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A NEW VALIDATED ANALYTICAL METHOD FOR THE ESTIMATION OF AMOROLFINE HYDROCHLORIDE USING UV SPECTROSCOPY IN BULK DRUG AND FORMULATION

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ABSTRACT

Amorolfine (or amorolfin), is a morpholine antifungal drug that inhibits -sterol reductase and cholestenol Δ-isomerase, which depletes ergosterol and causes ignosterol to accumulate in the fungal cytoplasmic cell membranes.This paper describes a simple, accurate, specific and validated method for the estimation of amorolfine hydrochloride in ointment form. A study was carried out of all the parameters established as per ICH guidelines to validate an analytical method for estimation. The method showed high sensitivity with reproducibility in results. The wavelength maxima (λ_{max}) was found to be 219 nm. The linearity for this method was found to be in the range of 05 – 30 µg/ml. The calibration curve (Fig -2) was drawn by plotting graph between absorbance and concentration. This method showed a correlation coefficient of 0.9997. The regression equation of the curve was Y= 0.0227x+0.02641. Method was successfully validated. In addition, this proposed method was simple, sensitive, Easy to apply and requires relatively inexpensive instruments. The proposed method can be used for routine analysis of amorolfine Hydrochloride in bulk as well as in the commercial formulations.

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INTRODUCTION:

Amorolfine (or **amorolfin**), is a morpholine antifungal drug that inhibits -sterol reductase and cholestenol Δ-isomerase, which depletes ergosterol and causes ignosterol to accumulate in the fungal cytoplasmic cell membranes. Marketed as **Curanail**, **Loceryl**, **Locetar**, and **Odenil**, Amorolfine is commonly available in the form of a nail lacquer, containing 5% amorolfine hydrochloride as the active ingredient. It is a topical solution for the treatment of toenail infections. Systemic treatments may be considered more effective.

It is used to treat onychomycosis (fungal infection of the toe- and fingernails). Amorolfine 5% nail lacquer in once-weekly or twice-weekly applications has been shown in two studies to be between 60% and 71% effective in treating toenail onychomycosis; complete cure rates three months after stopping treatment (after six months of treatment) were 38% and 46%. However, full experimental details of these trials were not available and since they were first reported in 1992 there have been no subsequent trials.

It is a topical solution for the treatment of toenail infections. Systemic treatments may be considered more effective.

It is approved for sale over-the-counter in Australia, Brazil, Russia, Germany and the UK, and is approved for the treatment of toenail fungus by prescription in other countries. It is not approved for the treatment of onychomycosis in the United States or Canada, but can be ordered from there by mail from other countries.

MATERIALS AND METHODS

Pharmaceutical grade of Amorolfine hydrochloride was kindly gifted from Optrix healthcare ltd, Hyderabad. Fungicros creams of Amorolfine hydrochloride was purchased from local Pharmacy. All the solvents and chemicals used were of analytical reagent grade and procured from Qualigens fine Chemicals (Mumbai).

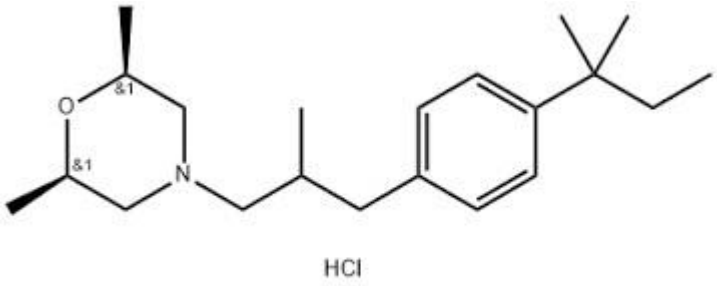

Fig. 1: Structure of Amorolfine hydrochloride.

Instruments:

Shimadzu AX - 220 digital balance, T 60- UV - Visible spectrophotometer with 1 cm matched quartz cells, Sonicator Sonica Ultrasonic cleaner model 2200 mH.

METHOD – SIMPLE UV- SPECTROSCOPY

The solubility of Amorolfine hydrochloride was determined in a variety of solvent ranging from non polar to polar using essentially a method of Schefter and Higuchi. The drug was found to be soluble in 0.1 N HCl, Chloroform, Glacial acetic acid 10 % , Distilled Water and freely soluble in Ethanol, Glacial acetic acid 20 % & 50 % . Considering the economic factor and sensitivity the drug were stable in Ethanol for 3 h, Ethanol was selected as the solvent for method.

Preparation of standard stock solution:

100 mg Amorolfine hydrochloride was accurately weighed and transferred into a 100 ml standard flask and dissolved with minimum quantity of Ethanol and made up to 100 ml with more Ethanol (1000 µg /ml).

