Research Article

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Kopexil vs Minoxidil: Exploring the Physicochemical Characteristics, Storage Stability, and Skin Permeability

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Conflicts of interest

The authors declare no conflict of interest.

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Funding acquisition: Hamed Hamishehkar, Farnaz monajjemzadeh.

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Abstract

Objective:

This study aimed to investigate the physicochemical properties, skin permeation behavior, and stability kinetics of Kopexil, a potential treatment for hair loss.

Methods:

Physicochemical characteristics of Kopexil were analyzed using Differential Scanning Calorimetry (DSC), Fourier-Transform Infrared Spectroscopy (FTIR), Ultraviolet-Visible (UV-Vis) spectroscopy, and High-Performance Liquid Chromatography (HPLC). Hydroalcoholic formulations (0.5%, 1%, and 2% Kopexil) were stored at 4 °C/60% RH, 25 °C/60% RH, and 40 °C/75% RH for 6 months, in accordance with ICH stability guidelines. HPLC method validation followed USP guidelines. In vitro skin permeation was assessed using Franz diffusion cells on full-thickness mouse skin. Tape-stripping followed by HPLC analysis quantified permeated drug levels. minoxidil served as a reference control in all assays.

Results:

DSC, FTIR, and UV-Vis spectra differentiated Kopexil from minoxidil. A validated HPLC method enabled precise analysis. Stability testing no physical changes in the formulations and $t_{90\%}$, which is the time required for the drug to retain 90% of its label claim was calculated. HPLC-UV analysis indicated $t_{90\%}$ values of 39-50 and 20-42 months in different concentrations and different storage conditions, for kopexil and minoxidil, respectively. Kinetics evaluations confirmed zero-order degradation kinetics, indicating concentration-independent drug loss. Kopexil demonstrated a superior skin permeation rate compared to minoxidil and less drug deposited within the stratum corneum confirmed by tape-stripping.

Conclusion:

Kopexil was successfully characterized by multiple analytical techniques, distinguishing it from minoxidil. The results showed that the Hydro-alcoholic formulations of kopexil has slightly higher $t_{90\%}$ values compared to minoxidil. Enhanced skin permeation of kopexil suggests potential advantage in topical alopecia treatment compared to minoxidil.

Keywords

Kopexil; Minoxidil; Alopecia; Hair Loss; stability test.

1. Introduction

Alopecia is a medical condition characterized by the partial or complete loss of hair in specific body areas. This condition predominantly affects the human scalp.[1] Various underlying diseases, including autoimmune diseases, endocrine abnormalities, chronic infections, and nutritional deficiencies, can cause alopecia.[2] The emotional aspects of hair loss can significantly diminish self-esteem and overall quality of life. The FDA-approved medications which are readily available for alopecia treatment are minoxidil and Finasteride.[3] Kopexil, with the chemical name 2,4-diamino-pyrimidine-3-oxide, known as aminexil, is a topical hair growth stimulant, widely used inn cosmetic products.[4] In addition, oral supplements containing amino acids, iron, selenium, and marine hydrolyzed collagen have been shown to improve hair growth in patients with androgenetic alopecia and telogen effluvium, offering a complementary approach to topical therapies.[5] The chemical structure of Kopexil closely resembles that of minoxidil (**Figure 1-A**), except for a piperidine substituent at C-6 (**Figure 1-B**).[6] Due to this similarity, Kopexil may share a similar mechanisms of action with minoxidil, and it has been shown that, both medications appear to extend the hair growth phase through non-hormonal pathways.[7] It has also been shown that Kopexil, softens hair follicles and enhances blood vessel dilation, thereby promoting blood flow to the hair follicles.[6]

Multiple studies have suggested Kopexil's effectiveness in reducing hair loss, treating perifollicular fibrosis, and delaying the natural aging process of hair roots.[6-8] Furthermore, it has been shown that kopexil enhances blood circulation to follicles, maintains tissue flexibility around hair roots and inhibits perifollicular fibrosis.[7,9,10] Both minoxidil and Kopexil affect the proliferation and differentiation of follicular keratinocytes, which increases the phase of active hair growth period. A study with 351 people who had alopecia found that using a 1.5% topical Kopexil solution decreased the amount of telogen hair and increased the amount of anagen hair compared to a placebo.[3] Additionally, observational studies support the tolerability and efficacy of Aminexil Clinical 5 (AC5) for mild alopecia cases.[3] In addition, combination therapy of Kopexil with SP94 has demonstrated additive effects in slowing hair loss.[3,11] Recent research has compared the efficacy of Kopexil and minoxidil in various

concentrations.[3,6,7,12] Most of these studies report similar or superior efficacy for Kopexil, as seen in the works of Amiri et al. (2023) and Jalilzadeh et al. (2024). However, it is noteworthy that Orasan et al. (2016), in an animal model, found minoxidil to show slightly higher efficacy. Such differences may result from variations in dosage, formulation, study model, sample size, and treatment duration. This variability underscores the need for standardized, head-to-head clinical trials to more precisely determine relative treatment performance.

