

Development of a UV-Vis method for minoxidil quantification in compounded capsules

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Abstract. Oral minoxidil has been used as a therapeutic alternative for the treatment of alopecia, particularly androgenetic alopecia. As the drug is administered at low doses, small variations in the active pharmaceutical ingredient content may alter drug exposure and affect its safety and efficacy, making quality control of compounded formulations relevant. This study aimed to develop and evaluate a UV-Vis spectrophotometric method for minoxidil quantification and to apply it to the determination of minoxidil content in compounded capsules obtained from pharmacies in the state of Pernambuco, Brazil. The method showed linearity, selectivity, precision, accuracy, and robustness compatible with the criteria considered in Brazilian Health Regulatory Agency Resolution RDC No. 166/2017. Formulations containing 0.5, 1.0, 1.5, 2.0, and 2.5 mg of minoxidil were evaluated. All formulations met the average weight determination criteria established by the Brazilian Pharmacopoeia. However, assay results showed substantial deviations from the declared content, ranging from 75.48% to 230.44%, with only one formulation meeting the acceptance range of 90% to 110%. The results indicate that mass uniformity alone does not allow assessment of active pharmaceutical ingredient content compliance and reinforce the importance of analytical methods and quality control in the evaluation of compounded medicines.

Keywords: Alopecia; UV-Vis spectrophotometry; Analytical validation; Quality control; Compounding pharmacy.

Introduction

Minoxidil was initially used in the treatment of arterial hypertension, being administered orally due to its peripheral vasodilatory action. During its clinical use, increased hair growth was observed as an adverse effect, a characteristic that subsequently led to its therapeutic application in the treatment of alopecias (Kearney et al., 2025).

In recent years, the use of low-dose oral minoxidil for the treatment of androgenetic alopecia has aroused increasing clinical interest, particularly in patients who exhibit an inadequate response or limitations to the use of the topical formulation. Its effect is related to the opening of ATP-dependent potassium channels, increased perifollicular blood flow, and modulation of the hair cycle, favoring the anagen phase and hair growth (Majewski et al., 2024).

The use of oral minoxidil for alopecia requires attention to the administered dose, since variations in the amount of the drug may alter patient exposure to the active ingredient. The drug exhibits a dose-related response, with the use of reduced

doses being recommended to minimize the occurrence of adverse events, especially those associated with the cardiovascular effects of the drug (Gupta et al., 2024).

The pharmacological effect of minoxidil depends on its conversion to the active metabolite by sulfotransferase enzymes, present mainly in the liver, in addition to tissues related to the hair follicle, this activation being fundamental for its therapeutic activity (Nascimento, 2021). Thus, achieving the appropriate dose of the drug contributes to adequate drug exposure during treatment. In this context, compounding pharmacies play an important role in providing individualized formulations; however, they require rigorous quality control processes to ensure that manipulated medications present adequate concentration of the active ingredient in the finished product and characteristics compatible with their therapeutic purpose (Tischner, Silva & Colacite, 2024). Although established requirements exist for the quality control of compounded medications, differences related to raw materials, weighing procedures, homogenization, and encapsulation

processes may influence the uniformity and final content of the formulations. Studies involving the evaluation of compounded products have demonstrated the importance of finished product analysis to verify its compliance and reliability (Poloniini, 2011; Guimarães, 2014).

Therefore, the quantification of minoxidil in compounded capsules becomes essential to assess the correspondence between the declared concentration and the content effectively present in the formulation. UV-Vis spectrophotometric methods present advantages related to operational simplicity, rapidity, and low cost, potentially representing a viable alternative for quality control analyses in routine laboratories. Thus, the present study aimed to develop and evaluate an analytical methodology by UV-Vis spectrophotometry for the quantification of minoxidil in compounded capsules obtained from different compounding pharmacies in the state of Pernambuco. Additionally, the study sought to determine the actual drug concentration in the formulations, compare the found values with the declared contents, and evaluate the obtained results against applicable quality criteria.

Material and methods

Compounded minoxidil capsules from different compounding pharmacy networks in the state of Pernambuco were evaluated. The establishments were not identified, being designated as Pharmacies A, B, C, D, and E.

