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# A Novel developed method for the estimation of Minoxidil in Pharmaceuticals Using a High-performance Liquid Chromatographic Process

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## Abstract

The purpose of this work is to create a precise and rapid RP-HPLC technique to measure Minoxidil in line with the International Conference on Harmonization (ICH) standards. The primary objective of the study is to: This study investigated the development and validation of an efficient, accurate, and quicker RP-HPLC technique for the detection of Minoxidil in compliance with the International Conference on Harmonization (ICH) recommendations. Methods: Specifically, it was Waters that was employed in the HPLC experiment. It was easier to isolate the medication utilising an Ion Pac zorbax 300-SCX Agilent Column with a length of 5 metres and a diameter of 4.6 millimetres, which was used in this study. The mobile phase was composed of Methanol, water, and acetonitrile in a volume ratio of (70:20:10) v/v with perchloric acid at pH 3 at a flow rate of 1 millilitre per minute, while the stationary phase was composed of acetonitrile and perchloric acid at pH 3. According to the data, the minoxidil had a preservation duration of 3.47 0.01 minutes after being applied to the skin. The R2 value was 0.9998 in the concentration range of 5-25 g/ml, indicating that the process was linear in the concentration range. It was found that the system's LOD and LOQ had concentrations of (1.980 and 5.980) g/ml, respectively. Precision of the method and system was predicted, and the findings were expressed as a percentage of the standard deviation (RSD), which were found to be well within the bounds of the projected precision. The fact that minoxidil recovery was in the region of 96-100 percent proved the accuracy of the technology used to analyse it. When performed in accordance with the International Conference on Harmonization (ICH) recommendations, the suggested RP-HPLC process was determined to be reliable. The following procedure can be used to do routine diagnostic analysis on a patient.

**Key Words:**

Chromatographic Method for Minoxidil, Exertion Degeneration, FT-IR for Minoxidil.

The suffering from high blood pressure or hair loss, minoxidil (2, 4-diamino-6-piperidinopyrimidine 3-oxide), also known as Rogaine, may be the answer. In the treatment of hypertension, it is an antihypertensive vasodilator. There are prescription and over-the-counter versions of this generic drug in tablet and topical liquid or foam form. One of the most common treatments for severe hypertension in patients who have not responded to at least two medications and a diuretic is the use of the medicine minoxidil. The use of

a loop diuretic with minoxidil to minimise salt and potassium retention is also common practise. In order to avoid the risk of reflex tachycardia, beta-blockers must be used with the medication. Minoxidil is officially known as 2,6-diamino-4-(piperidin-1-yl)pyrimidine 1-oxide [1-3] by the International Union of Pure and Applied Chemistry. For example, as shown in Figure 1

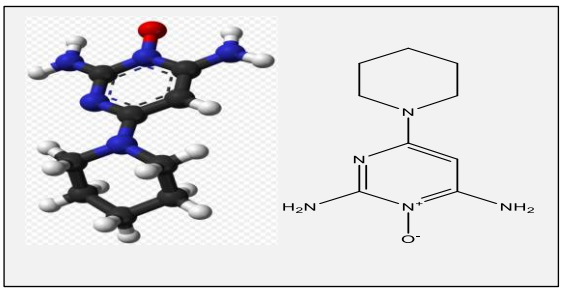



Figure 1: chemical stracture of Minoxidil

It's a white, crystalline powder with a slight odour, and it's used to treat hair loss. Alcohols and acidic solutions rapidly dissolve this tasteless and odourless material. It is not soluble in water, acetone, or alkaline solutions. Orally used as a peripheral vasodilator medication, it has helped people with resistant hypertension. However, it has been associated to hirsutism and fluids retention in high doses[4].