Table 1. Summary of Previous Studies Comparing kopexil and minoxidil

Study	**Formulation & Dosage**	**Duration**	**Population**	**Key Finding**
Amiri et al. (2023)[7]	Niosomal kopexil 1% vs. Niosomal minoxidil 2%	6 months	Males with androgenetic alopecia	Kopexil showed superior efficacy in reducing hair loss
Orasan et al. (2016)[6]	Kopexil vs. minoxidil (2% w/v)	Animal model	Rats	minoxidil showed slightly higher efficacy
Camacho et al. (2013)[12]	Kopexil + SP94 combination	6 months	180 males and females	Effective in slowing hair loss
Reygagne et al. (2021)[3]	Aminexil Clinical 5	Observational	International cohort	High tolerability and efficacy in mild alopecia
Jalilzadeh et al. (2024)[27]	Kopexil 5 % vs. minoxidil 5 %	Animal model	Mice	Kopexil showed higher efficacy

Minoxidil and kopexil degrade through pathways influenced by their molecular structures and environmental factors like pH, temperature, light, and oxygen. In minoxidil, water readily cleaves the N–O bond in the pyrimidine ring—especially in acidic

or basic conditions—forming 4-amino-6-hydroxypyrimidine derivatives. It also undergoes oxidative and photodegradation, with UV light converting N–O to N=O and oxygen oxidizing the piperidine ring or amine groups. Kopexil's pyrimidine ring is also sensitive to UV, but it is more water-stable. Overall, minoxidil may be less stable than Kopexil due to a greater susceptibility to hydrolysis and photodegradation. Thus, it's necessary to include protective packaging to increase stability, to incorporate UV stabilizers and antioxidants to stop deterioration and consequently extend the shelf life. Based on literature studies [6,7,9], this overview clarifies their stability profiles by offering a framework for further studies on the special degradation kinetics of Kopexil and minoxidil.

Since 1979, the Food and Drug Administration (FDA) guidelines, require a shelf life (or expiration date) expression, directly indicated on the container label. Similar requirements were approved in the European Union and all around the world. In 2003, the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) developed and published a second revised version, Q1A (R2), of the guidance document Q1A, which was first published in 1994.[13] Guidance document Q1A (R2) defines shelf life as the designated period within which a drug product is anticipated to maintain its quality and effectiveness according to the approved shelf-life specification, provided that the product is stored under the conditions specified on the container label".[14] To guarantee the quality and consistency of a pharmaceutical product throughout its storage period, it is essential to establish the product's shelf life based on comprehensive evaluations of its physical, chemical, and microbiological stability. The necessity of these tests is independent of the tests conducted to assure the overall efficacy of medical products.[13] The assessment of product stability should also consider potential risk factors such as precipitation, particle growth, alterations in the crystal structure, or any other characteristics of the active substance that might influence the thermodynamic stability and properties of the pharmaceutical product[15]. The stability program should incorporate stress testing as a part of its protocol to examine the product's response to severe conditions, such as temperature cycling, particularly when applied topically in formulations.[16]

Skin delivery offers a favorable alternative to oral administration due to its numerous advantages, which include the avoidance of gastrointestinal irritation, minimal systemic toxicity, less invasiveness, reduced risk of abuse, circumvention of first-pass metabolism, and provision of consistent plasma levels.[17] To ensure the safety, effectiveness, and appropriate formulation of topical products, it is crucial to conduct skin permeation testing on pharmaceutical products. Various parameters, including the physiological and pathological conditions of the skin, as well as the physicochemical properties of the active substance and carrier, significantly influence drug permeation into the skin.[18] The rate at which drugs permeate the skin can vary considerably among individuals, often differing by up to tenfold. Factors contributing to these variations include age, sex, race, anatomical location, and general health status. Physicochemical properties, molecular weight, and formulation-related factors are essential features that affect the skin permeation rate of any pharmaceutical product.[19] To ensure proper permeation into the skin, both the drug substance and its formulation require specific properties, including a distribution coefficient ranging from 10 to 1000, a molecular weight lower than 500 Daltons, and a melting point below 200 degrees Celsius.[20]

Although not approved by FDA, the European Commission has listed Kopexil in a recent cosmetic directive in Annex III, with a Maximum concentration in ready-for-use preparation up to 1.5% [21-23]. Literature review shows a recent trend in the treatments of hair loss using Kopexil, and the Cosmetic market also reveals the broad application of this ingredient in hair loss treatments.[24-27]

Due to the growing interest in utilizing Kopexil for alopecia treatment, the lack of similar studies on this subject, and its potential inclusion as an approved product in the future, our research team has taken the initiative to investigate its physicochemical properties and assess its skin absorption properties. In the study described here, we developed a simple and validated High-Performance Liquid Chromatography (HPLC) method to determine the stability of 0.5, 1, and 2% topical Kopexil hydro-alcoholic formulation according to the guidelines of the International Conference on Harmonization (ICH).[28] Additionally, a comparative skin permeation test by test stripping between minoxidil 5% and Kopexil 5% was conducted to investigate the partitioning of APIs through the skin

barrier and their deposition within the stratum corneum. This study provides a more detailed insight on the stability of Kopexil and may help pharmaceutical industries to develop suitable preparations.

2. Materials and Methods

Ethanol, Hydrochloric acid, sodium hydroxide, sodium hydrogen phosphate, and HPLC grade solvents (Acetonitrile and methanol) were obtained from Merck Chemicals Co. (Darmstadt, Germany). Kopexil was kindly gifted from the Food and Drug Administration (FDA) of Tabriz University of Medical Sciences, and minoxidil was purchased from Sigma Aldrich (St. Louis, IL).