Formulations from five establishments were evaluated, with a single batch of each formulation being assessed. Formulations with different declared concentrations of minoxidil were included.

Methanol (MeOH), used as solvent in the analytical procedures, was of analytical grade. As a reference substance, minoxidil base (secondary standard) was used, supplied by the company Iberoquímica Magistral, with a declared content of 99.7% on a dry basis, manufacturer's lot 01601032301001 (Iberoquímica lot 23030128).

Development of the analytical methodology

The quantitative determination of minoxidil content in the compounded capsules was performed by UV-Vis spectrophotometry, and analytical performance parameters of the method were evaluated, including selectivity, linearity, precision, accuracy, and robustness, according to the criteria established by Resolution RDC No. 166/2017 of the Brazilian National Health Surveillance Agency (ANVISA).

The evaluation of these parameters had a preliminary character, aiming to verify the applicability of the spectrophotometric method under the established experimental conditions, not characterizing a complete analytical validation. The analyses were performed on a UV Mini 1240 UV-Vis spectrophotometer (Shimadzu), using quartz cuvettes with a 1 cm optical path.

Initially, a minoxidil stock solution was prepared at a concentration of 1 mg/mL by

dissolving 10 mg of the standard in 10 mL of methanol. From this solution, working solutions at different concentrations were prepared by successive dilution in ultrapure water. The solutions were analyzed in triplicate by UV-Vis spectrophotometry at a wavelength of 288 nm. The analytical curve was constructed by relating the mean absorbance values obtained with the respective minoxidil concentrations, using linear regression by the least squares method.

Determination of the average capsule weight

The determination of the average capsule weight was performed according to the methodology described in the Brazilian Pharmacopoeia, 7th edition (Brasil, 2024).

Four capsules of each formulation were evaluated. Initially, the capsules were individually weighed containing the encapsulated material. Subsequently, they were emptied and weighed again. The content weight was obtained by the difference between the mass of the filled capsule and the mass of the empty capsule, and the average content weight was subsequently calculated. Due to the limited availability of commercial samples, a reduced number of units was used in relation to that recommended for complete pharmacopoeial assays. The evaluation was conducted as an exploratory analysis of the mass uniformity of the available formulations.

Preparation of sample solutions

The preparation of the sample solutions was adapted from the minoxidil monograph described in the United States Pharmacopeia (USP) and from the studies by Torrado-Salmeron et al. (2022) and Guareschi et al. (2024). After determining the average content weight of the capsules, the contents were pooled and homogenized. A mass of content corresponding to the average weight of the formulation was transferred to a 10 mL volumetric flask, to which 8 mL of methanol were added. The solution was subjected to an ultrasonic bath for 10 minutes to favor complete solubilization of the analyte. Subsequently, the volume was made up with methanol, followed by homogenization and filtration using a hydrophilic PTFE membrane filter with 0.45 µm porosity (Agilent). An aliquot of approximately 1 mL of the obtained solution was diluted in 10 mL of ultrapure water for subsequent spectrophotometric analysis.

Determination of minoxidil content

The minoxidil content in the capsules was determined from the concentration obtained by the analytical curve, considering the average content weight of the capsules. The content calculation was performed considering the experimental concentration found, dilution factor, and declared amount of minoxidil in the formulation. The results were expressed as % of the declared content.

Statistical analysis

The obtained data were organized and analyzed using GraphPad Prism software, version 8.0.1 (244) (GraphPad Software, San Diego, CA, USA). Linear regression analyses and statistical procedures were performed for the evaluation of analytical parameters, considering a significance level of 5% ($p < 0.05$).

Results and discussion

Development of the analytical methodology

The linearity of the spectrophotometric method for the determination of minoxidil base was evaluated by constructing an analytical curve using five concentration levels (5–25 $\mu\text{g/mL}$), analyzed in triplicate. The mean absorbances obtained were used to construct the calibration curve (Figure 1).