The treatment of androgenic alopecia with minoxidil has been discovered to be a unique use in dermatology (male pattern hair loss). By causing vasodilation and enhancing local irrigation and blood flow, the medicine has been applied topically in this case in order to encourage hair growth. ethanol and propylene glycol, or a combination of the two substances, are commercially available topical formulations that comprise 2 percent Minoxidil (20 mg mL-1) and a carrier of 2-nonyl1- 1,3 dioxolane. Although the United States Pharmacopeias has established a reference method for Minoxidil quantification using liquid chromatography, this method is not capable of giving appropriate resolution between Minoxidil and related compounds in a topical formulation, resulting in inconsistencies. Other methods for determining it in pharmaceutical formulations and human plasma have been proposed, including HPLC with UV detection and electrochemical detection, a solid-phase column with UV detection and electrochemical detection, differential pulse polarography, gas chromatography, radioimmunoassay, and electrolysis[5–6]. A key limitation of the field is that, as far as we know, no analytical method (including the reference method) exists that can consistently separate and quantify all of the known Minoxidil-related chemicals. A topical formulation of Minoxidil and all of its known related components cannot be efficiently separated and reliably quantified using a documented HPLC method[7,8], and no published HPLC methodology implies that Minoxidil is stable.

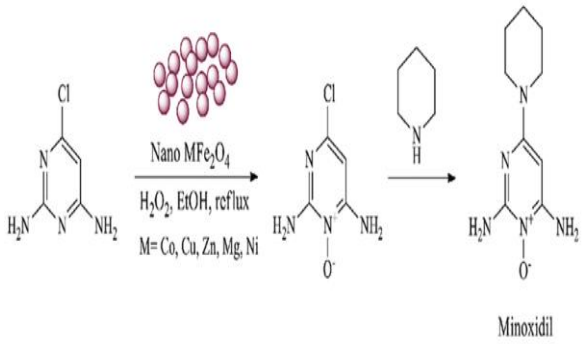
For the analysis of stability samples, regulatory bodies propose using stability indicating processes (SIMs). To develop the related pollutants under stressed settings, stress studies are needed, along with the creation and validation of strategies to do so. We provide here what we believe to be the first RP-HPLC approach in the world to add a stability indicator for the detection of Minoxidil and the quantification of all known Minoxidil-related chemicals. If you utilise the new method, you will be able to separate all of the molecules that are bonded together. The International Conference on Harmonization (ICH) (Validation of Analytical Procedures: Text and Methodology Q2) recommended that this method be verified.

Methods for determining the concentration of minoxidil have been described in this paper[11] utilizing high-performance liquid chromatography (HPLC). They came up with the idea and worked on developing the reversed-phase ion pairing technique.

## Synthesis of Minoxidil [12-14]

In recent years, the creation and characterisation of nano ferrites has allowed for the fulfillment of several scientific and technological criteria. This is especially true in the environmental and medicinal domains. Ferrites have been produced using a variety of techniques, including coprecipitation, hydrothermal, and solid-state. When it comes to nanoparticles with high crystallinity, the solid-state process is the most basic and cost-effective method of fabrication. The N-oxidation by $H_2O_2$ of 6-chloro-2,4-diaminopyrimidine in ethanol under reflux conditions was carried out with micro spinel ferrites acting as catalysts in the experiment. The goal of this research is to better understand nanomagnetic materials and their applications in functional group transformations. Ultimately, minoxidil was synthesized from piperidine,

2,6-diamino-4-chloropyrimidine N oxide, and a nucleophilic method, yielding a product with a high purity and yield (as shown in Scheme 1).).




Scheme 1. synthesis of Minoxidil

## Study's Purpose

Using an ultraviolet (UV) detector, the researchers set out to create and test a reverse phase high performance liquid chromatography approach for measuring Minoxidil in pharmaceuticals.

