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ANALYTICAL METHOD VALIDATION FOR IMPROVEMENT OF TRANSGUAL PROPERTIES AND DEVELOPMENT OF ANTIFUNGAL NAIL LACQUER A TOPICAL SOLUTION OF AMOROLFINE HYDROCHLORIDE DRUG BY HPLC

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Abstract

Nail lacquer have a potential role as a drug carriers for the topical treatment of disease such as onchomycosis, a common condition.Our aim was therefor to develop a systemic approach to the formulations of a simple drug in a topical solutions.Topical monotherapy of nail infection is limited by poor drug permeability into human nail plate. Numerous substance and methods are applied to improve the antifungal agent delivery across the nail plate. This work aimed to evaluate the effect of chemical and physical enhancers on the accumulation and permeation of Amorolfine hydrochloride through human nail clippings. Amorolfine hydrochloride is an inhibitor of the action of Squalene monooxygenase, Delta (14) reductase and D7-D8 isomerase and an antifungal agent. 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. A simple, specific, sensitive, robust, accurate and precise reverse-phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for simultaneous determination of Amorolfine Hydrochloride Validation parameters, such as system suitability, accuracy, inter-day and intra-day variances, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), robustness and stability were assessed following the standards set by the International Conference on Harmonization (ICH). The isocratic elution of Amolorfine Hydrochloride is carried out under ambient conditions using Potassium dihydrogen phosphate buffer (pH =5.0), acetonitrile and Tetrahydrofuran (40:30:30, v/v/v) as mobile phase flowing through a Promosil C18 column at a flow rate of 1.0 mL/min. The average retention time of Amolorfine Hydrochloride is 6.5 minutes.. The LOD and LOQ of Amolorfine Hydrochloride were 0.23µg/ml.The regression coefficient (r2) was 0.998. The relative standard deviation (RSD) was less than 2% for precision.

Keywords:Amorolfine.HCl, Onchomycosis, Squalene Monooxygenasse, Isomerase, International Conference on Harmonization, System Suitability.

INTRODUCTION

The fungal disease of the nail, Onychomycosis is the single most prevalent disturbance of the nail. It is characterized by discolouration, brittleness, and the thickening of the infected nail.and is caused predominantly by dermatophytes but also by Non-dermatophytes Molds and Yeats[1].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.[2]. Currently approved approaches for e 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. [3] Amorolfine is a new topical antifungal of the Phenyl-propyl Morpholines which is used in superificial fungal infections.[4] Antifungal succeptibility tests generally provide limited useful clinically data regarding the succeptibility of an isolated organism from a patient to a particular antifungal agent.[5] Minimum inhibitory concentration (MIC) values depend heavily on experiment condition such as medium used ,pH, inoculum size and duration of incubation. Moreever, correlation between invitro susceptibility and clinical efficacy is extremely variable.[6]

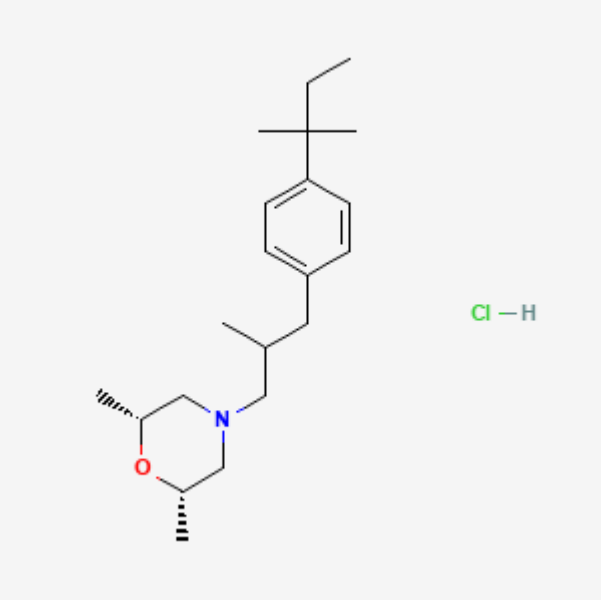
Investigational medicinal product (IMP)	Amorolfine Hydrochloride.
	Procedures of validation are based on the ICH harmonized tripartite guideline. "Validation of analytical procedures : text and methodology Q2/R1"
Analytical part	Analytical method : HPLC with UV detector Substance to be measured : Amorolfine Hydrochloride. Parameters of validation : - Specificity - Linearity and range - Precision(Repeatability, intermediate precision) - Accuracy - Solution stability - Robustness - System suitability

DRUG PROFILE

Drug profile :

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

Structure:



Amorolfine Hydrochloride

IUPAC NAME

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

Empirical Formula: C21H36ClNO.