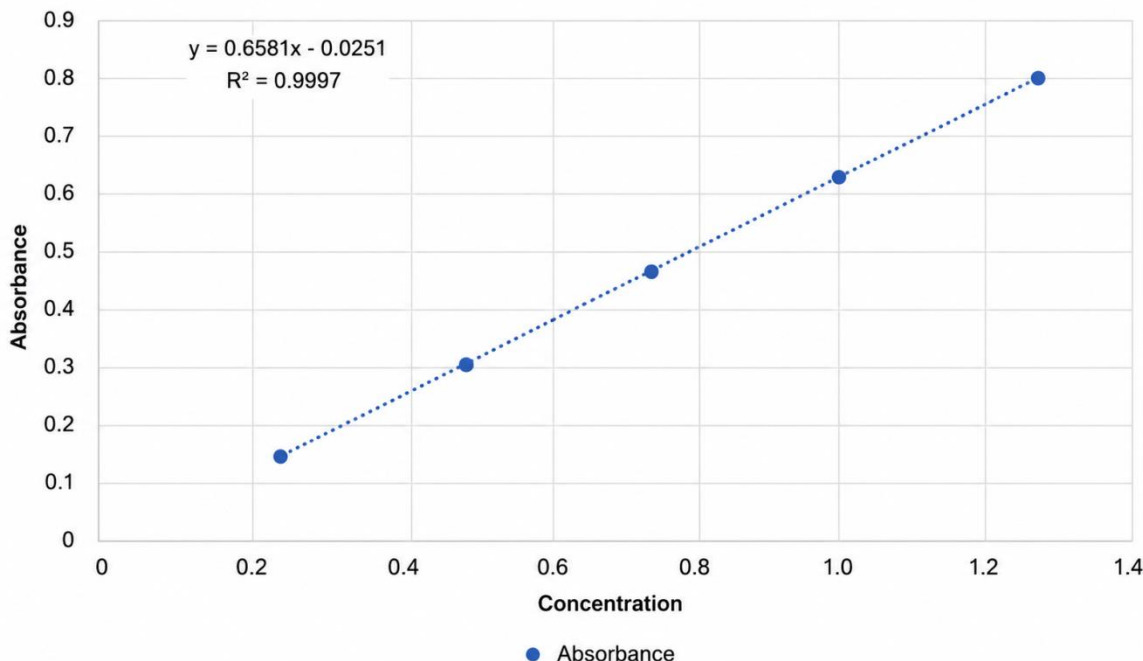


Figure 1. Analytical curve obtained for the spectrophotometric determination of minoxidil base by UV-Vis..

The linear regression model presented a coefficient of determination (R^2) of 0.9997 and a correlation coefficient (r) of approximately 0.994, demonstrating a high correlation between analyte concentration and the spectrophotometric response. These results meet the criteria established by ANVISA RDC No. 166/2017 and the ICH Q2(R2) guideline, which recommend correlation values above 0.99 for linearity assessment.

Analysis of variance (ANOVA) for the regression demonstrated statistical significance of the model ($F = 1020$; $p < 0.0001$), indicating that the slope of the line differed significantly from zero. The obtained intercept (-0.0246) presented a 95% confidence interval between -0.0615 and 0.0123, including zero, indicating no statistically significant difference of the intercept from the origin point.

The lack-of-fit test yielded $p = 0.9736$, demonstrating no evidence of inadequacy of the linear model to the experimental data. Furthermore, the low residual standard deviation ($Sy.x = 0.0282$) indicated reduced dispersion of the experimental values relative to the regression line.

Homogeneity of variances was assessed by Cochran's test, yielding an experimental value of $C = 0.4510$, which is lower than the critical value ($C_{crit} = 0.684$). Thus, no significant heterogeneity of variances was observed, allowing the use of unweighted linear regression.

Graphical analysis of residuals demonstrated random distribution around the reference line, with no systematic trend across the evaluated concentration range. Normality of residuals was confirmed by the Shapiro-Wilk test ($W = 0.961$; $p > 0.05$), indicating adequacy of the statistical assumptions applied to the model.

Collectively, the results demonstrate that the method exhibited satisfactory linear behavior in the evaluated range, being suitable for the quantitative determination of minoxidil base under the established experimental conditions. This result demonstrates adequate proportionality between concentration and analytical response throughout the studied range.