## Experimenting

### Tools

A high degree of computer processing power and ease of use distinguish the S-HPLC LC-100 series. Its extraordinary stability and dependability can be attributed to several different aspects, including the way electronic signals are organised and the internal electrical structure they're housed in. All three components are connected via fibre optic cable to an IBM compatible PC, which is then used to run the HPLC-UV system with a UV-100 quartz cell with a 1 cm beam wavelength. Two vacuum pumps and a UV indicator with variable frequency programmability, both compatible with Matlab R2003b and the PLS Toolbox, were included to complete the final product. Matlab UPVC and R2003b versions have also been provided for use in chemometric models. An integrating period had been established between summits. utilizing Angstrom Advanced Inc.'s LC solution programming package each compound in the sample was partitioned and the amount of each compound estimated utilizing an Ion Pac Zorbax 300-SCX Agilent Column with a high-pressure Ion Pac section. When the column was at normal temperature, the temperature had no effect on its stability. It was necessary to go through a millipore layer stream in the portable stage before inserting drug standard procedures and tablet test agreements into the HPLC framework.

### Reagents and Chemical

Industries Pharma Sigma-Aldrich has received approval for use in medical devices and medicines under the designation NO: 818FOOD37 and CAS NUMBER: 38304-91-5.

### Market Sample

In addition to Minoxidil®, Shambo®, this product contains 5 percent of Minoxidil® and Shambo® from OPALIA Pharma S.A., Z.I. Kalat El Andalous, 2022 Ariana Tunisie, batch number 047-Shambo.

### Set up the Samples for Measuring

#### Solution of HPLC grade (Sigma-Aldrich®).

In order to form a concentration of 5% of Minoxidil in Methanol, the stock content was calculated: solution of percloric acid and acetonitrile in the proportions 70/20/10 with a pH of 3.

We used percloric acid to synthesise Minoxidil (standard solution) to produce concentrations of 5, 10, 15, 20, and 25 g/ml in Methanol, water, and acetonitrile (in a 70:20:10) volume ratio.

### The Sample Modernization

PLS configuration set was streamlined to include Minoxidil-OPALIA Pharma S.A.® tablets in order to conduct sample modernisation. These tablets were recognised as being the same as the basic Minoxidil - 5gm/100ml tablets and were so included. It was recognised that 3 not known divergences in tests covering numerous subgroups of all were given as an agreed justification for finishing the fundamental alteration. When results from external authorization tests were included, the predictive ability of this revamped instance was examined. After this was completed, the attend test reinvigorating for each section was calculated using the generated tactic RP-HPLC with 3 concentrations of the additional free refreshing [17,18].

### Getting ready for the mobile process

Methanol and acetonitrile were mixed together in a 50:50 volumetric ratio to achieve this stage. Ultrasonic water bath was used to heat the liquid phase for 10 minutes before vacuum filtering through a 0.45m filter [19].

### Preparation of Eleuent

The diluting agent was also the mobile phase. pH 3 perchloric acid is combined with (70:20:10) v/v methanol, water, and acetonitrile.

### Preparation Minoxidil Standard Solution

In a 1000 ml volumetric flask, 200 mg of Minoxidil was diluted to the required volume using solvent (Mobile phase). 12.5ml of the aforementioned solution was used to dilute 100 ml of diluent, resulting in a concentration of 25 g/ml [19].

### Preparation of Minoxidil Sample Solution

A conical flask containing 5 gm/100 ml of Minoxidil shampoo was used for the experiment. An initial sample of 12.5 ml was changed to a 100 ml volumetric flask and diluted with dilution to fill the remaining capacity (by the eluent). The materials were broken down during the course of 30 minutes of incubation time. Using a solvent to dilute 12.5ml of this solvent to 25ml resulted in a final concentration of 25 g/ml, which was exceedingly low compared to other methods [20].

## Results

### Selection of Wavelength for Method Development

It was necessary to prepare a standard solution containing 1000 mg/ml of Minoxidil, and then perform concentration experiments to achieve a stock solution containing 100g/ml with a mixture of Methanol: water: acetonitrile in the ratio of (70:20:10) v/v with perchloric acid at pH3 and a standard solution containing 1000 mg/ml of Minoxidil. The wavelength was determined by inspecting the previously described standard medicine alternative between 190 and 300 nm.. According to the scanning results, the wavelength with the highest absorption was 254 nm. As a direct result, the wavelength of 254 nm was selected as the recognition wavelength for the RP-HPLC investigation (Figure 2).

