Scientia Pharmaceutica

Communication

Compounded Hair Solutions and Foams Containing Minoxidil: Does the Color Change Impact Stability?

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Abstract: An increasing number of pharmacies around the world are producing hair solutions and foams containing minoxidil for alopecia, commonly using ready-to-use vehicles such as TrichoSol™ or TrichoFoam™. However, it is paramount to determine the chemical and microbiological compatibility of these formulations so they can be safely implemented as vehicles of choice. Also, these products usually suffer from a change of color over time, which leads to many patients prematurely discontinuing treatment. As long-term treatment is recommended, this study aimed to assess the physical–chemical and microbiological stability and investigate the color change of compounded minoxidil formulations. For that, HPLC analyses and antimicrobial effectiveness testing were conducted in a bracketed study covering concentrations from 1.0% to 7.0% of minoxidil. HPLC, pH, and metals in 5.0% minoxidil compounded products were determined using ICP-MS to evaluate the mechanisms involved in their color change. The stability of the products varied from 120 to 380 days. The color change was remarkably noticeable, but apart from this parameter, no other quality attribute was affected throughout this period, including minoxidil content, which presented only minor fluctuations. No precipitation was observed, and pH was relatively stable. It is not expected that this yellow color will impact effectiveness. Finally, we created an indicative color chart of the behavior of minoxidil in the studied vehicles.

Keywords: minoxidil; alopecia; hair solution; hair foam; personalized medicine

Citation: Polonini, H.C.; Silva, C.C.V. Compounded Hair Solutions and Foams Containing Minoxidil: Does the Color Change Impact Stability? *Sci. Pharm.* **2023**, *91*, 39. https://doi.org/10.3390/scipharm91030039

Academic Editor: Nilesh Patel

Received: 1 May 2023
Revised: 24 July 2023
Accepted: 2 August 2023
Published: 15 August 2023

1. Introduction

Alopecia is partial or complete hair loss from one or more areas of the body, most commonly affecting the scalp [1]. It can be related to systemic conditions, such as autoimmune or endocrine diseases, chronic infections, and nutritional deficiencies [2]. In addition, alopecia can have an acute onset or be a slowly progressive disease. Androgenetic alopecia, also known as male or female pattern hair loss, is the most prevalent form of progressive hair loss, affecting 70% and 50% of middle-aged men and women, respectively [3]. It is characterized by non-scarring, progressive hair follicle miniaturization with a pattern distribution [4]. Clinically, it manifests as thinning along the scalp vertex and bitemporal hairline with relative sparing of the occipital scalp. It can also cause the frontal hairline in men to recede [5]. Contrarily, in women, it clinically manifests as diffuse hair thinning over the central scalp, but the frontal hairline is usually preserved [6]. Besides the clinical aspect, several studies have reported that alopecia affects patients' quality of life, self-esteem, and psychological well-being [7]. Therefore, early diagnosis and treatment are crucial for optimizing the course of the disease.

Minoxidil, currently the first-line treatment, was first introduced as an oral antihypertensive medication due to its potent vasodilatory properties [8]. However, hypertrichosis was found to be a common side effect among patients, leading to the development of a topical formulation that promotes hair growth [9]. Its exact mechanism of action remains unknown, but the conversion of minoxidil to its active derivative, minoxidil sulfate, seems an essential step in the medication's effectiveness [10]. Although topical minoxidil has a

good safety and efficacy profile, many patients prematurely discontinue treatment [11]. Undesirable hair texture, scalp irritation, and the frequency of application are associated with this poor level of compliance [12–14].

Most minoxidil topical formulations include excipients such as parabens, propylene glycol, artificial colorants, and high concentrations of alcohol, potentially leading to scalp irritation and undesirable side effects [15]. In addition, different minoxidil concentrations have been prescribed, and variable treatment responses have been reported [16]. Furthermore, previous studies with topical and oral minoxidil showed that products change color over time. Although the results suggest that efficacy is not affected, this might also lead to poor compliance [17,18].

TrichoSol™ and TrichoFoam™ are ready-to-use topical vehicles for preparing hair solutions and foams, respectively, within the context of compounding pharmacies. They were developed to avoid hazardous excipients [19,20] and to allow pharmacies to compound a wide range of minoxidil topical formulations. However, it is paramount to determine the chemical and microbiological compatibility of these formulations so they can be safely implemented as a vehicle of choice. Also, as long-term treatment is recommended, this study aimed to assess the physical–chemical and microbiological stabilities and investigate the color change of compounded minoxidil formulations. To the best of the authors' knowledge, this is the first evaluation of the behavior of minoxidil in both vehicles, with regard to color changes and physical–chemical and microbiological stabilities.

2. Materials and Methods

2.1. Reagents, Reference Standards, Materials, and Equipment

The methods listed in this study were carried out according to previously published in-house protocols [21–23]. Minoxidil (batch number 21A26-B080-078536), TrichoSol™ (batch number FG392/21), and TrichoFoam™ (batch number FG76/21) were obtained from Fagron. High-performance liquid chromatographic (HPLC)-grade reagents (Panreac, Barcelona, Spain) were used. Ultrapure water obtained with an AquaMax-Ultra 370 Series (Young Lin, Anyang, Republic of Korea) (18.2 MΩ·cm resistivity at 25 °C) was used throughout the experiments [24]. The minoxidil reference standard was a primary USP (Rockville, MD, USA) reference material. Iron, manganese, and chromium standards for inductively coupled plasma–mass spectrometry (ICP-MS) are certified reference materials obtained from NSI Lab Solutions (Raleigh, NC, USA). All volumetric glassware and the analytical balance used were calibrated.