Linearity is a fundamental parameter in quantitative UV-Vis spectrophotometric methods, since concentration determination depends on the proportionality between absorbance and concentration described by the Beer-Lambert Law. Thus, the obtained results indicate that the method exhibits performance compatible with the purpose of minoxidil quantification under the evaluated conditions (Brasil, 2024; Torrecilla et al., 2021).

Furthermore, UV-Vis spectrophotometry presents advantages related to operational simplicity, rapidity, low cost, and good sensitivity for quantitative analyses, characteristics that favor its application in routine laboratories when associated

with appropriate analytical validation (Brasil, 2017; Santos et al., 2023).

Selectivity assessment

The selectivity of the method was investigated by means of the standard addition assay, with evaluation of the matrix effect present in

the compounded formulations. The results demonstrated a significant difference between the slopes of the curves obtained for the standard and for the sample (t-test; $t_{\text{calc}} = 34.67$), indicating matrix influence on the analytical response (Figure 2).

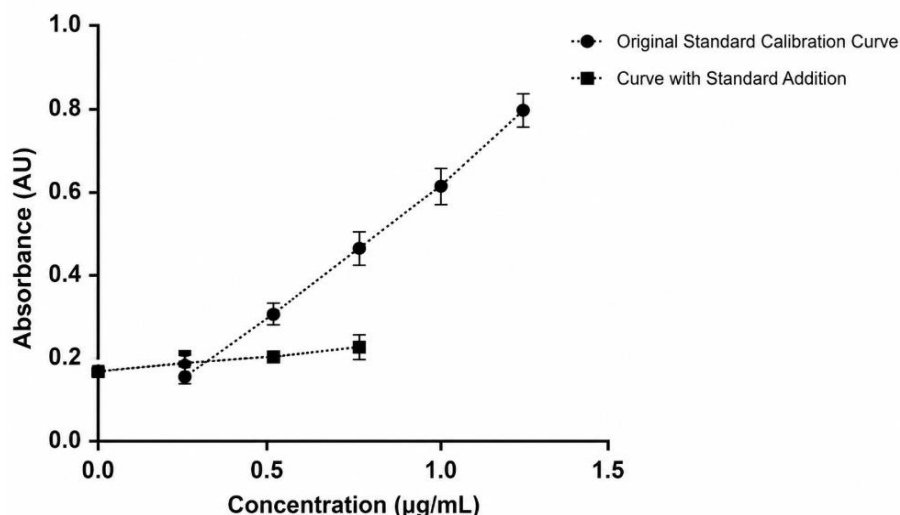


Figure 2. Assessment of matrix effect by comparison of analytical curves obtained for minoxidil standard and sample containing pharmaceutical matrix.

The presence of this effect may be related to the variable composition of excipients used by compounding pharmacies, whose individual composition was not available for the preparation of a specific matrix blank.

Despite the observed influence, the use of the standard addition strategy allows this effect to be minimized, since calibration occurs within the sample matrix itself. Thus, the method demonstrated applicability for the quantification of minoxidil in the evaluated compounded formulations, considering the compensation of matrix influence through this analytical approach.

The observed interference is compatible with the nature of compounded formulations, in which differences in the qualitative and quantitative composition of excipients may modify the spectrophotometric response of the analyte. In such cases, the use of standard addition constitutes an adequate alternative to minimize matrix effects during quantification.

Precision

The precision of the method was evaluated by means of repeatability assays, considering three concentration levels corresponding to 80%, 100%, and 120% of the working concentration. The obtained results are presented in Table 1.

The RSD values obtained ranged from 0.21% to 2.50%, indicating low dispersion among the determinations performed. For the low level (80%), a mean concentration of 1.1145 µg/mL was

obtained, with an RSD of 0.21%. At the intermediate level (100%), a mean concentration of 1.3064 µg/mL and an RSD of 2.50% were observed, representing the highest variability among the evaluated levels. At the high level (120%), the mean concentration was 1.6195 µg/mL, with an RSD of 0.57%.

Overall, the results demonstrate that the method presented adequate repeatability under the evaluated conditions, with low variation among analytical replicates. These results indicate that the spectrophotometric procedure presents consistent behavior for the quantitative determination of minoxidil.