### A New Approach is Being Created

When the United States Pharmacopeia (USP) was confident that all aspects of the chromatographic process were taken into account, the procedure was developed. Type of column and concentration of the solvent system were dependent variables for laboratory testing procedures. The stream rate was kept constant at 1.0 ml/s, and the temperature was kept constant at 20oC throughout the duration of each trial. For the purposes of process validation, the chromatographic conditions depicted in Table 1 and Figure 2 are identical to those described in the preceding paragraph.

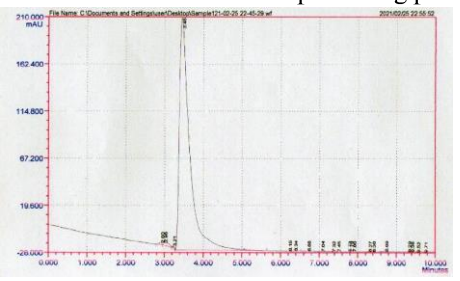



**Figure 2.** UV spectra of Minoxidil

**Table 1.** Experimental conditions of technique development

| Mobile phase | a mixture of Methanol: water: acetonitrile in the ratio of (70:20:10) v/v with perchloric acid at pH3 |
|---|---|
| Run time | 10 min |
| Column temperature | 20-25 °C |
| Detection wavelength | 254 nm |
| Flow rate | 1.0 ml/minute |
| Injection volume | 20 µL |

### Construction of Calibration Curve

Mobile phase (diluent) was used to create aliquots of various doses to produce concentrations of (5, 10, 15, 20, and 25) g/ml of a known absorption rate. In order to obtain the chromatograms, we used the most efficient experimental setups. It was possible to determine the concentrations of various chemicals using the chromatograms. Accordingly, a linear plot

was generated using an average standard solution for each time period.

## The Exertion Degeneration Studies [21-23]

Acidified and basic, oxidative, and heated attempts can be tested for degradation under conditions set by the International Conference on Harmonization (ICH). Table 2 displays the data.

Table 2: Results of forced degradation studies

| Type of degradation | Naproxen (60 µg/ml) | |
|---|---|---|
| | %Recovery | %Degradation |
| Undegraded | 100 | 1.110 |
| Acid | 95.412 | 0.953 |
| Base | 93.215 | 0.833 |
| Oxidative | 87.506 | 0.777 |
| Photolytic | 85.003 | 0.311 |
| Thermal | 75.710 | 0.606 |

## 1. Acid Degeneration

Five milligrammes of Minoxidil were dissolved in 100 millilitres of a liquid. It was placed to 70–80°C for 2–3 hours of reflux after 5 ml of 0.1 N HCl was added. Once the desired effect was achieved, the setup was destroyed with 0.1 N NaOH to finish the portable stage. Minoxidil's water can be electrolyzed and the outcome might be the creation of HCl. Hydrolysis, or the splitting of liquids, is one of these reactions. "Any acid or base stimulates amino hydrogenation." In [Figure 3].

