrithione	HPLC and HPLC-MS/MS	Column: MG C18 column (250 mm x 4.6 mm, 5 µm). Recoveries: were 85.8-104% (HPLC) and 87.6-107% (HPLC-MS/MS).	24
2	Zinc Pyrithione	LC-MS	Mobile phase: water Concentration: 3.5 and 10 mg L. Column temp.: 298 K.	25
3	Zinc Pyrithione	HPLC	Column: C18 column Mobile phase: methanol-water (3:2)	26

			Wavelength: 320 nm.	
4	Zinc Pyrithione	HPLC	Linear: 1.4-66 ng. coefficient variation: 0.9% for 17-ng	27

SERTACONAZOLE NITRATE [28-36]

Official method available for Sertaconazole nitrate. (British Pharmacopoeia) [28]

Sr.no	Drug	Method	Description	Ref.no
1	Sertaconazole nitrate	U.V spectrophotometry	Solvent: methanol Spectra range: 240nm-320nm Abs.: 260nm,293nm, and 302nm	28
2	Sertaconazole nitrate	TLC	Mobile phase: Ammonia, Toluene, Dioxin (1:40:60: v/v/v) application: 5µL detection: expose to iodine vapour for 30min	28
3	Sertaconazole nitrate	HPLC	Stationary phase: nitrile silica gel Mobile Phase: Acetonitrile, sod. Dihydrogen phosphate (37:63 v/v) Flow rate: 1.6 ml / min	28

			Detection: 220 Injection 20µL	

Reported method available for Sertaconazole nitrate [29-36]

Sr.no	Drug	Method	Description	Ref.no
1	Sertaconazole nitrate	RP-HPLC and UV	Column: C18 column Mobile phase: acetonitrile-water Flow rate: 1.8 ml/min Detection wavelength:260nm	29
2	Sertaconazole nitrate	RP-HPLC	Column: Spherisorb CN column (10 microns) Mobile phase: Acetonitrile:0.01 M sodium phosphate (37:63 V/V) wavelength: 260 nm	30
3	Sertaconazole nitrate	HPLC	Column: 10-microns Spherisorb CN column Mobile phase: acetonitrile and aqueous 0.01 M sodium phosphate (37:63, v/v). wavelength: 260 nm	31
4	Sertaconazole nitrate	LC-MS/MS	Column: C18 column Mobile phase: methanol: 0.2% formic acid (70:30, v/v,) Flow rate	32

			:0.2 mL/min.	
5	Sertaconazole nitrate	TLC (densitometry)	Mobile phase: chloroform-acetone-33 % ammonia (14:2:0.1 v/v/v). Wavelength: 302 nm	33
6	Sertaconazole nitrate	HPLC	Column: C18 column mobile phase: 0.2% formic acid aqueous solution (75:25, *v*: *v*); flow rate: 1.0 mL/min, Wavelength: 260 nm.	34
7	Sertaconazole nitrate	Capillary electrophoresis	Capillary: 50 µm, total length 80.5 cm. Temperature: 30 °C, applied voltage 30 kV	35
8	Sertaconazole nitrate	HPLC	Column: C18 column mobile phase: acetonitrile-0.01 mol. L-1 disodium hydrogen phosphate solution (37:63) flow rate was 1.0 mL/min wavelength: 260 nm.	36

Summary of literature review

Sr.no	Drug	Method
1	Zinc Pyrithione	HPLC-MS/MS- 1 HPLC-2 LC-MS – 1
2	Sertaconazole Nitrate	UV spectrophotometry- 1

		Capillary electrophoresis- 1 HPLC- 6 HPTLC – 2 LC-MS/MS - 1
3	Sertaconazole Nitrate + Zinc Pyrithione	Till date no method is found from literature review

>Method developed to determine the Zinc Pyrithion in Shampoo using HPLC and HPLC-MS/MS.where the samples washed by water to remove any surfactant and water soluble impurities,thereafter ultrasonic extracted by Acetonitrile-methanol for 30min and finally analyzed my MG C18 column(250 mm x 4.6mm,5µm.), RP-18e(100mm x 3mm x 2µm.plus APCI-MS/MS. Limits were detected as 0.015%(HPLC) and 0.003%(HPLC-MS/MS) and with limit of quantization of 0.05% and 0.01% respectively. good recoveries as 85.8-104%(HPLC) and 87.6-107% (HPLC-MS/MS) ,resulted in good linear relationship from 3.20µg.ml to 200µg.ml(HPLC) and 1.00µg.ml to 200µg.ml(HPLC-MS/MS) was obtained.Hence proposed method is applied succefully to analyse of Zinc Prithione,thus two established methods were rapid and reproducible with low interference.

