ANALYTICAL METHOD VALIDATION FOR IMPROVEMENT OF TRANSGUAL PROPERTIES AND DEVELOPMENT OF ANTIFUNGAL NAIL LACQUER A TOPICAL SOLUTION OF 5%W/V AMOROLFINE HCl BY HPLC

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Abstract- The two most common conditions of nail infections are noted as the nail disease which are Onychomycosis and nail psoriasis which affect the 14-18% of the population. Among them 50% of the nail disorder is affected by Onychomycosis. This work aimed to evaluate the effect of chemical and physical enhancers on the accumulation and permeation of Amorolfine hydrochloride through human nail clippings. In order to achieve the therapeutic benefit we designed and validate the method of Amorolfine Hydrochloride in the form of nail lacquer of 5%w/v which is sensitive, accurate, robust and proved to be the quality products formulations which can ensure the therapeutic efficiency better than any formulation which has not been studied till date. The development of lacquer formulation was guided by the quality by design approach to achieve the critical quality attributes needed to obtain the product of desired quality. Papain and Keratinase were used in the formulations of the nail lacquer in order to increase the permeability of the drugs in the nail clippings. Furthermore, The sample were detected at the photodiode Array using 220nm. The Mobile phase ratio of the Buffer : ACN: Tetrahydrofuran 4:3:3. The Buffer used was 0.05M KH2PO4 of PH 5.0 using orthophosphoric acid. This method was validated using ICH guidelines. Due to its high accuracy, Specificity, and precision the developed method can help in determination of Amorolfine Nail Lacquer and find out the efficacy rate of the drug by the hindrance of permeability coefficient of the drug in the nail bed. The average retention time of Amolorfine Hydrochloride is 4.3 minutes the regression coefficient (r2) was 0.99721. The relative standard deviation (RSD) was less than 2% for precision. Due to less retention time the product can be tested in the short period of time.

Keywords: Amorolfine.HCl, Onychomycosis, Therapeutic efficiency, International Conference on Harmonization, Relative Standard Deviation.

INTRODUCTION

The fungal disease of the nail, Onychomycosis is the single most prevalent disturbance of the nail. It is characterized by discoloration, brittleness, and the thickening of the infected nail and is caused predominantly by dermatophytes but also by Non-dermatophytes Molds and Yeats. Onychomycosis, the fungal infection of the nail unit, affects 14-18% of the of the general population worldwide. And has been called ‘stubborn clinical problems’. Currently approved approaches for the treatment for the Onychomycosis systemic therapy by means of oral treatment or topical application of antifungal formulations. Among them a novel Formulations of a nail lacquer a topical solutions of Amorolfine Hydrochloride is the effective means of treatment. Amorolfine Hydrochloride has a proven track record of treating mycotic infections in vivo Amorolfine is a novel topical antifungal of the Phenyl-propyl Morpholines class that is used to treat superficial fungal infections. A unique topical lacquer affects the human body's ability to absorb and pass through the in vitro finite dose model, nail. Antifungal susceptibility tests generally provide limited useful clinically data regarding the susceptibility of an isolated organism from a patient to a particular antifungal agent. This vehicle is an anhydrous/alcohol composition with a dual acrylate-silicone hybrid copolymer system that provides film forming and occlusion capabilities due to synergistic plasticizing components. In the in vitro finite dose model, a human nail plate is used, which is housed in specially designed diffusion cells that retain the nail at temperatures and humidity levels comparable to those encountered in real life. A little portion of the formulation. In comparison to the commercial control, we anticipate that this novel topical lacquer formulation will boost Amorolfine Hydrochloride
penetration into the nail. The severity of the initial fungal infection is critical for the following therapy response. Generally, topically applied treatments are unlikely to be effective if there is mycotic involvement in the nail matrix. The involvement of the nail matrix was one of the exclusion criteria for this reason. Clinically speaking, it is by no means obvious why systemic antifungal therapy should last as long as it does to promote healthy nail outgrowth from the matrix to the free edge.

**DRUG PROFILE**
Amorolfine Hydrochloride belongs to the class of pharmacological agents known as Antifungal agent.

**Structure:**

![Figure: 12 Structure of Amorolfine HCl](image)

IUPAC NAME: 
(2R,6S)-2,6-dimethyl-4-[2-methyl-3-[4-(2-methylbutan-2-yl)phenyl]propyl]morpholine;hydrochloride

**RESEARCH METHODOLOGY**

**Development of nail Lacquer of Amorolfine Hydrochloride and preparation of nitrocellulose**
About 10 gm (cotton) is added to 100 ml concentrated sulfuric acid and 50 ml of 70% nitric acid mixture and cooled to 5-10 °C to give cellulose nitrate. Then cotton was removed and washed in cold water and with sodium bicarbonate solution to remove all acid residues. It was then slowly dried at room temperature of 25 °C.