Selection of λ_{max} and stability studies:

The standard stock solution was further diluted with Ethanol to get 10 µg/ml concentration (1 ml to 100 ml). The solution was scanned between 200 and 400 nm using Ethanol as blank. From the spectrum obtained, 219 nm was selected as λ_{max} for the analysis of Amorolfine hydrochloride. Stability studies were performed and Amorolfine hydrochloride was found to be stable for 3 h and shown in Fig: 2.

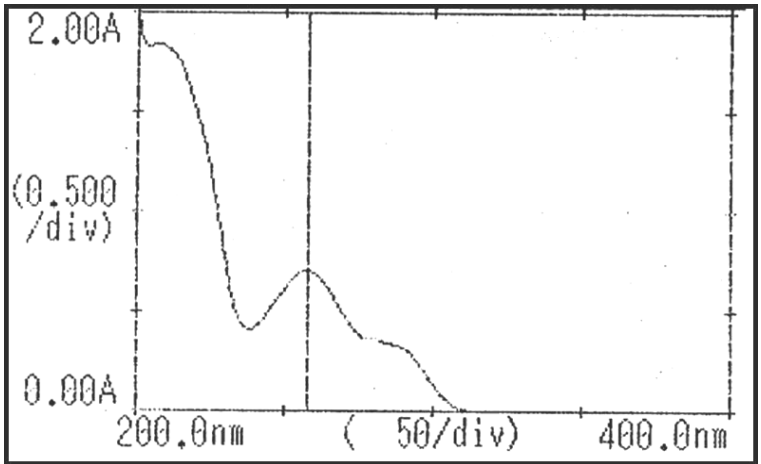

Fig. 2: UV Spectrum of Amorolfine hydrochloride in Ethanol (10µg/ml).

Calibration graph and linearity:

In this method, the aliquots (1-5 ml) of standard stock solution of Amorolfine hydrochloride were transferred into 100 ml standard flasks and made up to the mark with Ethanol. The absorbance was measured at 219 nm against Ethanol as blank. The sample solutions were found to be linear from 5-30 µg/ml. The calibration curve was plotted between concentration and absorbance and shown in Fig: 3.

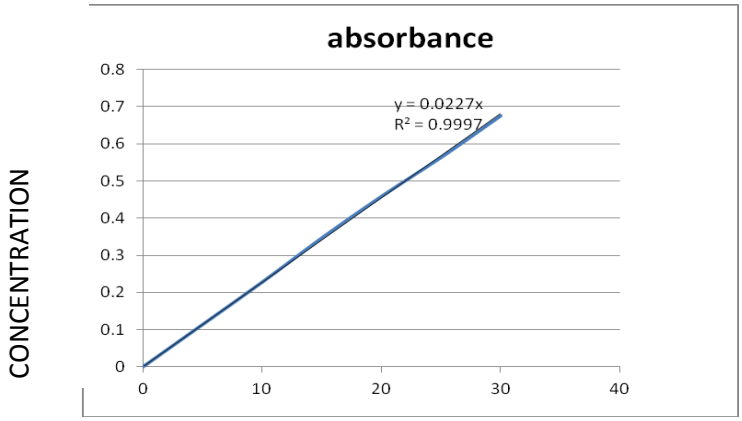

Fig. 3: CALIBRATION CURVE OF IN ETHANOL (10µg/ml).

Quantification of formulations:

Contents of the formulation (fungicros cream) containing 0.25% W/W of Amorolfine was accurately weighed to find out average weight. Cream equivalent to 100 mg of Amorolfine was transferred into 100 ml beaker, added little quantity of Chloroform. Then solution was makeup upto the mark with Ethanol. After the solution was filtered through Whatman filter paper No.41. From the clear solution, further dilution was made to bring a 10 µg /ml using Ethanol. The prepared solution was measured at 219 nm. The amount of Amorolfine was determined by using slope and intercept values from calibration graph.and shown in Table: 2.

TABLE 2: RESULTS OF ANALYSIS OF COMMERCIAL FORMULATIONS- AMOROLFINE HYDROCHLORIDE (0.25% %W/W) BY UV METHOD.

S.No	Labelled Amount mg (0.25% %W/W)	Amount found (mg)	% obtained	Average %	S.D	%RSD	S.E
1	245	245.08	100.03	100.00	0.1081663	0.108166	0.044158
2	245	244.62	99.84				
3	245	245.37	100.15				
4	245	245.04	100.01				
5	245	244.93	99.97				
6	245	245.06	100.02				

SD is standard deviation, % RSD percentage relative standard deviation.

*Average of six determinations.