> Zinc pyrithione has been widely used as one of the booster biocides in antifouling paints on the bottom of vessels. A direct analysis method for zinc pyrithione has been developed using LC-MS without trans-chelation and degradation. The addition of ammonium acetate in mobile water phase was effective in stabilizing zinc pyrithione in HPLC, and the optimal concentration was 20 mM. The lower temperature was favourable in preventing decomposition and transformation. The column temperature was set at 298 K. The temperatures of the drying gas and the vaporizer in the mass-selective detector were found to be 523 K. Under these conditions, a mass spectrum and chromatogram of zinc pyrithione in methanol were successfully obtained by LC-MS for concentrations between 3.5 and 10 mg L.

> A simple and rapid method for the determination of zinc pyrithione (ZPT) in antidandruff preparations by high-performance liquid chromatography (HPLC) has been developed. ZPT in samples was converted into a stable copper(II) complex by mixing with cupric sulphate solution followed by extraction with chloroform. HPLC was carried out on a Nucleosil 5 C_{18} column (15 cm × 4.6 I.D.) using methanol—water (3:2) as the mobile phase with UV detection at 320 nm.

The calibration graph was linear from 0.1–0.5 µg for ZPT. The recoveries from four shampoos were 98.0–100.6% with high accuracy.

> HPLC method developed to determine Zinc Pyrithion in commercial cosmetic products. Zinc Pyrithion was labeled with N-dansylaziridine and determined by RP HPLC. There was no interference by ingredients. Zinc Pyrithion was in linear range 1.4-66ng, Coefficient of variation was 0.9% for 17ng injections. Thus resulted in Zinc Pyrithion with high sensitivity and high selectivity and also traces amounts of Zinc Pyrithion adsorbed on human hair.

>The HPLC method and UV spectrophotometric method for the determination of Sertaconazole nitrate in formulations were simple, rapid, precise, accurate and sensitive. UV method being both cost and time saving alternative to HPLC method of analysis for sertaconazole nitrate. The HPLC method enables faster quantification of sertaconazole nitrate within run time of twenty minutes without interference of excipients, thus the method can be used for routine quality control of formulation containing sertaconazole nitrate.

> A high-performance liquid chromatographic method is developed for the determination of bulk sertaconazole nitrate and related compounds (potential impurities and degradation products) as well as a sertaconazole nitrate cream formulation. A 10-µm Spherisorb CN column is used along with a mobile phase consisting of acetonitrile and aqueous 0.01 M sodium phosphate (37:63, v/v). The sertaconazole nitrate peak is monitored at a wavelength of 260 nm, the retention time being 19.3 min. The detector response for sertaconazole nitrate is linear over the concentration range from 64 to 96 µg ml^{-1}. The method is found to be sufficiently selective for the reliable determination of related compounds, FI7001, FI-7009 and FI-7011, as indicated by same-day and between-day relative standard deviations (RSD) for replicate assays of 1.72% (n = 9) and 2.17% (n = 24), respectively. The application of this method to a cream formulation of sertaconazole nitrate is found to give a mean percentage recovery of 99.4% with RSD of 1.14% (n = 9); none of the cream vehicle peaks are found to interfere with the determination of sertaconazole nitrate.

>A capillary electrophoresis assay for the analysis of sertaconazole has been developed and validated. The in uence of buffer concentration, buffer pH, organic modi er, capillary temperature, applied voltage and injection time was systemically investigated in a fused silica capillary (i.d. 50 µm, total length 80.5 cm and effective length 72.0 cm). Optimum results were obtained with a 20 mM phosphate buffer (pH 4.0) containing 40% acetonitrile, capillary temperature 30 °C, applied voltage 30 kV and 3 s hydrodynamic injection at 50 mbar. The detection wavelength was set to 205 nm. Verapamil was used as internal standard. The method showed good selectivity, repeatability, linearity and sensitivity according to the evaluation of

the validation parameters. The method was applied to the determination of sertaconazole in pharmaceutical cream formulations.

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