Accuracy

The accuracy of the method was evaluated by means of recovery assays using three concentration levels (80%, 100%, and 120% of the working concentration), as presented in Table 2.

The mean recovery values obtained were 102.34%, 108.21%, and 111.96% for the low, medium, and high levels, respectively.

The 80% level presented a mean recovery of 102.34%, with low dispersion among the determinations. For the 100% level, a mean recovery of 108.21% was observed, while the 120% level presented recovery above the range traditionally described for quantitative methods (90–110%).

Table 1. Evaluation of the precision of the analytical method for the determination of minoxidil base in pharmaceutical formulations, at the 80%, 100%, and 120% levels.

Level	Flask identification	Measured absorbance	Calculated concentration ($\mu\text{g/mL}$)
Low (80%)	B1	0.710	1.1170
	B2	0.708	1.1140
	B3	0.707	1.1124
Medium (100%)	M1	0.856	1.3389
	M2	0.813	1.2735
	M3	0.835	1.3069
High (120%)	A1	1.035	1.6108
	A2	1.040	1.6184
	A3	1.047	1.6291

Table 2. Evaluation of the accuracy of the analytical method for the determination of minoxidil base at the 80%, 100%, and 120% levels.

Level	Flask	Absorbance (Y)	Theoretical concentration ($\mu\text{g/mL}$)	Corrected experimental concentration ($\mu\text{g/mL}$)	Recovery (%)
Low (80%)	B1	0.368	6.0	5.973	99.55
	B2	0.391	6.0	6.323	105.38
	B3	0.378	6.0	6.123	102.09
Medium (100%)	M1	0.539	7.5	8.572	114.29
	M2	0.493	7.5	7.873	104.97
	M3	0.495	7.5	7.903	105.37
High (120%)	A1	0.634	9.0	10.015	111.28
	A2	0.614	9.0	9.711	107.90
	A3	0.666	9.0	10.501	116.68

The greater variation observed at the high level may be related to the influence of the pharmaceutical matrix and to the inherent limitations of the spectrophotometric method employed,

especially considering that the evaluated formulations presented variable excipient composition.

Despite this variation, the results demonstrated proximity between experimental and theoretical values, indicating the method's capability to quantitatively estimate minoxidil under the evaluated conditions. However, the data should be interpreted considering the matrix influence observed in the selectivity assay.

In methods intended for quality control, accuracy constitutes one of the main parameters to ensure that the determined concentration adequately represents the actual drug content. Thus, the obtained results demonstrate the method's potential application for the quantitative

determination of minoxidil, although matrix influence should be considered during result interpretation (Brasil, 2017).

Robustness

The robustness of the method was evaluated by analyzing the influence of small experimental variations, including changes in the reading wavelength (287, 288, and 289 nm) and the stability of the analytical solution at different times after preparation (0, 2, 4, and 24 hours).

The results demonstrated low variation in the analytical response, with RSD values ranging from 0.00% to 1.86%, indicating maintenance of measurement precision under the evaluated alterations (Figure 3).