Recovery studies:

To the pre-analysed formulation, a known quantity of standard solution was added and the contents were mixed well, finally made up to the volume with distilled water. Absorbance was measured at 219nm. Amount present was calculated from slope and intercept. Then the % recovery was determined by using the following formula and shown in Table: 3.

$$\% \text{ Recovery} = \frac{N \sum xy - \sum x \sum y}{N \sum x^2 - (\sum x)^2} \times 100$$

TABLE- 3: RECOVERY STUDIES FOR FORMULATION - AMOROLFINE HYDROCHLORIDE (0.25% %W/W) BY UV METHOD.

Name of drug	Recovery levels	Concentration (µg/ml)	Amount recovered	% Recovery with SD
Amorolfine Hydrochloride	50 %	30	28.32	94.44±1.5061
	100 %	40	39.21	96.12±0.3181
	150%	50	49.58	99.16±1.8526

*Average of six determinations

Statistical Validation:

The obtained results were treated for statistical validation parameters [7-8] like Standard Deviation (SD) and Percentage Relative Standard Deviation (% RSD).

RESULTS AND DISCUSSION

The solubility profile of Amorolfine hydrochloride was determined as per procedure followed by Schefter and Higuchi. Using various polar to non polar solvents and from the solubility studies the category of solvents for Amorolfine hydrochloride was hereby confirmed as soluble in 0.1 N HCl, Chloroform, Glacial acetic acid 10 % , Distilled Water and freely soluble in Ethanol, Glacial acetic acid 20 % & 50 %

METHOD

Ethanol was selected as solvent for simple UV-method because of its easy availability, cost factor and high stability. The proposed method for estimation of Amorolfine hydrochloride in pure and in ointment dosage form were found to be simple and sensitive. The drug in Ethanol shows λ_{max} at 219 nm, with linearity range of 5 – 30 µg/ml.

The optical parameters [7-8] like Beer's law limits (05-30 µg/ml), Sandell's sensitivity (0.01028), correlation coefficient (0.9997), slope (0.0227), intercept (0.02641), limit of detection (2.08), and limit of quantification (6.93) were calculated for Amorolfine hydrochloride in Ethanol and produced in Table 1. Quantification of Amorolfine hydrochloride from ointment dosage form was performed and the amount present was determined by average of six replicate analyses and the amount in percentage purity is found to be 99.84 % to 100.15 % and shown in table 1.

TABLE1: OPTICAL CHARACTERISTICS OF AMOROLFINE HYDROCHLORIDE.

Parameters	Method Values
λ_{max}(nm)	219nm
Beer's law limit(µg/ml)	5 -30
Sandell's sensitivity (µg/cm^2/0.001 AU)	0.01028
Molar absorbtivity(L mol^{-1} cm^{-1})	0.905×10^3
Correlation Co-efficient (r)	0.9997
Regression equation (Y= mx+c)	Y = 0.0227 X + 0.02641
Slope(m)	0.0227
Intercept(c)	0.02641
LOD(µg/ml)	2.08 µg/ml
LOQ(µg/ml)	6.93µg/ml
Standard error of mean of regression line	0.2750

To evaluate the accuracy of the method and for knowing the interference from excipients recovery [7-8] study was performed. The Recovery of Amorolfine hydrochloride by UV- Spectroscopic [5-6] method was found to be 94.44 % to 99.16 % and the results are shown in Table 3. The values of co-efficient of variance were satisfactorily low and recovery was close to 100 % indicating reproducibility of the methods. The excipients in the formulation did not interfere in the accurate estimation of Amorolfine hydrochloride in tablet dosage form.

From the results, the UV-Spectroscopy method was found to be more precise. Since none of the spectroscopic method is reported for the estimation of Amorolfine hydrochloride in tablet dosage form, this developed method can be applied in industries for routine analysis of the Amorolfine hydrochloride in tablet dosage form.

CONCLUSION

The above method does not suffer from any interference due to common excipients. Therefore, it was shown that the proposed method could be successfully applied to estimate commercial pharmaceutical products containing Amorolfine. Thus, the above studies and findings will enable the quantification of the drug for future investigation in the field of analytical chemistry.

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LIST OF ABBREVIATIONS USED

% RSD Percentage Relative Standard Deviation
% - Percentage
λ - Lambda
µg - Microgram
λ_{max} - Absorption maximum
C - Degree Celsius
Fig - Figure
gm - gram
HCl - Hydrochloric Acid
IP - Indian Pharmacopoeia
LOD -Limit of Detection
LOQ -Limit of Quantification
mg - Milligram
min - minute
ml - Milliliter
mM - Millimole
M - Molarity
NaOH -Sodium hydroxide
ng - Nanogram
nm - Nanometer
r - Regression coefficient
S.D - Standard Deviation
S.E - Standard Error

Conflict of Interest:

No Conflict of Interest

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Authors' Contribution

All authors contributed in the research work

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