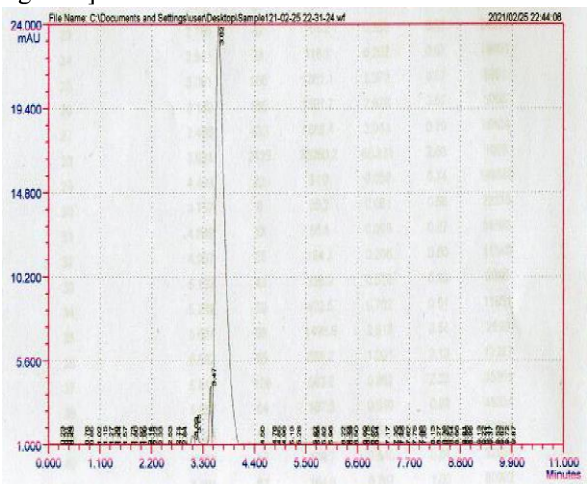



Figure 3. Acid degeneration

## 2. Base Degeneration

5 milligrammes of Minoxidil were combined with 100 millilitres of liquid, with the addition of foundations such as sodium hydroxide to regulate amine salts in the mixture. An amount equal to 2 cc of 0.1 N hydrochloric acid, or 2 to 3 litres of acid, was put into the container, which was then subjected to vigorous reflux for 2 to 3 hours. After the salt had been dissolved, 0.1 N hydrochloric acid was bled into the solution, and the solution was finished with phase water [Figure 4].

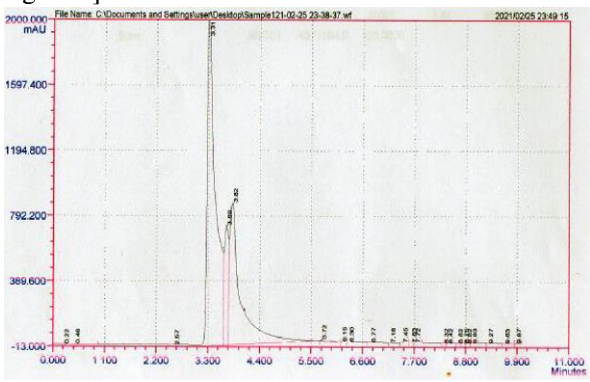



Figure 4. Base degeneration

## 3. Oxidative Degeneration

Five grammes of minoxidil and five millilitres of the 20 percent H2O2 formulation were added to the powder in a 100 millilitre flask. For two to three hours, the container was kept at a temperature close to that of reflux. Figure 5 shows that after applying pressure, the container had been properly compressed and was ready for transportation.

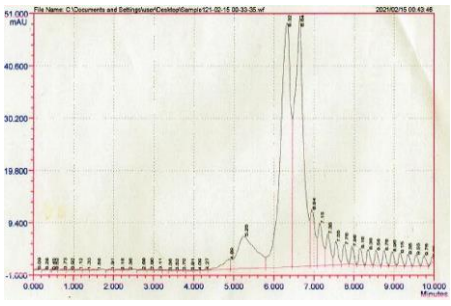



Figure 5. Oxidative degeneration

## 4. Photolytic Degeneration

In order to observe photo-degradation in this experiment, 5 grams of minoxidil was positioned in a Petri dish and was under the sun for 2-3 hours. A ml volumetric flask was used to dilute and swirl the tablet

once the pressure cooker had finished its cooking cycle. The solvent's frequency band is in the infrared region of the electromagnetic spectrum when scanning is taking place. Minoxidil can interfere with pharmaceutical preservatives to a certain amount when broken down in this way, as shown en in Figure. 6.

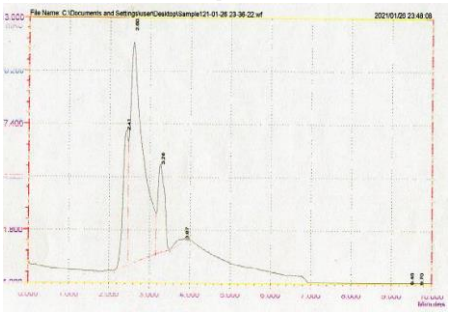


Figure 6. Photolytic degeneration

### 5. Thermal Degeneration

A glass plate with minoxidil zeolite was placed in a hot oven for 2–3 hours at 105oC and allowed to dry. The particles were placed in a graduated cylinder filled with 100 mL of water and deposited in the sink. When the temperature of the solvent rises over 100 C, it indicates that Minoxidil has been substantially completed, as illustrated in Figure 7. Thermal disintegration of the molecule is also demonstrated in Figure 7.