2.1. Preparation of formulation

To prepare the formulations, the precise quantities of the necessary active ingredients were accurately measured. These ingredients were separately dissolved in a solution of ethanol and water in a ratio of 70:30 under continuous stirring using an overhead stirrer until it yielded a homogenous dispersion. Ultimately, the total volume was adjusted to 100 mL using the same solution, and 0.5, 1, 2, and 5 % (w/v) hydro-alcoholic solutions of minoxidil and Kopexil were separately prepared.

2.2. Physicochemical characterization of minoxidil and kopexil

2.2.1. Differential Scanning Calorimetry (DSC)

The thermal behaviors of minoxidil and Kopexil were analyzed using a DSC-60 calorimeter (Shimadzu, Japan) under a nitrogen atmosphere to prevent oxidative degradation. Each accurately weighed sample (~2 mg) was placed in a hermetically sealed aluminum pan, with an empty aluminum pan serving as a reference. The samples were scanned over a temperature range of 25–300 °C at a heating rate of 10 °C min^{-1}. The instrument was calibrated using indium (Tm = 156.6 °C, ΔH = 28.4 J g^{-1}) and zinc (Tm = 419.5 °C) as standard references. Enthalpy calculations and thermogram analysis were performed using TA-60 software (version 1.51). The DSC curves were used to determine melting points, phase transitions, and potential thermal degradation patterns of the active compounds.

2.2.2. FTIR (Fourier transform infrared) spectroscopy

The infrared spectra of minoxidil and Kopexil were acquired using the potassium bromide disk preparation method using an FTIR spectrometer (MB- 100 series, Bomem, Canada).The spectrum was obtained by averaging ten consecutive scans of the same sample while maintaining a constant resolution of 4 cm^{-1}. The FTIR data were processed using the Grams/32 version 3.04 software developed by Galactic Industries Corporation, Salem, NH.

2.2.3. HPLC analysis of minoxidil and kopexil

The UV spectra of minoxidil and kopexil, at the same concentration as the dilution, were recorded using a spectrophotometer (Cecil 7400 series, UK) to confirm their spectral properties.

The HPLC analysis (KNAUER, Germany) was conducted using a high-resolution C18 column (CLIPEUS C18, 5µm, 250 × 2.1 mm, USA) at a temperature of 25°C. The mobile phase consisted of phosphate buffer (pH=6.8) and acetonitrile (80:20) and was maintained at a flow rate of 1.1 mL/min, at a temperature of 25°C. The Ultraviolet-Visible (UV-Vis) detector was set at a detection wavelength of 238 nm. The injection volume was 20 µL for all analyses.

Subsequently, a HPLC system coupled with PDA analysis (Agilent Technologies 1260 Infinity, USA), was used with the same column and mobile phase. PDA detection was performed from 200-400 nm (Agilent, USA) for pooled stressed samples.

2.2.3.1. Standard solution preparation

Standard solutions of Kopexil and minoxidil were prepared to enable accurate and reproducible HPLC analysis. In separate 100 mL volumetric flasks, 75 mg of Kopexil, 100 mg of minoxidil were accurately weighed and dissolved in 10 ml of methanol, selected for its ability to effectively solubilize both compounds due to their polar functional groups. Dilutions were made using acetonitrile and water (80:20 v/v), yielding stock solutions with concentrations of 750 µg/mL for Kopexil and 1000 µg/mL for minoxidil. From each stock solution, 1 mL was transferred to a 10 mL volumetric flask, and the volume was adjusted to 10 mL with the same solvent mixture (acetonitrile: water (80:20

v/v)). This dilution step resulted in standard working solutions with concentrations of 75 µg/mL for Kopexil and 100 µg/mL for minoxidil.

2.2.3.2. Sample preparation

The topical solutions were formulated separately with different concentrations of Kopexil (0.5%, 1%, and 2% Kopexil, w/v) and minoxidil (0.5%, 1%, and 2% minoxidil, w/v) in a mixture of ethanol and water (70/30) within a 60-mL bottle. To prepare a sample, 0.5 mL from these solutions was transferred to a clean, dry 100 mL volumetric flask. Then, diluted to a final volume of 100 mL using a solution of acetonitrile and water (80/20).

2.2.3.3. Validation of HPLC method

The validation of the method was conducted following International Council for Harmonization (ICH) guidelines. Various validation parameters were assessed, including linearity, accuracy, precision, limit of detection, and limit of quantification. System suitability of the proposed HPLC method was also evaluated.[29,30]

For the preparation of linearity solutions, Varying volumes of the stock solution (0.25, 0.5, 0.75, 1, 1.25, 1.5 mL) were carefully pipetted out and transferred into clean and dry 10 mL volumetric flasks. The contents of each flask were then diluted to a final volume of 10 mL using a diluent composed of acetonitrile and water in a proportion of 80:20. This dilution process resulted in a concentration range of 18.75-112.5 µg/mL for Kopexil and 25-150 µg/mL for minoxidil. The linearity of the method was evaluated using three separate series of solutions containing Kopexil and minoxidil at concentrations ranging from 18.75 µg/mL to 112.5 µg/mL and 25 µg/mL to 150 µg/mL, respectively. The resulting peak areas were plotted against the corresponding concentrations. The method precision or repeatability was assessed by injecting six working standard solutions and performing six sample injections. The peak areas of all the injections were recorded, and the standard deviation, percent relative standard deviation, and percent assay were calculated. For intra-day analysis, the samples were analyzed six times a day at 09:00 am, 11:00 am, 01:00 pm, 03:00 pm, 05:00 pm, and 07:00 pm, while inter-day

