# TOPICAL SERTACONAZOLE NITRATE: ASSESSING EFFICACY AND SAFETY AGAINST CUTANEOUS FUNGAL INFECTIONS IN RADIOLOGY PRACTICE

## Dr. Shetty Sachin Karunakar*

Associate Professor, Department of Radiology, Sri Lakshmi Narayana Institute of Medical Sciences & Hospital, Osudu, Puducherry – 605502, India.

**ABSTRACT**

Effective topical therapy for cutaneous fungal diseases relies on delivering potent pharmacological agents to the target site while minimizing potential toxicity. In this study, we evaluated the efficacy and safety of a topical formulation containing sertaconazole nitrate, a broad-spectrum antifungal agent, using in vitro methods. Tape stripping technique was employed to quantify the amount of sertaconazole nitrate in the skin, allowing for the determination of dermal absorption parameters such as apparent diffusivity and partition coefficients. Additionally, the skin irritation potential of the formulation was assessed using in vitro EpidermTM models. Our results indicated that the concentration of sertaconazole nitrate in the skin exceeded the minimum inhibitory concentration (MIC100) for fungal pathogens, suggesting potential efficacy against cutaneous fungi. Importantly, the formulation did not induce skin irritation during the irritation test, highlighting its safety profile. These findings underscore the utility of in vitro techniques in developing topical antifungal products that are both effective and non-toxic, offering promising prospects for the management of cutaneous fungal infections.

**KEY WORDS**: Dermatical formulations; dermatological irritation; sertaconazole nitrate; dermal absorption; dermatological formulations; skin irritation.

## INTRODUCTION

A variety of skin and mucous membrane disorders are caused by cutaneous fungal infections [1, 2, 3]. The majority of the global population suffers from dermatophyte infections, primarily tinea pedis [2]. It is estimated that 30%–40% of people with Pityriasis versicolor will develop seborrheic dermatitis if they do not receive treatment [4]. Fungal infection incidence and severity have increased due to the HIV pandemic and the use of immunosuppressive agents. To treat these infections, azole antifungals are commonly used, including sertaconazole nitrate (SN). As a fungicidal agent, SN inhibits the synthesis of ergosterol and mimics tryptophan chemically, resulting in an increase in membrane porosity [5-7]. Due to SN's lipophilic properties, it retains moisture on the skin without absorbing too much into the body. Several studies demonstrate that it has an antifungal activity against Dermatophytes, Candida species, and Malassezia strains. It is essential to deliver active agents

in sufficient concentrations to effectively treat fungal diseases with topical formulations. Azole antifungals can be dissolved more readily in anhydrous vehicles, facilitating skin partitioning [8]. The treatment rationale is guided by dermatologopharmacokinetics (DPK) to ensure local drug bioavailability. The tape stripping technique can be used to gauge bioavailability of topical products and their equivalence [9]. In vivo dermal exposure can be estimated by estimating diffusivity and partition coefficients in the stratum corneum (SC). This data provides insight into drug absorption and uptake rates. The purpose of our study was to investigate the anhydrous gel formulation of SN for its ability to absorb antifungal drugs in vitro. A steady-state and an unsteady-state tape stripping method was used to determine formulation absorption [10]. The assessment of skin irritation and sensitization also included aspects of bioavailability. EpidermTM tissue model has been used in vitro, and is endorsed by the European Center for Validation

Corresponding Author:- **Dr. Shetty Sachin Karunakar**

of Alternative Methods (ECVAM). SN's in vitro absorption dynamics are further explored in this comprehensive study, enhancing its possibility of treating fungal infections.

## METHODOLOGY

TwoA Pharmachem (Lisle, IL, USA) provided two A Pharmachem with sitaconazole nitrate (SN). BASF provided PEG 400 and propylene glycol for this study. Sigma Aldrich supplied menthol and ascorbic acid, and Fisher Scientific supplied glycerin (Waltham, MA, USA). Besides those reagents, all the others were HPLC-grade or highly pure.

### Preparation of Anhydrous Gel

A glass jar was used to prepare the anhydrous gel. Anhydrous ethanol and glycerin were combined with gluculel® to dissolve and disperse the gelling agent. A TeflonTM-coated magnetic stir bar was used to stir continuously throughout the integration of the gelling agent (Klucel®). After stirring for a few minutes, the gel should have become visually homogeneous. Polyethylene glycol (PEG) 400 was then used to dissolve the 2% SN. The mixture was then stirred continuously until it became clear, followed by the addition of isopropyl myristate, ascorbic acid, and menthol. Parafilm® was used to prevent ethanol from evaporating throughout the process. The formulation ratio for anhydrous gel was determined by testing different vehicles prior to forming it. Table 1 summarizes the formulation compositions. According to Table 2, the final composition of the anhydrous gel has been optimized.