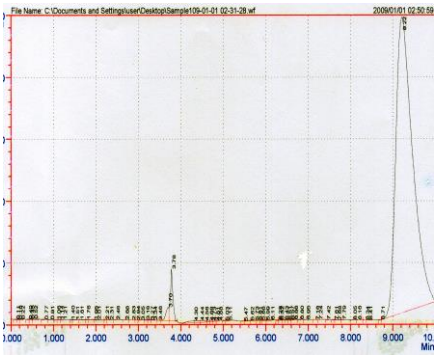


Figure 7. Thermal degeneration

## Infrared Spectrophotometer of Minoxidil []

The FT-IR spectra was captured on a KBr disc at the University of Basrah's College of Science using the FT-IR-84005582-SHIMZU. The data was then transferred to a computer.

### Standard Minoxidil

Figure 8 shows the FT-IR spectra of conventional Minoxidil, which has significant lines at 1668 and 1617 $cm^{-1}$, which are attributable to the carbonyl group. In addition, as illustrated in figure 8, a strong band at 1487 $cm^{-1}$ might be attributed to the stretching of aromatic C=C by vibration stretching.

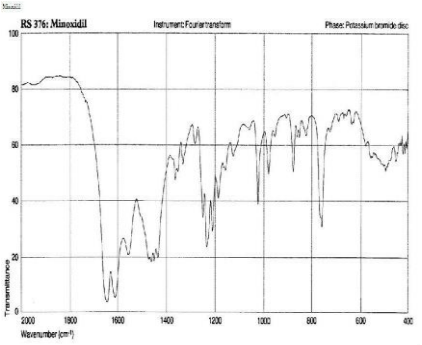



Figure 8: FT-IR for Standard of minoxidil

### Sample of Minoxidil

The FRIR spectra of the material was analysed. A few key peaks can be seen in the infrared spectrum of typical Minoxidil, which is depicted in Figure 9.

For example, the carbonyl group is responsible for a conspicuous band at 1620 $cm^{-1}$. Aromatic C=C vibration stretching is responsible for the existence of two strong bands at 1481 and 1450 $cm^{-1}$. C-N bond was assigned a medium-sized band at 1224 $cm^{-1}$, while an aromatic bending of the C-H bond was assigned a multiple-band spectrum spanning 979-763 $cm^{-1}$.

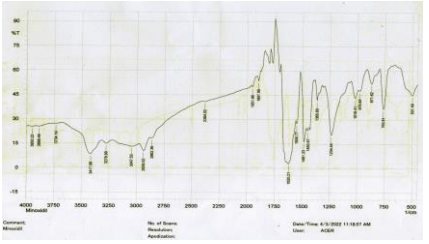


Figure 9: FT-IR spectrum of Minoxidil –Sample

## Discussion

### Validation

According to the ICU standards for the limit of quantification, the technique has been found to be effective for high specificity, scheme, predictability, accuracy, and finesse among an ICH [24].

### Specificity

Raising a defined combination of appropriate pollutants to a specific concentration demonstrated the mixture's integrity. It was determined that the precision of the measurements of minoxidil at a concentration of 25 g/mL was within the acceptable range.

## Development of HPLC method

When using chromatographic separation, the mobile phase includes a concentration of 15 g/mL of Minoxidil with a retention time of 3.45 0.01 minutes, with a range of 0% to 30%. The median detection limit of Minoxidil in the mobile phase is 2.5 0.1 in equation 3. This shows that the established HPLC process was designed with extreme precision.

## System Suitability

Prior to completing an uncertainty analysis, ensuring that a system is compliant is described as ensuring that the system is operationally acceptable before performing an uncertainty analysis. The Minoxidil dosage is 100 mg/ml [25] for six regurgitates, and the duration is six weeks. Minimum and maximum absorption and refractive indices must be within 2% of the mean plus or minus 2% of the average peak absolute standard deviation (means).