stability was analyzed for 6 consecutive days at 09:00 am. The peak areas of all the injections were measured, and the standard deviation, relative standard deviation, and accuracy in assay were computed. The obtained results were found to be within the specified acceptance criteria. To evaluate accuracy, the standard addition method was employed at three distinct levels, namely 50%, 100%, and 150%. The percentage recoveries of kopexil and minoxidil, were determined through this process. The limit of detection (LOD) and limit of quantification (LOQ) of Kopexil and minoxidil were determined by the calibration curve method. Solutions of both Kopexil and minoxidil were prepared and injected in triplicate. The average peak area of three analyses was plotted against concentration.

$$LOD = (3.3 \times Syx)/b \quad (1)$$

$$LOQ = (10.0 \times Syx)/b \quad (2)$$

LOD and LOQ were calculated using equations (1) and (2), where Syx is residual variance due to regression; b is the slope. System suitability parameters were established through the preparation of standard solutions containing minoxidil and Kopexil. These solutions underwent six injections, and parameters such as peak tailing, resolution, and USP plate count were subsequently assessed.

2.3. Physicochemical stability test

The physicochemical stability of hydro-alcoholic preparations of minoxidil and Kopexil (0.5, 1, 2 % each) was studied by subjecting them to stability conditions at 4 °C/60 % RH, 25 °C/60 % RH, and 40 °C/75 % RH as per ICH guidelines for 6 months. For estimating the shelf life, $t_{90\%}$, which is the time required for the drug to retain 90% of its label claim, was calculated. At predetermined time intervals, the concentration of the drug was quantified by a validated HPLC assay, expressed as the percentage of the labelled claim, and plotted against storage time. The obtained data for each temperature were fitted by linear regression to zero, first, second and third order kinetic models, and the dominant model for the kinetics of drug loss was selected according to the highest RSQ (Squared correlation coefficient) and the lowest MPE (Mean percentage error) values. Zero order kinetics was the best fitted model; thus, the degradation rate constant (k)

was then obtained from the slope of the regression line and $t_{90\%}$ values were calculated accordingly. Zero order indicates concentration-independent degradation kinetics. The formula used was as:

$C = C_0 - k_0t$

where:

C = Concentration

C_0 = Initial concentration

K_0 = Rate of elimination

t = Time

Accordingly, and the 95% confidence intervals (CI) for the $t_{90\%}$ values were calculated at each intended storage temperature.

2.4. *In vitro* study

2.4.1. Skin preparation

Skin samples were acquired from the black/BALB mice strain at the Animal Centre of the local University in Tabriz, Iran. The process involved obtaining full-thickness skin by surgically removing the underlying subcutaneous fat. Epidermal membranes were then obtained by immersing the full-thickness skin in 60 ◦C water for 1 minute, enabling the separation of the epidermis from the dermis. The subsequent extraction of the stratum corneum (SC) from the epidermal membranes was achieved through trypsin digestion. To accomplish this, the epidermis was left to float in a phosphate-buffered saline (PBS) solution containing 0.01% trypsin at 37 ◦C overnight, and the digested viable epidermis was delicately removed using cotton buds. At the same time, the remaining SC membrane was rinsed multiple times with distilled water. The isolated SC membranes were air-dried with absorbent paper, carefully placed between parafilm sheets, and covered with aluminum foil. All skin membranes were stored at -20 ◦C and utilized within one month [31].

Ethical approval for the study was obtained in advance from the local research ethics committee at Tabriz University of Medical Sciences, Tabriz, Iran, under reference number IR.TBZMED.AEC.1401.038. The study followed the guidelines outlined in the

"Guide to the Care and Use of Experimental Animals" by the Canadian Council on Animal Care.

2.4.2. Skin permeation study

In-vitro skin permeation investigations were conducted using full-thickness skin in Franz diffusion cells, with an effective diffusion area of 1.33 cm^2 and a receptor chamber capacity of approximately 3.4 mL. The skin was sectioned into discs and securely positioned between the donor and receptor compartments of the Franz cell, with the stratum corneum side facing the donor chamber. The receptor compartment, filled with pH 7.4 PBS, was immersed in a water bath at a controlled temperature of 35 ± 0.5 ◦C. The donor solution, comprising 1 mL of a hydroalcoholic formulation with either 5% w/v minoxidil or kopexil, was placed in the donor compartment. A layer of parafilm was employed to cover the donor compartment, thereby preventing evaporation.[31]

To evaluate permeation, 200 µL of the receptor phase was withdrawn at predetermined intervals, and an equal volume of fresh PBS was added. High-performance liquid chromatography (HPLC) was utilized to assess the minoxidil content in all samples.

After the completion of the Franz diffusion studies, any residual formulation on the exposed skin surface was meticulously removed by wiping it with a cotton bud. The skin was then subjected to tape stripping comprising the application of adhesive tape (D-Squame tapes, CuDerm Corp., Dallas, TX, USA) to the skin surface for approximately 5 seconds, followed by careful removal (20 times). Groups of five tapes were placed in separate vials and soaked overnight with 2 mL of methanol before analysis with HPLC. The quantity of kopexil/ minoxidil absorbed in the tapes corresponded to the amount of kopexil/ minoxidil deposited within the stratum corneum (SC).[31]

2.5. Statistical Analysis

Statistical analysis was conducted utilizing either a paired Student's t-test or ANOVA to determine the significance of the difference in the data.