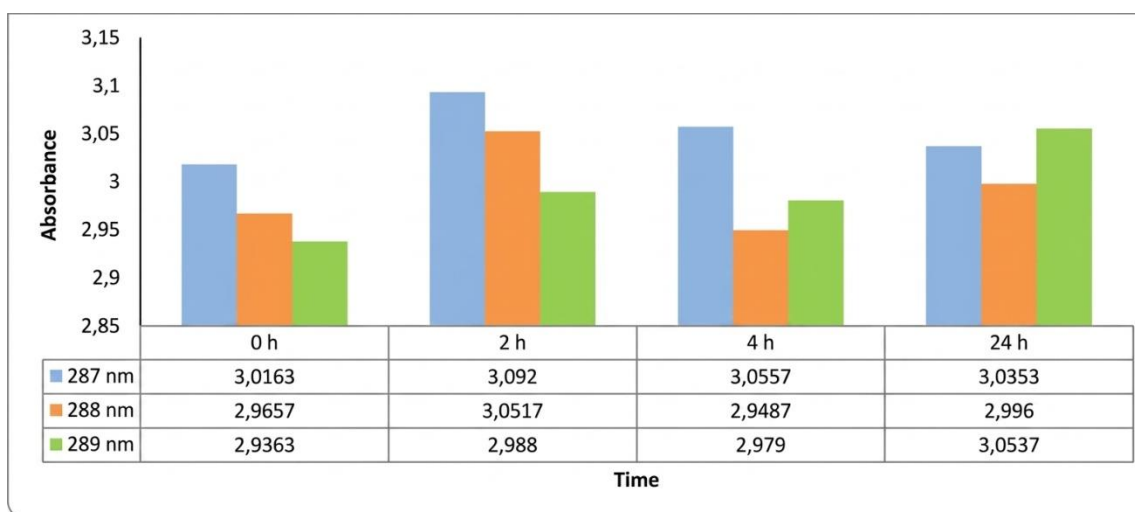


Figure 3. Influence of wavelength variation and reading time on the spectrophotometric response of minoxidil.

Wavelength variation did not promote relevant changes in the obtained absorbance, demonstrating that small modifications in the instrumental parameter did not compromise the analytical response. Likewise, the stability of the solution over 24 hours presented low variation, indicating that the solution remained suitable for analysis within the evaluated period.

These results suggest that the method presents satisfactory robustness against the tested experimental conditions, favoring its application in the quantitative determination of minoxidil in compounded formulations.

The observed stability against small experimental alterations reinforces the applicability of the method in routine analyses, an aspect particularly relevant for spectrophotometric methods intended for quality control, in which simplicity and reproducibility represent important advantages of the technique (Brasil, 2017; Santos et al., 2023).

Determination of average weight

The average weight determination assay was performed to evaluate the mass uniformity of minoxidil capsules from different compounding

pharmacies. The formulations were identified as Pharmacies A, B, C, D, and E, preserving the confidentiality of the establishments.

Due to the limited availability of commercial samples, four capsules of each formulation were evaluated, and the individual unit masses, average content weight, and standard deviation were determined. Although the number of analyzed units is lower than that recommended for complete pharmacopoeial assays, the evaluation allowed an exploratory analysis of the filling uniformity of the available formulations. The obtained results are presented in Table 3.

According to the Brazilian Pharmacopoeia (Brasil, 2024), capsules with an average weight below 300 mg must present a maximum individual variation of $\pm 10\%$, whereas those with a mass above 300 mg must present a variation of up to $\pm 7.5\%$.

All evaluated formulations presented individual values within the established limits, indicating compliance regarding mass uniformity. The lowest coefficient of variation was observed for the 2.5 mg formulation from Pharmacy E (0.82%), while the greatest variability occurred in the 2.0 mg

formulation from Pharmacy B (4.49%), although still within the criteria considered acceptable.

The low dispersion observed among the analyzed units suggests adequate uniformity in the capsule filling process, indicating consistency among the units produced by the different pharmacies evaluated. However, it should be noted that mass uniformity alone does not allow conclusions regarding content uniformity or minoxidil content, requiring quantitative evaluation of the drug to confirm the quality of the formulations.

This result reinforces the importance of performing complementary content assays, since capsules with adequate mass uniformity may present differences in the concentration and distribution of the active ingredient resulting from failures during weighing, homogenization, or encapsulation steps, particularly in compounded preparations produced with different raw materials and excipients. Thus, the association between average weight determination and content assays provides a more comprehensive assessment of the

final product quality, allowing verification of both filling uniformity and drug content compliance (Poloniini, 2011; Guimarães, 2014). Assay of minoxidil in compounded capsules obtained from different pharmacies in Pernambuco. Minoxidil content was determined in compounded capsules obtained from different compounding pharmacies in the state of Pernambuco, using UV-Vis spectrophotometry and an analytical curve previously established for drug quantification.

The analytical curve presented the regression equation: $y = 0.6581x - 0.0251$, in which y corresponds to absorbance and x to the concentration of minoxidil base ($\mu\text{g/mL}$).

The results allowed the evaluation of the correspondence between the experimentally found content and the declared concentration in the commercial formulations, enabling the identification of possible potency deviations in the different samples evaluated. The values obtained for all formulations are presented in Table 4.