During the concept analysis process, a buildup of Minoxidil at a concentration of 5 grammes per 100 millilitres revealed that counsel and assistance had been supplied. In the audit experience, it was discovered that the peak height area and resident time had a 2 percent RSD mistake, as indicated in Table 3 for the peak height area and residence time.

**Table 3:** Evaluation of the appropriateness of the minoxidil system

| Parameters | Value of Minoxidil | Recommended limits |
|---|---|---|
| USP plate count | 2544 | ⩾ 2000-2500 |
| USP tailing factor | 2.84 | ⩾ 1.0-2.0 |
| Peak area | 65418.9 (%RSD 0.201) | RSD ⩽ 1 |
| Retention time | 3.4±0.221 (%RSD 0.130) | RSD≈ 0.400 |
| Resolution | ~ 4.5 min | ⩾ 5 min |

## Linearity

Spectra of stock solutions with various concentrations (5 to 25 g/mL) were observed. By averaging the estimated values, the linearity plot was produced using the documented chromatograms to identify the mean quantities of chromatographic peaks. To put it another way, the correlation coefficient was found to be 0.9998. Tables 3 and 4 and Fig. 10 show that minoxidil is linear, as demonstrated in Fig. 10.

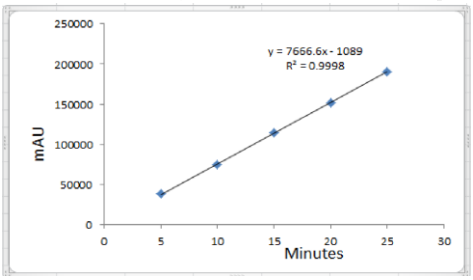



**Figure 10:** Calibration plot of Minoxidil

## Limit of Detection and Limit of Quantification

The LOD level may be determined under the alleged laboratory circumstances, but this cannot be guaranteed in the samples. Lowest possible precision and accuracy can only be achieved by using the lowest feasible level of precision (LOQ). According to the following formulas [26], LOD and LLOQ were determined as follows: Table 4 shows the method's great precision with LLOD=3.3SD/S and LLOQ=10SD/S.

**Table 4:** Data about Minoxidil's linearity regression can be found in Table 4.

| R2 | 0.9998 |
|---|---|
| Standard error | ~0.01 |
| Standard error estimate | ~0.01 |
| Intercept | ~1089 |
| Slope | 7666.6 |
| LLOD (µg/ml) | 1.98 |
| LLOQ (µg/ml) | 5.98 |

## Accuracy

The accuracy of a forecast is determined by how close the projected return is to the actual value. Recovery of analyte can be estimated and represented by the symbol R. Three consecutive analyses (n=3) of the Minoxidil solution (5, 10, and 15 g/ml) were performed on the Minoxidil solution in order to test the calibration curve that had been produced for each of the proposed methods. In order to determine whether or not the proposed technique was accurate, the results

from the experiment were analyzed utilizing the formula [percent Recovery = (Recovered concentration /Injected concentration) multiplied by 100]. In accordance with Table 5, departures from this aim of more than one-tenth of a percentage point are not regarded acceptable. The results of the accuracy tests are summarised in Table 5.

Table 5: the accuracy tests

| Claimed conc. (µg ml–1) | Found conc. (µg ml–1) | Recovery±RSD |
|---|---|---|
| 5 | 4.9 | 98±0.101 |
| 10 | 9.8 | 98±0. 102 |
| 15 | 15 | 100±0.104 |
| 15 µg $ml^{-1}$ for drugs (Minoxidel Minoxidil – OPALIA Pharma S.A., Z.I. Kalaat El Andalous 2022 Ariana Tunisie,-5000mg) | 14.8 | 98±0.113 |

## Precision

The proposed model was used to do six measurements at a Minoxidil concentration of 15 g/100 mL, and the RSD % was calculated from the measurements' results. The RSD accuracy was deemed inadequate by the United States Pharmacopeial Convention [28] since it was less than 2%. The results of precision studies are shown in Table 6.