Molecular Weight: 354.0

Melting Point: 205°C to 211°C

Solubility: Practically insoluble in water.

PHYSICAL PROPERTIES

Nature: Amorolfine Hydrochloride is white to off-white powder.

Storage: Store in a well-closed container, protected from light. Store at 25°C (77°F

CLINICAL PHARMACOLOGY:

Mechanism of Action:

The Fungicidal or fungistatic activity of amorolfine depends primarily on its ability to inhibit the formation of ergosterol, a component of the fungal cell membrane.Alteration in membrane sterol content lead to changes in membrane permeability which subsequently affect fungal metabolic processes. Within this sterol biosynthetic pathway amorolfine interferes with two enzymes Delta 14 Reductase and D7-D8 isomerase, leading to depletion of ergosterol .These effects are both time and concentration dependent ; observed changes in sterol patterns correlate with inhibition of fungal growth.Although amorolfine exhibits significantly higher affinity for the isomerase.than the reductase enzyme.,the earlier codition of the reductase enzyme within this pathway gives it greater importance. At a molecular level amorolfine doesn't primarily affect cellular respiration or synthesis of DNA , RNA ,Protein or carbohydrate at inhibitory drug concentration. [7]

Pharmacokinetic Properties: No Pharmacokinetic studies have been performed for this product. Amorolfine when administered topically to the surface of the nails, penetrates into the nail substance and reaches effective antifungal concentrations.

Precutaneous absorption : The precutaneous absorption of new antifungal agent Amorolfine Hydrochloride measured through both skin and nail using invitro technique .Application of a 5% concentration in either an ethanol

or Methylene chloride lacquer resulted in permeation rates higher in the nail.[9] Following topical application ,an active concentration of amorolfine is retained in the skin for several days. The bioavailability of topical amorolfine is 4-10 % and the drug is excreted in urine and faeces .Nevertheless,the plasma concentration of active ingredient is reportedly below the sensitivity limit even after long term treatment. Nail lacquers have been used as a cosmetic for a very longtime to protect nails and for decorative purpose .Conventional nail lacquers generally consists of solvents film forming polymers , resins which increase adhesion of the film to the nail plate.[10]

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:**
  - **(a) Column:** 4.6 mm x 250 mm; 5.0 µ (C18)
  - **(b) Column temperature:** 40˚ C
  - **(c) Wavelength:** 220 nm
  - **(d) Flow rate:** 1.0 ml/minute
  - **(e) Injection volume:** 10.0 µl
- **Suitability requirements:** In the chromatogram obtained with the standard solution, the relative standard deviation of 5 replicate injections should not more than 2.0%; tailing factor should not more than 2.0 and column efficiency should not less than 2000 theoretical plates.
- **Standard preparation:** Weigh about 28 mg of Amorolfine Hydrochloride WS and transfer it to the 50 ml volumetric flask. Add 25 ml of diluents and sonicate for 15 minutes to complete dissolution of the material. Cool to room temperature and make up the volume with diluents up to the mark. Further dilute 5 ml of this solution to 50 ml with diluents. Mix well and filter through 0.2 µm filter paper. (50 mcg/ml of Amorolfine**)**
- **Sample preparation:** Pipette out 5 ml of sample equivalent to 250 mg of Amorolfine in 100 ml volumetric flask. Add 50 ml of diluents. Sonicate for 5 minutes. Cool to room temperature and make up the volume with diluents up to the mark and then stir mechanically for 15 minutes. Further dilute 2 ml of this solution to 100 ml with diluents. Mix well and filter through 0.2 µm filter paper. (50 mcg/ml of Amorolfine)

METHOD VALIDATION

Validation of the method will be performed by investigation and documentation of Specificity, Linearity and range, Precision (Repeatability, Intermediate precision), Accuracy, Solution stability and Robustness.

❖ **SPECIFICITY**

Specificity will be investigated by analyzing following solutions :

- Blank: Mobile phase
- Placebo: Placebo
- Standard solution: WS of Amolorfine Hydrochloride.
- Sample solution: Amorolfine HCl Nail Lacquer 5%w/v

Acceptance criteria

Specificity will be accepted if:

- Amorolfine HCl is detected separately from matrix components.

❖ **LINEARITY AND RANGE**

Investigation

Linearity will be investigated by :

- Analyzing a standard curve over the range of 80 % to 120 % of Amolorfine HCl relative to the declared content of Amolorfine Hydrochloride in the IMP.
- Calculation of slope, intercept and correlation coefficient by linear regression.

Acceptance criteria :

Linearity will be accepted if :

- The correlation coefficient is ≥ 0.98.