3. Results and discussion

3.1. DSC results

Results related to the thermal analysis of kopexil and minoxidil are all shown in Figure 2. DSC gives information regarding the melting characteristics and crystalline nature of

the drug. The DSC thermograms of kopexil (A, blue curve) and minoxidil (B, red curve) exhibit distinct thermal behaviors that differentiate the two compounds. Kopexil shows two sharp endothermic peaks at 142.89 °C and 212.36 °C, corresponding to its melting transitions and possible polymorphic or crystalline phase changes.[23] Similar endothermic events are seen in other drugs like mebendazole and phenazone.[32,33] In contrast, minoxidil displays a single prominent endothermic peak at 171.22 °C, consistent with its melting point as reported in the literature[34] , followed by a broad thermal event at higher temperatures, likely related to decomposition. The differences in the number, position, and shape of the endothermic peaks reflect variations in crystalline structure and thermal stability between the two compounds, providing a clear basis for their physicochemical distinction.

3.2. FTIR results

The FTIR spectra of kopexil **(Figure 3. A, Violette line)** and minoxidil **(Figure 3. B, red line)** reveal similarities and distinct differences in their IR absorption peaks. Both compounds show strong amine stretching bond corresponding to amine (-NH) stretching in the 3300–3600 cm^{-1} range, with kopexil showing peaks at 3521.9 cm^{-1} and 3405.1 cm^{-1}; in comparison, minoxidil exhibits absorption at 3406.2cm^{-1} and 3323.1cm^{-1}. Additionally, both molecules display weak N-H stretching vibrations, with kopexil peaking at 3646.8cm^{-1} and minoxidil at 3647.3 cm^{-1}. Bending of NH_2 substituted to pyrimidine ring in both minoxidil and kopexil, as observed in kopexil at 1628.9 cm^{-1} and minoxidil at 1655.8cm^{-1}.Specific NC_2=N-R stretching of pyrimidine ring is observed, with kopexil peaking at 3119.2 cm^{-1} and minoxidil at 3164.9cm^{-1}. A major distinction between the two compounds is observed in the nitroso (-N=O) region, where kopexil exhibits absorption at 1209cm^{-1} and 1268 cm^{-1}, while minoxidil shows peaks at 1298cm^{-1} and 1325cm^{-1}. Furthermore, minoxidil contains a substituted imine (-N=C-R) functional group at 1563cm^{-1}, which is absent in kopexil. minoxidil also shows a piperidine ring at 1440cm^{-1}, which is not observed in kopexil (**Figure 1. S**). These findings highlight the structural differences between minoxidil and kopexil and underscore the utility of FTIR spectroscopy in their differentiation and characterization (Table 2).

Table 2. Comparative Analysis of FTIR Spectra of kopexil and minoxidil

Functional Group/Feature	**Kopexil (cm^{-1})**	**Minoxidil (cm^{-1})**	**Remarks**
Amine (-NH) stretching	3521.9, 3405.1	3406.2, 3323.1	Both compounds show strong amine stretching bands
N-H stretching	3646.8 (weak)	3647.3 (weak)	Weak NH_2 bands in both minoxidil and kopexil
NH_2 substituted to the pyrimidine ring	1628.9 (strong)	1655.8 (strong)	Bending of NH_2 substituted to the pyrimidine ring in both minoxidil and kopexil
NC_2=N-R stretching of the pyrimidine ring	3119.2 (broad)	3164.9 (broad)	Specific to the pyrimidine ring in both minoxidil and kopexil
Nitroso (-N-O) stretching	1209,1268	1298, 1325	Specific to Nitroso in both minoxidil and kopexil
Substituted imine (-N=C-R)	-	1563	Unique to minoxidil; not observed in kopexil. A Piperidine ring has been substituted to pyrimidine ring
Piperidine ring	-	1440	Unique to minoxidil; not observed in kopexil. kopexil lacks the Piperidine ring.

3.3. UV-visible spectra results

Given the chemical structures of kopexil and minoxidil, with aromatic primary and tertiary Nitrogens (**Figure 1**), acid solution (0.1 M HCl) was selected as the solvent. Examination of the UV-visible spectrum of kopexil (as depicted in **Figure 2-A. S**) revealed that the maximum wavelengths were at 205 nm and 248 nm in a 0.0016% solution in 0.1 M HCl. As depicted in **Figure 2-B. S,** the maximum wavelengths of minoxidil UV absorption were at 205 nm, 229 nm, and 284 nm in a 0.0016% solution in 0.1 M HCl, an observation that was completely aligned with previous studies [9]. Based on the spectra

depicted in **Figure 2. S** and previous studies, the adjusted wavelength for HPLC-UV detection analysis was kept at 238 nm.

