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PRE-FORMULATION STUDIES FOR FORMULATION AND DEVELOPMENT OF ETHOSOMAL GEL OF OXICONAZOLE NITRATE

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ABSTRACT

We perform pre-formulation evaluation of Oxiconazole nitrate in this study to develop new formulation of ethosomal gel for antifungal activity. Pre-formulation is a group of studies focused on a new drug candidate's physicochemical properties, which could influence drug efficacy and the production of a dosage form. The program has shown that valuable information can be collected before selecting a compound as a candidate for a type of solid-dosage. The analysis of pre-formulation is to improve the elegant dosage form by determining the kinetic rate profile, compatible with the other ingredients, and to determine the new drug's physicochemical parameter. The pre-formulation also provides information about the organoleptic property, solubility, melting point and drug-related partition coefficient, drug stability, partition coefficient among these properties. All the findings and results showed that the nitrate of Oxiconazole serves as a suitable candidate for a topical drug delivery system that may boost bioavailability.

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INTRODUCTION

The pre-formulation formed in the late 1950s and early 1960s as a result of a change of focus on the production of consumer pharmaceutical products [1]. Nearly all the drugs are sold as tablets, capsules or both. It is important to examine the physical and chemical properties of the drug substance alone or in combination with excipients in the appropriate form before the production of the main dosage type [2]. It can be described as an examination of, and when combined with excipients, the physical and chemical properties of a drug material. The overall goal of pre-formulation research is to generate useful information for the formulator in developing stable and bioavailable dosage forms that can be generated in bulk. Pre-formulation investigations are designed to provide all required data, in particular physicochemical, physico-mechanical and bio-pharmaceutical properties of drug substances, excipients and packaging materials [3, 4].

Need/Importance of Dosage Form:

1. To provide reliable drug delivery in a safe, efficient and convenient way.
2. To guard against harmful climate, such as oxygen or moisture.
3. To protect against gastric acid and after oral administration of the result. Example: Tablet with enteric coating.
4. To mask the drug's odour and taste.
5. To provide porous or insoluble solubility and stabilization for that liquid preparation. E.g., suspension.
6. To provide drug action which is regulated by pace. Example: continued release and regulated tablet release, such as aspirin. [2]

MATERIAL AND METHOS:

Oxiconazole nitrate was procured from Yarrow Chem Products, Mumbai, India. Carbopol 934, HPMC-K4M, Chloroform and Methyl Paraben were procured from S.D. Fine Chem. Ltd, Mumbai, India. Ethanol was obtained from Qualigens Fine Chemicals Mumbai, India. All other reagents used were analytical grade.

Objective of Preformulation:

To formulate elegant dosage types. It provides comprehensive information to establish the best drug delivery system for the formulator. This is the first step before the raw material is formulated or formed into a dosage type.

Pre-formulation Parameters:

1) Organoleptic properties
3) Solubility analysis
3) Melting Point
4) Loss on drying
5) Partition co-efficient
6) Identification Tests
7) Analytical Methodology
8) Compatibility Study of Drug and Excipient [2]

Organoleptic Properties:

A typical pre-formulation scheme should start with the drug product description. The new drug's color, odour, and taste must be reported using concise terminology. To avoid confusion among scientists using different terms to describe the same property, it is important to establish a standard terminology to describe such properties [5].

Solubility:

Solubility can be defined as the ability of a solvent (solid, liquid, or gaseous chemical substance) to dissolve in a solid, liquid, or gaseous solvent to form a solution identical to the solvent. A substance's solubility is the quantity of solute that will dissolve in a given amount of solvent at a given temperature. It is an important parameter for the design of dosage forms. [6]

Procedure:

About 10 mg of the drug was accurately weighed and transferred to 5 different 10ml volumetric flasks. Various solvents (methanol, ethanol, chloroform, acetone, and water) were added to the flask respectively and the solubility was determined.