Table 6: the Precision tests

| Claimed conc. (µg ml-1) | Intraday | | Interday | |
|---|---|---|---|---|
| | Found (µg ml-1) | Recovery±RSD % | Found (µg/ml) | Recovery±RSD % |
| 5 | 4.9 | 98±0.101 | 4.8 | 98±0.111 |
| 10 | 9.8 | 98±0.102 | 9.5 | 96±0.103 |
| 15 | 15 | 100±0.104 | 15 | 100±0.103 |
| 20 | 19.8 | 99±0.100 | 19.5 | 98±0.109 |
| 25 | 25 | 100±0.140 | 25 | 100±0.139 |
| 15 µg $ml^{-1}$ for drugs (Minoxidel – OPALIA Pharma S.A., Z.I. Kalaat El Andalous 2022 Ariana Tunisie,-5000mg) | 14.8 | 98±0.106 | 14.8 | 100±0.108 |

The means and standard deviations, or calculus, of the individual investigations are displayed in Tables 6 and 7. It was discovered that accuracy was less than two percent.

## Robustness

Using modest variations in fluid velocity and buffer/solution concentration, the calibration accuracy was verified and can be shown in Table 9. Measurement of an observational procedure's ability and self-resiliency when applied to a normal operation is measured in terms of how easy it is to use. The flow velocity and molarity of the initial solution were found to have changed by less than 2%.

Analytical conditions must be carefully monitored and included into the approach as necessary to ensure the method's robustness. As demonstrated in tables 3 and 7, the injection amounting resilience was examined by making a single, noticeable alteration to the concentration of natural buffer and organic eluent velocity (about 10.2 ml/s) [29,30].

Table 7: Minoxidil analytical system and analyst

| Parameter | Minoxidil | | |
|---|---|---|---|
| | Discovered | Recovery | RSD |
| System | 15 µg/ml | 100 % | 0.110 % |
| Analyst | 15 µg/ml | 100 % | 0.111 % |

# The Applications of Method

For the scientific technique of Minoxidil-OPALIA Pharma S.A., Z.I. Kalaat El Andalous 2022 Ariana Tunisie, BATCH: 047 -Shamboo, we used commercial shampoo that contains 5% of Minoxidil Shamboo®.

In terms of Minoxidil concentration, the standard version was determined to be 100 percent 2.0 percent, while the limited spss version was found to be 99 percent 1%. This confirms the accuracy and precision of the formulas for Minoxidil results. Table 8 summarised the findings.

Table 8: Minoxidil tablet assay results

| Analyte | Labeled claim (mg) | Found (mg) | Mean (mg) | %Recovery | %RSD |
|---|---|---|---|---|---|
| Standard Naproxen | 15 | 15 | 15 | 100 | ±0.104 |
| Minoxidel Minoxidil – OPALIA Pharma S.A., Z.I. Kalaat El Andalous 2022 Ariana Tunisie,-5000mg | 15 | 14.8 | 14.9 | 99.8 | ±0.106 |

## Conclusion

The verification criteria for the enhanced RP-HPLC technique were extraordinarily stringent. The estimated SDS has a margin of error of less than 2 percent, according to the data. T is the abbreviation for Minoxidil has an elimination half-life of 3.47 minutes, according to theoretical calculations. The precision of the mean extraction technique for Minoxidil was found to be 1.980 percent, with a maximum of 5.980 percent. This is evidenced by the low LOD and LOQ values, which demonstrate the method's ability to differentiate between signals and noise. Assuming that the well-established RP-HPLC technology can achieve these requirements, it could be employed for routine testing in the future.

## Competition for Disclaimers of Interest

Authors said they had no conflict of interest in the project. That's hardly a reason to file a lawsuit against a firm, after all. Overall, the study's purpose is to improve the way the general population responds to medication In addition, the research study's costs were paid for entirely by the authors.

## Refrenses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