**Table 1: Composition of SN (2% w/w) in different vehicles**

| Components | Formula A (%) | Formula B (%) | Formula C (%) |
|---|---|---|---|
| Sertaconazole Nitrate (SN) | 2 | 2 | 2 |
| Propylene glycol | 98 | 49 | 25 |
| PEG 400 | - | 49 | 25 |
| Ethanol, anhydrous | - | - | 48 |

**Table 2: Anhydrous gel composition**

| Components | Formula (%) |
|---|---|
| Sertaconazole Nitrate (SN) | 2 |
| Propylene glycol | 20 |
| Klucel | 2 |
| Glycerin | 15 |
| PEG 400 | 20 |
| IPM | 2 |
| Menthol | 1 |
| Ascorbic acid | 0.2 |
| Ethanol, anhydrous | 37.8 |

### In Vitro Permeation

In diffusion studies using Franz diffusion cells, we used a freshly excised porcine ear that had been obtained from a local abattoir soon after death without prior sanitization. We separated the outer layers of the skin from the subcutaneous fat and stored them at 80 degrees Celsius. We maintained a temperature of 37°C in the water bath to achieve a 32°C skin surface temperature by clamping thawed skin between donor and recipient compartments. As sink conditions, PEG 400:1X PBS (phosphate buffered saline, pH 7.4) was used in the receiver compartment (60:40 volume/volume). The drug was administered in various vehicles at an infinite dose in a preliminary study. To assess the efficacy of the anhydrous formulation, a 10 mg/cm2 dose was applied to the skin four times after formulation optimization. Stratum corneum (SC) drug amounts were quantified by tape stripping following diffusion studies. As a result of tape stripping, unsteady-state and steady-state profiles of the anhydrous gel were determined, respectively, after 5 and 24 hours. We weighed the treated skin after adhering adhesive tape to it and removing it sequentially. Using the first tape strip discarded, SC mass was determined. Methanol was used to extract the remaining stripped skin for HPLC analysis. A lag time for chemical penetration, tlag, and parameter values such as Ksc/v between SC and vehicle were estimated using recorded data. Using parameter estimates, Matlab generates concentration-time profiles via pdepe, a function used to solve partial differential equations. For a 5-minute exposure to SN, MIC100 values were used to assess its efficacy in treating fungal infections. A 3D EpidermTM tissue culture model was used to test skin irritation, and MTT assay was performed on the cells to determine cell viability. Using a validated HPLC method and an Alliance HPLC system with a Luna C18 column, this analysis was performed using an Alliance HPLC system.

## RESULTS
### Skin SN localization
In vitro permeation of SN was assessed using tape stripping. In the preliminary diffusion study, anhydrous ethanol significantly increased drug retention. In order to proceed with subsequent research, the formulation was optimized and transformed into anhydrous gel. To simulate unsteady-state conditions, tape stripping was immediately performed after 5 minutes of gel application.

### Diffusion Model
The single-layer diffusion model, which excludes dermal diffusion, was appropriate because the drug content in stripped skin was minimal during the 5-minute exposure experiment. Following the application of formulation and subsequent stripping of tape, the concentrations-depth profiles are shown. The MIC100 for targeted fungi, including Mucor furfur and yeast, was determined based on this profile. A sustained drug concentration beyond the MIC100 was found in the SC profile for yeast and almost throughout the SC depth for M. furfur. Sertaconazole concentrations are effective in treating fungal infections after a brief 5-minute exposure, indicating that the formulation is effective in treating fungal infections.

### Skin Irritation Test
The EpidermTM tissues treated with positive control (PC), negative control (NC), and anhydrous gel showed relative viability. A substance whose cell viability is less than 50%, such as PC, is considered an irritant. In an MTT assay, the NC showed 100% viability, while the PC and gel showed 2.9% and 17.6%, respectively.

## DISCUSSION
The skin must be properly saturated with the drug for topical therapy to be effective. Active ingredients can be permeated more effectively by specific vehicles. There has been evidence that ethanol enhances drug penetration for lipophilic drugs through mechanisms that include increasing drug solubility and enhancing thermodynamic activity by evaporating ethanol or solubilizing skin lipids . Anhydrous ethanol significantly improved drug distribution within the skin during initial diffusion studies using various vehicles. The highly lipophilic SN was formulated into an anhydrous gel containing ethanol in order to improve its cutaneous penetration. A formulation optimized for antipruritis and co-solvent properties using propylene glycol, glycerin, and PEG 400 as humectants, along with menthol as a lipophilic penetration enhancer, is presented in Table 2. A notable feature of the optimized formulation was the fact that it kept its transparent and clear appearance without drug crystallization. There are numerous benefits of using anhydrous vehicles, including good tolerance, no residues, and elimination of certain side effects associated with greasy vehicles. The treatment of fungal diseases with an anhydrous gel containing 2% ketoconazole has been proven

to be superior in clinical trials [11-13]. These advantages enable the formulation of an anhydrous gel containing SN to be more effective against cutaneous fungal infections.