Melting Point:

The melting point for a capillary tube is determined. The term "melts down ..." indicates that, unless otherwise specified, the temperature at which the product is totally melted as shown by the solid's disappearance will be within the range of ±4 ° C from the stated value. [7]

Procedure:

1. Take 5-6 cm of fine capillary length. Seal the one end by horizontally inserting the end of the capillary tube into the extreme edge of a low, steady Bunsen flame for a few seconds, rotating the capillary mean while.
2. Take a small volume of the material, the melting point of which has to be calculated on a porous board, and powdered it with a spatula.
3. Introduce the powdered compound into the capillary tube by inserting and gently spinning the open end of the capillary tube into the powdered compound. Push the capillary tube softly against the porous plate, so that it sinks into the closed end. Perform the presentation and taping process three or four times.
4. The thermometer bulb is moistened with conc. Sulphuric acid or paraffin liquid and attaches the capillary to the lower end of the thermometer.
5. Place the capillary tube thermometer in the melting point system containing at least two thirds of its volume of liquid paraffin so that the capillary's closed end stays below the liquid paraffin surface.
6. Then gently heat the beaker and write down the temperature from time to time and eventually note down the temperature at which the sample started to melt till all sample melts completely.

Loss on Drying:

Loss on drying is a commonly used test method for determining a sample's moisture content, though it may sometimes refer to the loss of any volatile sample material.

Procedure:

Drying loss was measured directly by moisture balance in IR. The instrument was first modified by spinning knob. About 5 grams of medication was correctly measured. The temperature was set for 5 minutes at 100 ° C to 105 ° C and constant readings were taken by setting the knob and calculating the percent moisture.

Partition Coefficient:

The partition coefficient directly affects drug permeability by biomembrane, and can be approximated by calculating drug partition coefficient in n-octanol / water. The logarithm of the unionized solute concentration ratio in the solvents is called log P. The log P value is also known as a measure of lipophilicity.

The lipophilicity of an organic compound is generally described as a partition coefficient; log P, which can be defined as the unionized compound concentration ratio, at equilibrium, between organic and aqueous phases as in equation.

$$Po/w = (\text{C oil/water})\text{equilibrium}$$

Or

$$\log P = \frac{(\text{un ionized compound})\text{org}}{(\text{un ionized compound})\text{aq}}$$

The partition coefficient is expressed in a logarithmic scale, therefore a log P = 0 means that both the compound and the partitioning solvent are equally soluble in water. If the compound has a log P = 5 then the compound in the partitioning solvent is 100,000 times more soluble. A log P = -2 means the compound is 100 times more water soluble, that is to say very hydrophilic. The study of the partition coefficient used n-octanol as the oil phase, and the aqueous phase was the phosphate buffer 7.4. The two phases were combined in equal quantities and saturated on a mechanical 24-hour water bath shaker at 37±2 ° C. At 2000 rpm, the saturated phases were separated by centrifugation. Equal volume of both phases was put in conical flasks and added to every 100 mg of medication. The flasks were shook for 6 hours at 37±2 ° C to attain full partitioning at 100 rpm. The two phases were separated by centrifugation at 1000 rpm for 5 min and were then analyzed for drug [8, 9].

Identification Tests:

Identification of drug was also confirmed by UV-VIS and IR spectroscopy.

UV Spectral Analysis:

The theory of UV-visible spectroscopy is to remove electromagnetic radiation from the 200-800 range and to induce higher energy states [10].

Procedure:

Accurately weighed 10 mg of Oxiconazole nitrate and dissolved in 100 ml of methanol in 100 ml of volumetric flask and prepared suitable dilution to make it to a concentration of 10 µg/ml and recorded the spectrum in U.V spectrophotometer (LABINDIA UV 3000 +) in the range of 200-400 nm to find the λmax.

Infra Red Spectral Analysis:

The infra Red Spectroscopy of the drug was carried out to ascertain identity of drugs.

Procedure:

A pellet about 1 mm in diameter of each drug was prepared by compressing 3-5 mg of a 100-150 mg potassium bromide drug in KBr press. The pellet was placed in 80 IR compartment and scanned with a FTIR – Brukers Alpha between wave number 4000-600 cm^{-1}. [11]

Analytical Methodology:

A) Preparation of Standard Stock Solution:

About 10 mg of Oxiconazole nitrate was precisely weighed and transferred to 100 ml volumetric flask. The medication was dissolved in 1 ml of methanol, and the amount was made with water up to the mark to provide a 100 µg / ml stock solution.

B) Preparation of Blank Reagent Solution:

In 40 percent aqueous methanol (v / v), the blank reagent solution is prepared by adding 3ml of 0.1 M citric acid solution (pH 2.3) to 2 ml of 0.1 percent Methyl Orange (w / v). The reaction mixture was than extracted by shaking with 10 ml of dichloromethane. The extraction was carried out three times until the solution was apparent. The organic extracts were collected in a 50 ml volumetric flask and diluted to volume with dichloromethane. In each volumetric flask, approximately 0.2 g anhydrous sodium sulphate was added, slowly shaken for about 1min, and filtered.