Table 3. Determination of the average weight of compounded minoxidil capsules.

Formulation	Declared dose	Average content weight (mg)	Standard deviation (mg)	RSD (%)	Result
Pharmacy A	0.5 mg	156.9	5.1	3.25	Compliant
Pharmacy B	2.0 mg	403.2	18.1	4.49	Compliant
Pharmacy C	0.5 mg	188.1	5.6	2.98	Compliant
Pharmacy D	1.0 mg	148.3	2.9	1.96	Compliant
Pharmacy E	1.0 mg	108.3	3.2	2.95	Compliant
Pharmacy E	1.5 mg	186.3	4.3	2.31	Compliant
Pharmacy E	2.5 mg	242.7	2.0	0.82	Compliant

Table 4. Assay of minoxidil in compounded capsules obtained from different pharmacies in Pernambuco.

Formulation	Declared dose	Found content (%)	Evaluation
Pharmacy A	0.5 mg	84.21	Below specification
Pharmacy B	2.0 mg	230.44	Above specification
Pharmacy C	0.5 mg	110.25	Above upper limit
Pharmacy D	1.0 mg	155.72	Above specification
Pharmacy E	1.0 mg	100.61	Compliant
Pharmacy E	1.5 mg	81.76	Below specification
Pharmacy E	2.5 mg	75.48	Below specification

According to criteria used for the evaluation of drug content, values between 90% and 110% of the declared content are considered adequate for pharmaceutical products. Considering this range, only the 1 mg formulation from Pharmacy E presented compliance regarding minoxidil content. The remaining formulations presented deviations from the declared value, including both results below specification (Pharmacies A and E at the 1.5 and 2.5 mg concentrations) and values above the established limit (Pharmacies B, C, and D).

The greatest discrepancy was observed in the 2 mg formulation from Pharmacy B, which presented a content corresponding to 230.44% of the declared value, indicating a concentration approximately 2.3 times higher than that specified on the label. Conversely, the 2.5 mg formulation from Pharmacy E presented the lowest relative content (75.48%), characterizing a substantial reduction in the expected amount of the drug.

The variability observed among different formulations demonstrates that compounding pharmacies may present relevant differences in the final content of the active ingredient, reinforcing the importance of implementing analytical quality control strategies for monitoring the potency of compounded medications.

Content control becomes especially relevant for oral minoxidil, since the drug is employed at low doses, so that small variations in the active ingredient content may represent relevant differences in the dose effectively administered to the patient. These variations may alter drug exposure and influence the occurrence of adverse effects, particularly cardiovascular effects, described for oral minoxidil (Gupta et al., 2024; Jiménez-Cauhe et al., 2025).

Although the present study does not allow inferring direct clinical consequences, significant deviations from the declared content may compromise the achievement of the intended therapeutic dose and affect treatment predictability.

Considering that the treatment of alopecia depends on maintaining adequate drug concentrations during continuous use, monitoring the quality of compounded formulations represents an important strategy to increase therapeutic reliability and contribute to patient safety (Majewski et al., 2024; Jiménez-Cauhe et al., 2025).

Conclusion

The present study enabled the development and evaluation of a UV-Vis spectrophotometric method (288 nm) for the quantitative determination of minoxidil in compounded capsules. The methodology presented satisfactory analytical performance regarding the evaluated parameters, including linearity, precision, and robustness, demonstrating potential for application in quality monitoring of formulations containing minoxidil. However, the selectivity assessment evidenced the influence of the pharmaceutical matrix, indicating the

need for complementary studies with greater control over the composition of the excipients present in the formulations. Thus, performing complete analytical validation using samples with known composition may confirm the definitive applicability of the method for different matrices.

All evaluated formulations presented compliance regarding the average weight determination assay, according to the criteria established by the Brazilian Pharmacopoeia, 7th edition. Nevertheless, the assay demonstrated significant variations in minoxidil content among the analyzed samples, with results both below and above the declared value, evidencing relevant differences in the quality of the evaluated compounded formulations.

The results reinforce the importance of implementing simple and accessible analytical strategies for the monitoring of compounded medications, contributing to greater quality control and to the reduction of deviations between the declared dose and the amount effectively present in the formulations.

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