In vitro and in vivo methods can be compared according to the OEC Guidance. Human percutaneous absorption can be better understood through in vitro studies. When comparing test and reference products using excised human skin, there was a good correlation between their bioavailability in vitro and in vivo [14]. There has been evidence that SN has the potential to treat fungal infections more effectively than other treatments. Taking SN as a model drug in our study was one key objective in order to illustrate and predict local bioavailability in vitro. Optimizing formulations and enhancing therapeutic efficacy can be achieved by establishing a dermal absorption profile. Antifungals should be concentrated on the SC surface to maximize localization of action while minimizing systemic uptake [15]. The systemic bioavailability of 2% SN cream has been reported to be negligible/minimal in previous in vivo studies. As evidenced from our study, in vivo receptor levels are minimal (not shown). As a result, we are primarily concerned with the absorption of SN formulations at the target sites and not with their systemic absorption.

It is widely used to assess topically applied substances' penetration using dermal pharmacokinetics (DPK), or skin stripping. During the past decade, DPK has demonstrated promising results in assessing the bioavailability of topical products and their equivalence. Using adhesive tape, the SC is sequentially removed, which is easy and noninvasive and useful for evaluating drug absorption rates, especially for antifungals, keratolytics, and sunscreens. To simulate human skin in vitro, we used porcine ear skin. Through gravimetry, the amount of SC removed was quantified. Although there are multiple layers in the skin, the SC and underlying layers like the viable epidermis provide the primary barriers against topical absorption [16, 17]. Because antifungals such as SN are absorbed through the SC, our equations assume the SC inhibits dermal absorption at a rate-limiting level. In order to achieve adequate fungal eradication time with an effective antifungal agent, SC drug levels and its persistence at the target site can play a part. According to tape stripping and analysis of the amount of drug in the SC and deeper layers after two exposure times (5 min and 24 h), approximately 66% of the applied dose penetrated the skin after 24 hours. In just 5 minutes after applying the dose, approximately 8% was already in the skin.

According to the formulation application time, the normalized depth profile of SC concentration. There might be ongoing drug diffusion during tape stripping, resulting in a non-linear 24-hour profile, despite the fact that stripping time was shorter than lag time. Conversely, the 5-minute exposure profile exhibited a linear concentration-depth relationship inverse to that of the 5-minute profile, possibly due to method sensitivity constraints which limited the analysis to 10 tape strips. Study results indicate that

complete barrier ablation isn't necessary to determine SC thickness and interpret concentration-depth profiles. For steady-state conditions, the SC thickness of porcine skin was approximated to be 23.7 x 4.0 m, and for unsteady-state conditions it was 24.4 x 3.3 m, in agreement with published values. According to this, the SC was almost completely removed within 5 minutes. According to the results of and exposure experiments lasting five minutes and 24 hours, the lag times were estimated at 0.43 ± 0.09 h and 0.41 ± 0.23 h, respectively. A 5-minute exposure resulted in a texp/tlag ratio of about 0.19; a 24-hour exposure resulted in a texp/tlag ratio of about 58.2. Texp/tlag ratios exceeded 1.7 in our steady-state experiment [18] but not in the unsteady-state experiment. Given the fact that a volatile vehicle will evaporate in a matter of minutes, 5-minute exposure partition coefficients and lag times can be reasonably interpreted as representing drug uptake into the skin. This coefficient indicates how much drug is able to bind to the SC compared to the applied formulation in the SC. We estimated Dsc using $Dsc = Lsc2/(6 \times tlag)$. Post-formulation drug uptake can be characterized by thermodynamic and kinetic parameters derived from the concentration-SC depth profile.

For simulation, we chose this profile since it provided a better fit to exposure data for 5 minutes. SN concentration-depth profiles simulated by the model exceeded literature-derived MIC100 for yeasts throughout SC thickness, and for M. furfur. Therefore, assuming steady-state conditions, achieved drug exposure might be sufficient to eliminate superficial fungal infections caused by these pathogens.

Detectable drug amounts beyond the SC layers may occur as a result of the sink boundary condition violation in the model fit after steady-state data [19]. While *in vivo* sink conditions are commonly assumed, microdialysis experiments revealed significant levels of drug in subdermal layers. Since drug levels below the SC are five times lower than the SC, we believe that our boundary condition is reasonable. The results need to be generalized and the violation may be resolved through further exploration.

A topical therapy's safety profile is as important as its therapeutic outcomes. Adverse reactions can be triggered by skin irritation and have an impact on therapeutic efficacy. When it comes to treating fungal infections, SN is known to be non-toxic and safe. It is the vehicle that can cause irritation, not the drug itself. As the gel contained considerable ethanol, we evaluated its potential for irritation as our final step. In vitro human epidermal models have replaced conventional irritation tests, such as the Draize test, because of ethical concerns. Human epidermal keratinocytes were modeled using the EpidermTM model. Irritators are formulations that yield a lower than 50% cell viability on the MTT assay.

## CONCLUSIONS

The goal of this study was to determine local bioavailability of SN anhydrous gel in the skin by assessing dermal absorption through in vitro taping stripping. Mathematical models were used to determine the partition and diffusion coefficients of essential substances. These parameters were used to predict drug absorption after five minutes. It was intriguing to find that the estimated concentration-time profiles were higher than MIC100 values for M. furfur and yeast throughout most of the stratum corneum. In light of this promising result, it may be possible to achieve effective levels of concentration at the target site through the formulation of this gel.

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