C) Preparation of Working Standard Solution:

A 3ml of 0.1 M citric acid solution (pH 2.3) was added from stock solutions of 1,2,3,4,5,6,7 ml Oxiconazole nitrate aliquots. 2ml of 0.1 per cent Methyl Orange (w / v) was then added to 40 per cent aqueous methanol (v / v). The reaction mixture was extracted by shaking with 10 ml of dichloromethane. The extraction was carried out three times before the solution was apparent. In each volumetric flask, approximately 0.2 g anhydrous sodium sulphate was applied, shaken slowly for about 1min, filtered, and the first portion of the filtrate discarded. Using U.V spectrophotometer (LABINDIA UV 3000 +), the absorbance of the resulting solution was measured at 427 nm after about 3 min. (12)

Compatibility Study of Drug and Excipient:

A) Physical change:

Drug-excipient compatibility studies represent an important step in the pre-formulation in the production of all dosage types. Potential physical and chemical reactions between drugs and excipient may have an effect on the chemical, physical, therapeutic properties and dosage form stability.

Procedure:

Studies on compatibility of drug-excipients were conducted for one month. The drug with excipients Lecithin, Carbopol 934 and Cholesterol was subjected for one month in the stability chamber to storage at room temperature and elevated temperature at 40±2 ° C/75±5 percent RH. The samples were taken after 7, 14, 21 and 30 days to test for physical changes such as discolouration, odor etc.

B) FTIR Study:

FT-IR spectra were recorded on a FTIR spectroscopy using the Brukers Alpha instrument at a frequency range of 400-4000 cm-1 with a resolution of 4 cm^{-1} using the method of potassium bromide discs. Individual samples as well as the product and excipient mixture were crushed, thoroughly mixed in a mortar with potassium bromide for 3-5 min and compressed into a disk by applying a pressure of 5 tons in hydraulic press for 5 min. The sample concentration of potassium bromide will range from 0.2% to 1%. The pellets were put in light path and for proof of any interactions, spectrum was collected and reviewed.

RESULTS AND DISCUSSION:

Preformulation Studies:

Organoleptic Properties:

It was done by appraising sensory characters such as taste, appearance, odor, etc. Table 1 shows the organoleptic assessment of oxiconazole nitrate. The physical appearance of the drug with official reports was found to be similar.

Table 1: Organoleptic Evaluation of Oxiconazole nitrate.

Experimental	Property Studied	Standard Values	Result
Organoleptic Property	Colour	Nearly white Crystalline powder	White crystalline powder
	Odour	Odourless	Odourless
	Taste	Slightly bitter	Slight bitter

Determination of Solubility (At room temperature):

Oxiconazole solubility in watery and non-aqueous solvents was determined. It is soluble in methanol; it is sparingly soluble in ethanol, chloroform and acetone; and it is partially water soluble. The results of the solubility studies are summarized in table 2.

Table 2: Solubility Studies of Oxiconazole nitrate.

S. No.	Solvent Used	Standard solubility	Result
1.	Methanol	Soluble	Soluble
2.	Ethanol	Sparingly soluble	Sparingly soluble
3.	Chloroform	Sparingly soluble	Sparingly soluble
4.	Acetone	Sparingly soluble	Sparingly soluble
5.	Water	Very slightly soluble	Slightly soluble

Determination of Melting Point:

The range of melting point defined by the melting point device is given in table 3. The melting point of the drug sample was found to be within the range of 137 ºC to 139 ºC, which is almost the same as normal melting point i.e.137 ºC-138 ºC and confirms the drug sample identification. DSC test (PerkinElmer Thermal Analyzer) was verified the mean melting point. The sample drug DSC thermograph is given in figure 1.

Table 3: Melting point range of Oxiconazole nitrate.