3.4. Validity of the HPLC assay

The HPLC retention times of kopexil and minoxidil were found to be 2.3 min (**Figure 4. A**) and 3.4 min (**Figure 4. B**). In **Figure 4. C** samples of both drugs were mixed together and injected to the system. All quantifications were made based on individual injection of each sample of minoxidil or kopexil. The method for kopexil was linear in the concentration range of 0.25–4 μg mL^{-1} with R^2 of 0.999888, and the *LOD* and *LOQ* values of 0.031 and 0.92 μg mL^{-1}, respectively. The method for minoxidil was linear in the concentration range of 0.25–4 μg mL^{-1} with R^2 of 0.99251, and the *LOD* and *LOQ* values of 0.03 and 0.1 μg mL^{-1}, respectively. Calibration plots and regression equations are provided in the supplementary material (**Figure 3. S and 4. S**). The relative standard deviation was less than 2 % in both cases. The mean recovery of kopexil ranged between 98 and 101 %. PDA analysis revealed peak purity values of 0.9989% and 0.9995 for kopexil and minoxidil pooled stressed samples respectively. Results are shown as supplementary material (**Figure 5. S and 6. S**). System suitability parameters of the developed method are represented in Table 3.

Table 3. System suitability parameters of the developed HPLC method

System suitability parameters	Kopexil	Minoxidil
Retention Time (Rt)	2.3 min	3.4 min
Capacity Factor (k')	0.21	0.79
Theoretical Plates (N)	235	289
Relative Standard Deviation (RSD)	0.445	0.37
Signal-to-Noise Ratio (S/N)	8340	5000
Tailing Factor (T or TF)	1.125	1.13
Resolution (Rs)	1.57	
Selectivity Factor	3.75	

3.5. Physicochemical stability results

For shelf life calculations, $t_{90\%}$, which is the time required for the drug to retain 90% of its label claim, was calculated as an estimation.

3.5.1. Kopexil

The variations of kopexil concentration as a function of storage time are shown in **Figure 5**. The results of a six-month physical stability evaluation of the formulation showed no detectable variations in color, odor, and aggregation in the formulations of the kopexil. Using the zero-order model, $t_{90\%}$ values and its 95% confidence intervals (CI) were calculated at each intended storage temperature and at certain concentration values. Data are presented in table 4. The results showed that the kopexil content was within the range, in 39 to 50 month' time period based on different initial concentrations and different storage conditions. It should be mentioned that all results obtained for $t_{90\%}$ as a shelf-life estimation are predictive estimates and in accordance with ICH Q1A (R2), results must be confirmed through long-term real-time stability studies at target storage conditions before a definitive shelf life is assigned.

3.5.2. Minoxidil

The variations of minoxidil concentration as a function of storage time are shown in **Figure 6**. The results of a six-month physical stability evaluation of the formulation showed no detectable variations in color, odor, shape, and aggregation in minoxidil solutions. The results showed that the minoxidil content was within the range, in 20 to 42 months' time period, based on different initial concentrations and different storage conditions. The calculated shelf lives and CI values are represented in Table 4. As mentioned previously, in accordance with ICH Q1A (R2), results must be confirmed through long-term real-time stability studies at target storage conditions before a definitive shelf life is assigned.

Table 4. Table 4. Predicted shelf life for each formulation based on stability testing.

Drug	Percentage in the formulation	Temperature (°C)	Shelf-life (months)	95 % CI (Lower limit percentage)	95 % CI (Upper limit percentage)
Kopexil	0.5	4	49.3	98.9	101.1
		25	45.2	96.1	103.9
		40	39.2	96.5	103.5
	1	4	44.6	96.5	103.5
		25	42.2	95.1	104.9
		40	50.3	96.7	103.3
	2	4	39.0	95.8	104.2
		25	49.1	99.2	100.8
		40	42.2	98.3	101.7
Minoxidil	0.5	4	42.2	90.2	109.8
		25	42.8	97.8	102.2
		40	36.9	97.3	102.7
	1	4	38.6	99.3	100.7
		25	28.9	87.5	112.5
		40	20.1	95.5	104.5
	2	4	27.25	88.0	112.0
		25	26.90	99.6	100.4
		40	30.41	84.6	115.4

3.6. Determination of skin absorption of kopexil solution

For skin permeation measurements, percentage of the drug passed through the skin into the reservoir compartment of the Franz cell, was plotted versus time (hours) (**Figure 7).** Both products followed zero-order kinetics with R^2 = 0.9928 and k = 0.3888 for minoxidil and R^2 = 0.9928 and k = 0.4697 for kopexil.

The amount of kopexil released after 2, 4, 8, and 24 h is represented in **Figure 7**. The corresponding flux rates attributed to kopexil and minoxidil were 0.4697 cm^{-2} h^{-1} and 0.3888 cm^{-2} h^{-1}, respectively. According to the results, the permeation amount of kopexil 5% hydroalcoholic solution was 1.2 times greater than that of minoxidil 5% hydroalcoholic solution (p-value = 0.048, t-test).

According to the results of tape stripping studies, the amount of kopexil 5% solution deposited within the stratum corneum (described fully at 2.4.2), was 1.1 times less than that of the minoxidil 5% solution.

These findings may indicate that the developed formulation of kopexil has a greater absorption in comparison to minoxidil and delivers the active ingredients across the skin efficiently (p-value = 0.041, t-test). In a previous study it was shown that the percutaneous absorption rate of the niosomal formulation of kopexil achieved better results than the Niosomal formulation of minoxidil.[7]

In that study, an intra- and inter-group comparison was made between the percutaneous absorption rates of gel formulations and the niosomal formulations of minoxidil and kopexil. The results indicated that both Minoxidil and kopexil nano-niosomes exhibited percutaneous absorption rates 3 to 11 times higher than the control group using the gel formulation. Moreover, when comparing the 24-hour percutaneous absorption rates specifically for minoxidil nano-niosomes and kopexil nano-niosomes, it was observed that the kopexil nano-niosomes demonstrated a higher absorption rate of 253.78 $\mu g/cm^2$.[18] Based on the log (P) (octanol-water partition coefficient) values of kopexil (-2.09) and minoxidil (1.24), it can be concluded that the higher absorption of kopexil, as compared to minoxidil, may be attributed to cellular absorption pathways that are receptor-mediated and independent of passive diffusion across the cellular membrane.[35] These mechanisms include facilitated diffusion, active transport, endocytosis, and pinocytosis.