**Formulation of nail lacquer**
Amorolfine Hydrochloride and Nitrocellulose were combined, and the necessary amount of Ethanol was used to dissolve the mixture. A magnetic stirrer was used to dissolve the needed amount of the mixture of Amorolfine Hydrochloride and nitrocellulose in ethanol at a consistent speed. Papain, Glycerine, Ethyl acetate, BHT and the needed quantity of keratinase were carefully combined to create clear solution, which had a volume of 100ml. The prepared nail lacquer was poured into a glass bottle with a small mouth and a plastic screw lid. After that we develop a method for testing the Amorolfine HCl which is precise, accurate and sensitive analytical method for the quantification of Amorolfine Hydrochloride in Amorolfine Hydrochloride 5% w/v nail lacquer.

**Optimization of chromatographic conditions:**

**Mobile phase:** 40 volumes of Buffer; 30 volumes of Acetonitrile and 30 volumes of Tetrahydrofuran. Mix and adjust pH to 5.0 with dilute phosphoric acid.

**Buffer:** Dissolve 6.8 gm of Potassium dihydrogen phosphate in 1000 ml of water.

**Diluents:** Methanol

**Chromatographic conditions:**
- Column: 4.6 mm x 250 mm; 5.0 µ (C18)
- Column temperature: 40 °C
- Wavelength: 220 nm
- Flow rate: 1.0 ml/minute
- Injection volume: 10.0 µl

**Standard preparation:** Transferring 25 mg of amorolfine hydrochloride WS to the 50 ml VF after weighing it. And
to completely dissolve the substance, add 25 ml of diluents and sonicate for 15 minutes. Using diluents, further dilute 5 ml of this solution to 50 ml. Mix thoroughly and run through 0.2-micron filter paper. (Amorolfine 50 mcg/ml)

**Sample preparation:** Pipette out 5 ml of material, which is equal to 250 mg of amorolfine, into a 100 ml volumetric flask. Pour 50 ml of diluents in, for sonication

**RESULTS AND DISCUSSION**

**Percentage content of different Formulation as a nail Lacquer**

The percentage of drug content for all lacquers was determined to be adequate and ranged from 93.6 to 102.01%. The lowest percentage of drug content was 93.6% (F0), and the highest percentage was 102.01% (F3). Having a drug content of more than 90% in the formulation verifies that the methods of formulation and the ingredients chosen are not having an impact on the stability of the drug. A high medication content also ensures that a successful therapeutic outcome can be anticipated.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>% Drug Assay</th>
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<tbody>
<tr>
<td>F0</td>
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<tr>
<td>F1</td>
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<tr>
<td>F3</td>
<td>102.01</td>
</tr>
<tr>
<td>F4</td>
<td>100.36</td>
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**Percentage of drug Assay of different formulations**

The analytical method validation of F4 was done. This formulation was better in all ways because it contains permeation enhancers like papain, Keratinase, BHT and its percentage content was 100.36%. The papain and keratinase can breakage the sulfhydryl group of nail keratin and increase the permeability of drug in the nail surface so healing of the nail fungus can be easily eradicated so F4 was better to validate than other four formulations.

**VALIDATION PARAMETERS**

**Specificity of Amorolfine Hydrochloride**

The different sample of Blank (Diluents), Blank (Mobile phase), placebo, standard and sample were injected, only standard and sample area were plotted in the chromatogram at retention time of 4.35 min and 4.346 min as a major peaks. Area of other peak other than the major peak is detected of Placebo as the two different excipients.

**System suitability of Amorolfine Hydrochloride**

The system suitability complies as the %RSD value is less than 2.0% of the retention time, area of the major peak, height, tailing factor is less than 2.0, and column efficiency is greater than 2000 theoretical plates.

**Repeatability of Amorolfine Hydrochloride**

In the repeatability three different concentrations of sample were prepared. The concentration range of 8mcg/ml, 10mcg/ml, and 12 mcg/ml. These concentrations were assayed and was found to be consistent results. The %RSD of Repeatability was found to be 0.3265.

**Intermediate precision of Amorolfine HCl**

In the Intermediate precision, also known as within-laboratory precision measure of the variation in test results within the same laboratory under different conditions over a short period of time. It assesses the reproducibility of the method within a specific laboratory, accounting for factors such as different days, analysts, instruments. The table shows that Amorolfine Hydrochloride nail lacquers cannot be affected the test results by changing analyst, Instrument and different days. The % RSD was found to be 0.2257 which shows that intermediate precision meaning produces consistent and reproducible results under different conditions within the laboratory.