S. No.	Melting Point		Result
	Onset	Complete	
1.	136	139	137-139
2.	137	139	
3.	137	139	

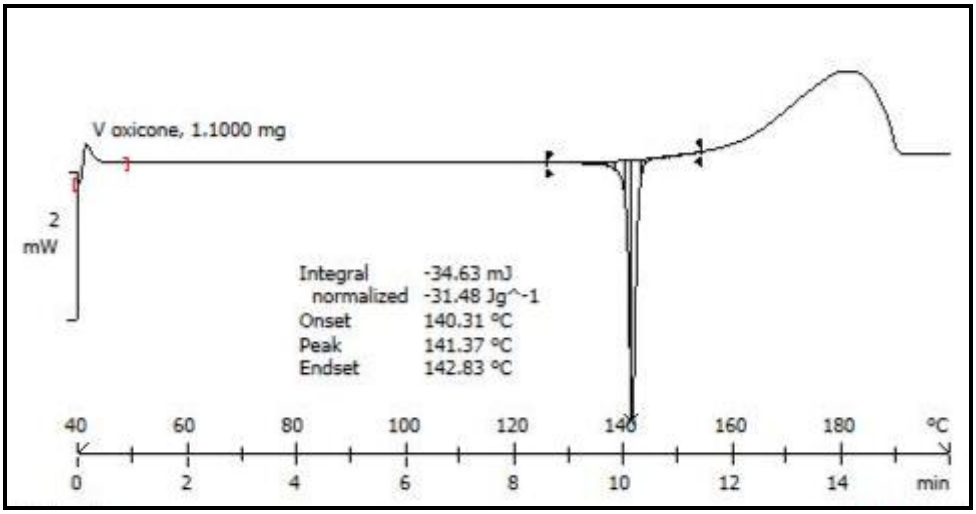

Fig. 1: DSC Thermogram of pure Oxiconazole nitrate.

Loss on Drying:

The drying loss of the drug sample was found to be no more than 0.2 percent, which was not more than 0.5 percent in line with the pharmacopoeial specification.

Determination of Partition Coefficient:

Oxiconazole's partition coefficient value was found to be 5.62±0.001 which is identical to the reported value, i.e., 5.84 and this revealed its lipophilic character. The estimation of the partition coefficient is ascertained in table 4.

Table 4: Calculation of partition coefficient of Oxiconazole Nitrate.

Water : Octanol System (25:25)	Calculated Partition coefficient	Calculated log P	Reported log P [logKow (Partition Coefficient)]
Water Octanol	416869.38	5.62±0.001	5.84

Identification Tests:

Identification of drug was also confirmed by UV-VIS, IR spectroscopy.

UV Spectral analysis (Determination of λmax):

The UV spectrum of Oxiconazole nitrate in methanol was scanned and λmax was found to be 220 nm respectively. The UV spectrum report is given in figure 2 as given below.

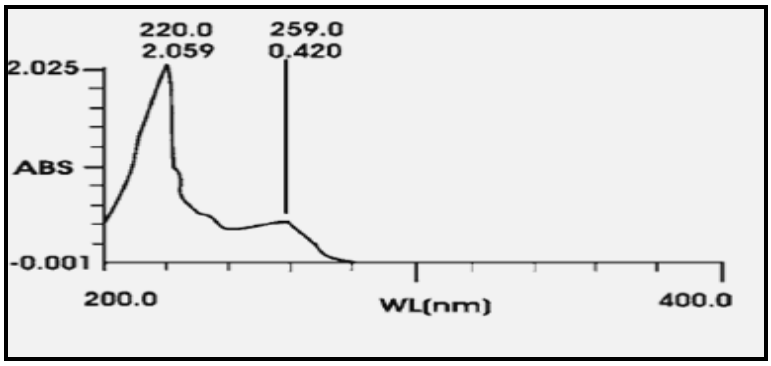

Fig 2: UV Spectrum Report of Oxiconazole Nitrate.

Infra Red Spectral Analysis:

IR Spectra of Oxiconazole nitrate in their pure form was recorded. The FTIR spectrum of sample drug is shown in figure 3. The IR spectrum interpretation of Oxiconazole nitrate is given in Table 5.