❖ **PRECISION (REPEATABILITY AND INTERMEDIATE PRECISION)**

Precision will be investigated by:

- Analyzing three different concentrations (80%, 100% and 120% of Amorolfine relative to the declared content of Amolorfine HCl in the IMP; n= 3 of each concentration) within one day compared with the 100% standard solution (Repeatability).
- Analyzing one concentration (100% of Amorolfine HCl relative to the declared content of Amorolfine HCl in the IMP; n= 3 of each concentration) at different instruments, analysts and days compared with the 100% standard solution (Intermediate precision).

Acceptance criteria

Precision will be accepted if :

- The relative standard deviation (RSD) of repeatability is ≤ 2.0%.
- The relative standard deviation (RSD) of intermediate precision is ≤ 3.0%.

ACCURACY

Accuracy will be investigated by :

- Analyzing three different concentrations (80% to 120% of Amorolfine relative to the declared content of Amorolfine HCl in the IMP; n= 3 of each concentration) prepared by spiking the known concentrated sample of active ingredient in the placebo sample.
- Calculation of the mean value of the percentage deviation of the back calculated concentration from the declared value (Recovery).

Acceptance criteria

Accuracy will be accepted if :

- The mean value of the percentage deviation of the back calculated concentration from the declared value (Recovery) is between 98.0% and 102.0%.

SOLUTION STABILITY

Solution stability will be investigated by:

- Analyzing solutions of Amolorfine HCl (concentrations: 100%; n = 3) in comparison to the fresh prepared solutions.

(i) Initially prepared samples (3 concentrations for storing at room temperature and 3 concentrations for storing in refrigerator at 2-8^{0}C)

(ii) 3 concentrations of stored samples at room temperature after 24 hours.

(iii) 3 concentrations of stored samples at refrigerator at 2-8^{0}C after 48 hours.

Acceptance criteria

Solution stability will be accepted if:

- The mean value of the stored solutions is between 97.5% and 102.5% in comparison to the fresh prepared solutions.

❖ ROBUSTNESS

Investigation

Robustness will be investigated by:

- Analyzing solutions of Amorolfine HCl (System suitability solution: 100 %; n = 3) and considering the influence of :
  - ✓ Three different flow rates (1.45 ml/minute; 1.5 ml/minute and 1.55 ml/minute)
  - ✓ Two different columns (4.6 mm x 15 cm; C18 and 4.6 mm x 25 cm; C18)
  - ✓ Three different compositions of the mobile phase in comparison to the test method conditions.

Acceptance criteria

Robustness will be accepted if :

- The relative standard deviation (RSD) of robustness is ≤ 2.0%.

❖ DATA EVALUATION

For the different calculations, Microsoft Excel will be used.

System suitability test

The system suitability test will be accepted if :

- The relative standard deviation (RSD) of the peak areas of six subsequent injections of the standard solution of Amolorfine Hydrochloride is not higher than 2.0%.
- The column efficiency is not less than 2000 theoretical plates.
- The tailing factor is not more than 2.0

❖ REVALIDATION CRITERIA

Analytical method require revalidation if:

- Changes in the protocol are only permitted if there will be a need.
- The method parameters have to be changed to maintain the original performance and the change is outside the tolerance allowed by USP.
- New compounds are analyzed that are not within the methods original scope.
- The sample matrix changes.

❖ DEVIATIONS

Any deviation occurring during the activity shall be recorded and assessed whether it has any GMP criticality. GMP non-critical deviations can be justified whereas GMP critical deviation may require investigation and corrective actions. Appropriate justification, investigation, corrective action and verification of effectiveness of corrective action shall be recorded.

DISCUSSION AND CONCLUSION: This study suggested that the nail lacquer a novel formulatios is more suitable for delivery to the human nails,because it penetrates into the finger nails via the hydrophilic pathway.Since the nail is mainly constituted of hydrophilic materials ,drug hydrophilic properties are a prerequisite for drug penetration . The formulation constitute of Ethyl acetate ,butyl acetate and ethanol which evaporates after the application of nail lacquer Diffusion of topically applied urea into nails resulting in the separation of the nail plate from the nail bed. Loosening of the nail bed from the nail plate, arising from the absorption of noxious chemicals present in earlier study that nail cosmetics has also been reported (Sulzberger etal.1948). It must be stressed ,however ,that nail permeability is generally poor and drug flux through the nail plate is low.The secondary peaks or related substances in the formulation decreases due to the compatible formulation used in the 5% Amolorfine.HCl Nail lacquer in the formulations.Due to less degradation of the product ,there is less chances of isomerism of the molecules in the nail lacquer than in the other dosage forms which has not been studied till date.

The HPLC method developed for the estimation of 5% nail lacquer of Amorolfine Hydrochloride was found to be specific, precise, robust, linear and accurate.

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