4. Conclusion

This study aimed to assess the physicochemical characteristics, stability, and skin permeability of kopexil in comparison to minoxidil for topical alopecia therapy. Our research indicates that kopexil possesses unique thermal and spectroscopic characteristics, enhanced skin permeability, and suitable stability. DSC, UV and FTIR analysis revealed physicochemical characteristics of kopexil and minoxidil, successfully. The primary finding indicated that hydro-alcoholic formulations of kopexil (0.5%, 1%, 2%) exhibited stability for more than six months under diverse conditions, with a

conservative shelf life of two years at 25°C. It is worth mentioning that predicted stability values derived from stability studies require confirmation by real-time long-term stability testing under ICH Q1A (R2) guidelines before being considered definitive. The study revealed kopexil's superior skin permeation rate compared to minoxidil *in vitro*, indicating improved delivery efficacy. Despite these findings, long-term degradation mechanisms and clinical efficacy of kopexil remain inadequately researched, revealing a significant knowledge deficit. Future research may evaluate the in vivo efficacy and degradation products of kopexil across various formulations to advance its medicinal utility.

5- References

1. Jamerson TA, Aguh C. An approach to patients with alopecia. *Medical Clinics* 2021;105(4):599-610.

2. Qi J, Garza LA. An overview of alopecias. *Cold Spring Harbor perspectives in medicine* 2014;4(3):a013615.

3. Reygagne P, Mandel VD, Delva C, Havlíčková M, Padlewska K, Khalil R, et al. An anti-hair loss treatment in the management of mild androgenetic alopecia: Results from a large, international observational study. *Dermatologic Therapy* 2021;34(6):e15134.

4. Zhang S, Zhou H, Chen X, Zhu S, Chen D, Luo D, et al. Microneedle delivery platform integrated with codelivery nanoliposomes for effective and safe androgenetic alopecia treatment. *ACS Applied Materials & Interfaces* 2024;16(13):15701-17. doi: https://pubs.acs.org/doi/10.1021/acsami.3c16608?goto=supporting-info

5. Milani M, Colombo F, GFM-O-Trial Investigators Group: Chiara Baraldo MB, Paolo Chieco , Laura Colonna , Mandel Victor Desmond , Maria Cristina Fiorucci. Efficacy and tolerability of an oral supplement containing amino acids, iron, selenium, and marine hydrolyzed collagen in subjects with hair loss (androgenetic alopecia, aga or faga or telogen effluvium). A prospective, randomized, 3-month, controlled, assessor-blinded study. *Skin Research and Technology* 2023;29(6):e13381.

6. Orasan MS, Bolfa P, Coneac A, Muresan A, Mihu C. Topical products for human hair regeneration: A comparative study on an animal model. *Annals of Dermatology* 2016;28(1):65-73. doi: https://doi.org/10.5021/ad.2016.28.1.65

7. Amiri R, Mohammadi S, Azizi S, Pardakhty A, Khalili M, Aflatoonian M. Evaluation of efficacy and safety profile of niosomal kopexil 1% lotion compared to niosomal minoxidil 2% lotion in malepattern alopecia. *Iranian Journal of Dermatology* 2023;26(2):68-73. doi: https://doi.org/10.22034/ijd.2022.308979.1442

8. Rambwawasvika H, Dzomba P, Gwatidzo L. Alopecia types, current and future treatment. *J Dermatol Cosmetol* 2021;5(4):93-9.

9. Rizwana I, Prakash KV, Mohan GK. Simultaneous estimation of minoxidil and aminexil in bulk and pharmaceutical formulations by rp-hplc method. *Oriental Journal of Chemistry* 2015;31(1):277-84.

10. Nestor MS, Ablon G, Gade A, Han H, Fischer DL. Treatment options for androgenetic alopecia: Efficacy, side effects, compliance, financial considerations, and ethics. *Journal of cosmetic dermatology* 2021;20(12):3759-81.

11. Camacho F, Camacho-Serrano F, Giménez JM, Hernánez MG, Padillo JP, Cejudo MP, et al. Tratamiento de las alopecias de patrón masculino y femenino. Eficacia clínica del aminexil y sp94 en dos series de 180 pacientes, hombres y mujeres. *Medicina Cutánea Ibero-Latino-Americana* 2013;41(1):18-33.

12. Camacho F, Camacho-Serrano F, Giménez JM, Hernánez MG, Padillo JP, Cejudo MP, et al. Treatment of alopecias of male and female patterns. Clinical efficacy of aminexil and sp94 in two surveys of 180 patients, men and women. *Medicina Cutánea Ibero-Latino-Americana* 2013;41(1):18-33. doi: https://doi.org/10.4464/MC.2013.41.1.5046

13. Yasmeen A, Sofi G. A review of regulatory guidelines on stability studies. *The Journal of Phytopharmacology* 2019;8(3):147-51.