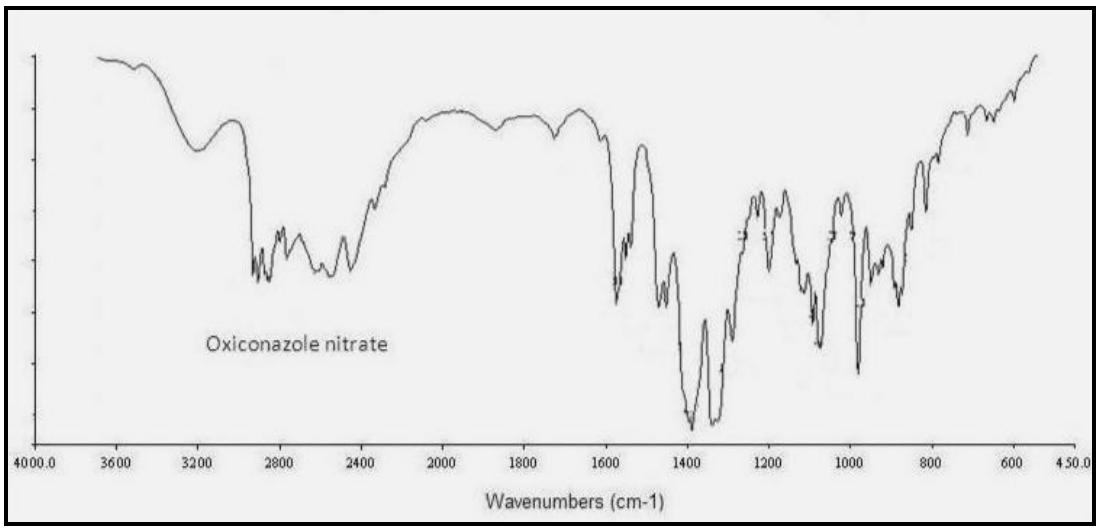

Figure 3: IR Spectra of Oxiconazole nitrate.

Table 5: IR spectrum interpretation of Oxiconazole nitrate.

S. No.	Functional group	Wave number observed (cm^{-1})
1	C-H (methylene, CH_2)	2959
2	C=N	1474, 1455
3	N-O (of cis isomer)	1330-1384
4	C-H (aromatic)	3139-3059

The IR spectrum of the drug sample was in agreement with the standard IR spectrum of pure oxiconazole nitrate mentioned in the official monograph.

Analytical Methodology:

Preparation of Calibration Curves:

Oxiconazole nitrate calibration curve showed the graph obeyed Beers Lambert Law at concentration range (2-20µg / ml). The equation of regression was found to be: y = 0.043x-0.028 and also a strong coefficient correlation of 0.999 was observed. The mean absorbance of different oxiconazole concentrations in distilled water was shown in Table 6. Figure 4 provided a comparison of mean concentrations of oxiconazole in distilled water (µg / ml) versus mean absorbance (nm) of different concentrations.

Table 6: Readings for calibration curve of Oxiconazole nitrate.

S. No.	Concentration(µg /ml)	Absorbance at 427nm
1.	2	0.061±0.0013
2.	4	0.14±0 .0021
3.	6	0.236± 0.0009
4.	8	0.326± 0.0011
5.	10	0.408± 0.0032
6.	12	0.5±0.0014
7.	14	0.5823± 0.0026

* Each value is average of three separate determinations ±SD.

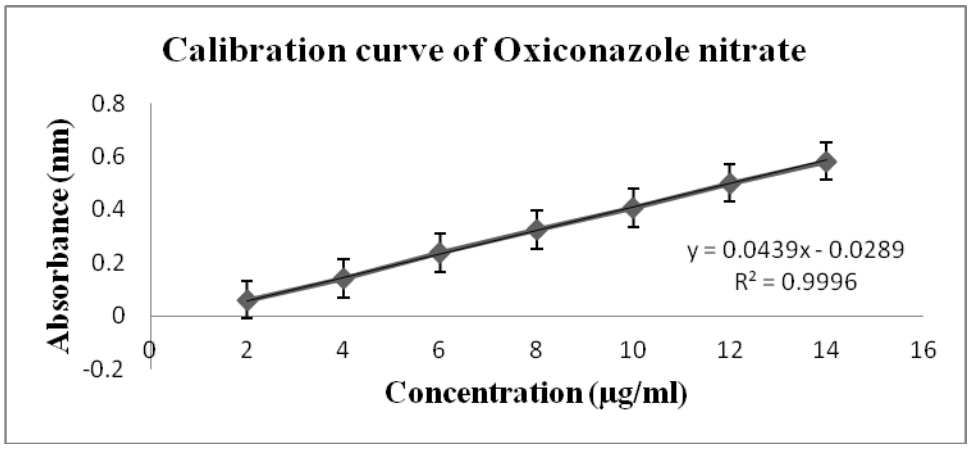

Fig 4: Calibration curve of oxiconazole nitrate.

Compatibility Study of Drug and Excipient:

Physical Change:

No physical changes such as discolouration; improvements in texture etc have been found during the study of compatibility.