**Accuracy of Amorolfine HCl**

In this different chromatographic results were calculated and the data of three different level of addition were added in
the three different tests and the amount spiked was found accurate. The maximum percentage recovery was found to be 100.89% and minimum percentage recovery was 100.16%. It presents balanced view of the product including potential benefits and risks. It ensures information provided to healthcare professional and consumer is reliable, truth and robust data.

**Robustness of Amorolfine HCl**

In our study the changes was done by changing flow rate of 1.05 ml/min and 0.95 ml/min and in the column dimensions only. In a robustness study, specific parameters or conditions are intentionally altered to evaluate their impact on the method's performance. These parameters may include factors such as pH, temperature, mobile phase composition, flow rate, column dimensions, and sample preparation techniques. The results of a robustness analysis are typically analyzed using statistical methods. The statistical analysis helps determine the significance of the observed variations and assesses their impact on the method's performance. Key statistical measures such as standard deviation, was assessed and found to be 0.3443. Robustness analysis is carried out by comparing the obtained results under varied conditions to predefined acceptance criteria or specifications. Robustness analysis can help identify critical parameters that significantly affect the method's performance.

**Solution stability of Amorolfine HCl**

First of all initial sample at room temperature and after 24 hours of storing it was tested successfully and percentage recovery was found within the limit of 97.5% to 102.5%. Secondly, another sample was stored at a temperature of 2-8°C and sample was tested initially and after 48 hours. Percentage recovery of sample at refrigerator was within the limit of 97.5% to 102.5%. The storage conditions play a crucial role in solution stability. Factors such as temperature, light exposure, humidity, and the presence of reactive substances can influence the stability of a solution. It is important to define and control the storage conditions to accurately assess the solution's stability. Stability testing involves subjecting the solution to predetermined storage conditions and monitoring its characteristics over time. This testing can include visual inspection, chemical analysis, and physical measurements to evaluate changes in appearance, pH concentration, degradation products, or other relevant parameters. Stability testing is conducted against pre-established acceptance criteria.

**Linearity of Amorolfine HCl**

In this different concentrations of 8.10 mcg/ml, 9.20 mcg/ml, 10 mcg/ml, 11.20 mcg/ml, 12.10 mcg/ml of standard solutions of Amorolfine Hydrochloride chromatographic results were plotted and the relationship of different concentrations was linear and within the range. R² indicates the proportion of variability in the response variable that can be explained by the linear relationship with the concentration of the analyze. Higher values of R² (closer to 1) indicate a stronger linear relationship. Coefficient of regression was found to be 0.99721 which is nearly 1. The linearity criteria are met, it demonstrates that the method can accurately quantify the analyze within the specified concentration range the implications of linearity on the method's suitability for its intended application and the reliability of the obtained results.

**CONCLUSION**

The goal of the current study was to develop and validate simple, sensitive, accurate, and reproducible for the estimation of Amorolfine Hydrochloride in a lacquer form, the RP-HPLC method was devised. These techniques were validated in accordance with ICH recommendations, and the outcomes met their requirements. As a model medicine,
Amorolfine Hydrochloride was used, and formulations were made using the keratolytic agent Keratinase and the permeation enhancer papain so the permeability of the drug can be achieved in the site of application and fungicidal activity can be achieved. Furthermore, content of Amorolfine Hydrochloride in Amorolfine HCl 5%/w/v in nail lacquer is found out by developing a method. As a result, this approaches are validated and suitable for routine analysis.

- The Specificity complies as the secondary peaks are not detected in Blank solution and related with Placebo solution in sample solution have the resolution greater than 1.5 with respect to the respective major peaks.
- The System suitability complies as the % RSD value is less than 2.0% of retention time, area of the major peak, height; tailing factor is less than 2.0; and column efficiency is greater than 2000 theoretical plates.
- The Repeatability complies as the %RSD value is with in the limit NMT 2.0% i.e., 0.3265%.
- The Accuracy complies as the range is within the limit 98.0% to 102.0% (i.e., 100.16% to 100.89%).
- The Robustness complies as the % RSD is within the limit NMT 2.0% i.e., 0.3443%.
- The Intermediate precision complies as the % RSD value is within the limit NMT 3.0% i.e., 0.2257%.
- The Solution stability complies as the % recovery is within the limit (97.5% to 102.5%) i.e., 100.07 % to 100.15% at room temperature and 99.83% to 99.92% at refrigerator.
- The Linearity complies as the correlation coefficient is 0.99721 which is greater than 0.98.
- The low percentage RSD value in the recovery studies indicates that the procedures are correct and that the excipients in the dosage forms have no effect on the formulation.

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