14. Capen R, Christopher D, Forenzo P, Ireland C, Liu O, Lyapustina S, et al. On the shelf life of pharmaceutical products. *AAPS PharmSciTech* 2012;13:911-8.

15. Zilker M. The stability of finished pharmaceutical products and drug substances beyond their labeled expiry dates: Universität Würzburg; 2019.

16. Laccourreye O, Maisonneuve H. French scientific medical journals confronted by developments in medical writing and the transformation of the medical press. *European Annals of Otorhinolaryngology, Head and Neck Diseases* 2019;136(6):475-80.

17. Zaid Alkilani A, McCrudden MT, Donnelly RF. Transdermal drug delivery: Innovative pharmaceutical developments based on disruption of the barrier properties of the stratum corneum. *Pharmaceutics* 2015;7(4):438-70.

18. Souto EB, Fangueiro JF, Fernandes AR, Cano A, Sanchez-Lopez E, Garcia ML, et al. Physicochemical and biopharmaceutical aspects influencing skin permeation and role of sln and nlc for skin drug delivery. *Heliyon* 2022;8(2).

19. Bachhav YG, Patravale VB. Formulation of meloxicam gel for topical application: In vitro and in vivo evaluation. *Acta pharmaceutica* 2010;60(2):153-63.

20. Guillot AJ, Martínez-Navarrete M, Garrigues TM, Melero A. Skin drug delivery using lipid vesicles: A starting guideline for their development. *Journal of Controlled Release* 2023;355:624-54.

21. Vincenzi C, Marisaldi B, Tosti A, Patel B. Effects of a new topical treatment containing several hair growth promoters in women with early female pattern hair loss. *Skin appendage disorders* 2019;5(3):146-51.

22. Garre A, Piquero J, Trullas C, Martinez G. Efficacy and safety of a new topical hair loss-lotion containing oleanoli c acid, apigenin, biotinyl tripeptide-1, diaminopyrimidine oxide, adenosine, biotin and ginkgo biloba in patients with androgenetic alopecia and telogen effluvium: A six-month open-label prospective clinical study. *J Cosmo Trichol* 2018;4(1):1-6.

23. Commission E. Cosing of substance: 2,4-pyrimidinediamine, 3-oxide. CosIng; 2009 [cited 2009]; Available from: https://ec.europa.eu/growth/tools-databases/cosing/details/28108.

24. Antypenko L, Gladysheva S, Vasyuk S. Development and validation of uv spectrometric method for estimation of aminexil (kopexil) in bulk. *RECIPE* 2016;19(2):182. doi: https://doi.org/10.5021/ad.2016.28.1.65

25. Mfuh AM, Larionov OV. Heterocyclic n-oxides-an emerging class of therapeutic agents. *Current medicinal chemistry* 2015;22(24):2819-57. doi: https://doi.org/10.2174/0929867322666150619104007

26. Cao J, Wu B, Yuan P, Liu Y, Hu C. Advances in research of hydrogel microneedle-based delivery systems for disease treatment. *Pharmaceutics* 2024;16(12):1571.

27. Jalilzadeh S, Hamishehkar H, Monajjemzadeh F. Kopexil vs minoxidil: In vivo comparative study on hair growth, hair growth promoting factors and toxicity. *BioImpacts* 2024;15(1):30484-.

28. Siddiraju S, Sahithi M. Stability indicating rp-hplc method development and validation for the simultaneous determination of aminexil and minoxidil in pharmaceutical dosage form. *Annales pharmaceutiques françaises* 2015;73(2):114-22. doi: https://doi.org/10.1016/j.pharma.2014.10.005

29. Ojha A, Bhargava S. International council for harmonisation (ich) guidelines. Regulatory affairs in the pharmaceutical industry: Elsevier; 2022. p. 47-74.

30. Epshtein N. System suitability requirements for liquid chromatography methods: Controlled parameters and their recommended values. *Pharmaceutical Chemistry Journal* 2020;54(5):518-25.

31. Abd E, Benson HA, Roberts MS, Grice JE. Minoxidil skin delivery from nanoemulsion formulations containing eucalyptol or oleic acid: Enhanced diffusivity and follicular targeting. *Pharmaceutics* 2018;10(1):19.

32. Bolmatenkov DN, Nizamov II, Sokolov AA, Notfullin AA, Solomonov BN, Yagofarov MI. Polymorphism and phase-transition thermodynamic properties of phenazone (antipyrine). *Molecules* 2025;30(13):2814.

33. Swanepoel E, Liebenberg W, Devarakonda B, De Villiers M. Developing a discriminating dissolution test for three mebendazole polymorphs based on solubility differences. *Die Pharmazie-An International Journal of Pharmaceutical Sciences* 2003;58(2):117-21.

34. Makky AM, S. El-leithy E, Hussein DG, Khattab A. A full factorial design to optimize aminexil nano lipid formulation to improve skin permeation and efficacy against alopecia. *AAPS PharmSciTech* 2023;24(1):40. doi: https://doi.org/10.1208/s12249-023-02500-3

35. Kawaguchi R, Yu J, Ter-Stepanian M, Zhong M, Cheng G, Yuan Q, et al. Receptor-mediated cellular uptake mechanism that couples to intracellular storage. *ACS chemical biology* 2011;6(10):1041-51.