FTIR Study:

FTIR spectra of ' pure substance ' and ' drug stuck ethosomes ' were contrasted with the study of product incompatibility with excipient and conditions of reaction. Key peaks of excipient-trapped drug have been contrasted with peaks of pure drug in order to know whether they are compatible with each other. Figure 5 shows overlays of FTIR spectra of pure and trapped material.

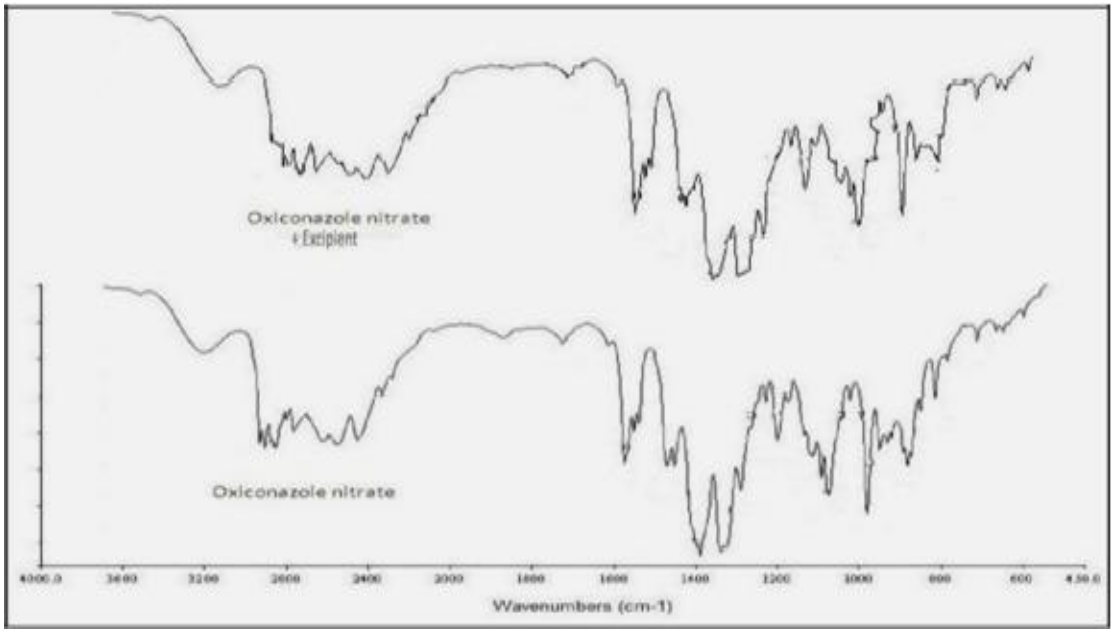

Fig 5: Compatibility study of Oxiconazole nitrate and excipient by FTIR.

Principle peaks of drugs have been found retained; peak extension may be due to convergence of polymer system and product peaks in ethosomal formulation. From the spectral analysis it was found that there was no significant change in the peaks of pure drug and drug polymer mixture in the FTIR spectrum of pure Oxiconazole nitrate. Hence it can be concluded that no unique interaction between the drug and the polymers was observed.

CONCLUSION

Pre-formulation experiments have a major role to play in predicting problems with formulation and finding rational directions in both liquid and solid drug formation technologies. The most effective salt production and stability studies in solution would show the effectiveness of parental or other dosage type and can classify method of stabilization. It was inferred from the above pre-formulation studies that the excipients were photo-stable, and pre-formulation studies were passed. Excipients are ideal for formulating the Oxiconazole Nitrate ethosomal gel.

ABBREVIATIONS

Table 7: list of symbols and abbreviations.

S.No.	Symbol/ abbreviation	Description
1.	%	Percentage
2.	±SD	Standard deviation
3.	µg	Microgram
4.	^{0}C	Degree Celsius
5.	Cm	Centimeter
6.	i.e.	That is
7.	mg	Milligram
8.	Min	Minutes
9.	ml	Milliliter
10.	mm	Millimeter
11.	nm	Nanometer
12.	PEG	Polyethylene glycol
13.	Rpm	Revolution per minute
14.	λ	Wavelength
15.	λ_{max}	Maximum wavelength
16.	DSC	Differential Scanning Calorimetry
17.	FTIR	Fourier-transform infrared spectroscopy

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CONFLICT OF INTEREST:

Nil

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