HPLC analyses were performed on a qualified and calibrated chromatography system (Young Lin, Anyang, Korea) comprising a quaternary gradient pump, a photodiode array (PDA) detector, a 96-vial programmable autosampler, a column oven compartment, a variable sample loop up to 200 mL, and a software controller (Clarity). The chromatographic determinations were developed in-house. An L1(C18), 250 × 4.6 mm, at 25 °C was used for separation (Phenomenex). The column was connected to a pre-column with the same packing (4.0 × 3.0 mm, 5 µm) from the same vendor as the columns. The mobile phase comprised a mixture of methanol, water, and acetic acid (70:30:1, *v*/*v*/*v*), at a 0.5 mL/min flow rate. Samples and standards were diluted to a 10 µg/mL concentration in the mobile phase and injected at a volume of 20.0 µL. Detection was conducted in UV mode at 254 nm. Mobile phases and receptor media were filtered through a 0.45 µm filter membrane (RC-45/15 MS; Chromafil, Düren, Germany) and degassed using an ultrasonic apparatus (Model 1600A; Unique, Indaiatuba, Brazil) for 30 min immediately before use.

ICP-MS analyses were performed on a qualified and calibrated 7700x system (Agilent, Tokyo, Japan). Analysis conditions followed a previous method published by our group [25]: argon flux = 15 L min^{-1}, plasma frequency = 26.99 MHz; gas mode: no gas (no collision cell used) based on mass $_{26}$Fe, $_{52}$Cr, and $_{55}$Mn; sample uptake = 40 s at 0.3 rps; rinse between samples = 30 s with water at 0.5 rps followed by 30 s with 1% nitric acid at 0.5 rps. Tuning solution, blanks and calibration checks were performed to guarantee accuracy. Samples (n = 3) were diluted at 0.1% (*v*/*v*) in 1% Suprapur® nitric acid.

2.2. Forced-Degradation Studies: Stability-Indicating Characteristics

API samples were subjected to the following stress conditions to validate the capacity of the HPLC method to determine any possible degradation product generated during the storage of the tested samples:

(1) Dilution in acid (0.1M HCl, at 25 °C);
(2) Dilution in base (0.1M NaOH, at 25 °C);
(3) Exposure to ultraviolet (UV) light at 365 nm (at 25 °C);
(4) Heating to 70 °C;
(5) Dilution in H_2O_2 35% (*v*/*v*) (at 25 °C).

These solutions were prepared at the working concentration for the API using serial dilution from a stock solution and suitable diluents. The stock solutions were mixed via sonication for 10 min, and the final solutions were filtered (15 mm regenerated cellulose syringe filters, with 0.45 µm pore size) before injection into the HPLC system. Any extraneous peaks found in the chromatograms were labeled. A resolution of 1.5 between the peaks of the degradation products and the API was considered a complete separation. Also, a discrepancy greater than 2% between the stressed sample peak and the standard, non-stressed sample peak was considered indicative of API decomposition.

2.3. Validation of the High-Performance Liquid Chromatography Method

The validations of the chromatographic listed in this study were conducted according to in-house protocols [21–23]. These protocols follow the guidelines from the USP and the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) [26,27].

The specificity of the methods was determined using the solutions mentioned in "Forced-degradation Studies: Stability-indicating Characteristics" and by conducting HPLC analyses of a standard solution, TrichoSol™ or TrichoFoam™ blank solutions, and a mobile phase/diluents blank solution. The acceptance criterion was defined as a percentage of a discrepancy between the peak areas lower than 2%. In addition, the specificity of the method was obtained through a comparison of standard chromatograms with and without the matrix. All analyses were run in triplicate.

To ensure precision, the test assessed the dispersion degree among the series of measurements obtained by the same analyst (repeatability) and between two analysts in two days (within-lab variations, intermediate precision) for solutions of the API at working concentrations. Repeatability was determined using the consecutive analyses of six replicates by one analyst for one day. Different analysts also performed an intermediate precision in six replicates but on two different days. An injection precision of <5% relative to the coefficient of variation was considered appropriate.

The same analyst performed accuracy measurements by injecting the chromatographic samples to which the matrix was added (at the same concentration levels performed for the linearity test; n = 3 for each concentration level). The results are expressed as a percentage of recovery, compared with the analytical curve obtained from linearity.

For linearity, the test was conducted by plotting three standard curves, each constructed from the API concentrations listed in Table 1, to assess the linear relationship between the concentration of the API and the obtained areas. For this purpose, the data for each concentration range of the curve, after fitting via the ordinary least squares method, were evaluated using analysis of variance (ANOVA) and subjected to the least-squares method to determine the correlation coefficient of the calibration curve.

The limit of detection (LD) and limit of quantification (LQ) were determined from three standard calibration curves and calculated as shown in Equations (1) and (2), respectively:

$$LD = (3.3 \times \sigma)/IC \quad (1)$$

$$LD = (10 \times \sigma)/IC \quad (2)$$

where IC is the mean slope of the analytical curves and σ is the standard deviation obtained from the noise estimate from the analysis of white samples (at least 10).

Table 1. Summary of validation results of the (ultra) high-performance liquid chromatographic methods.

Parameter		Minoxidil in TrichoSol™	Minoxidil in TrichoFoam™	Minoxidil in Hydroalcoholic Solution
Linearity	Range (μg/mL)	72.10–133.90	72.10–133.90	72.10–133.90
	Analytical curve	y = 92.581x − 1145.055	y = 92.581x − 1145.055	y = 92.581x − 1145.055
	r	0.9995	0.9995	0.9995
	ANOVA's Significance of Regression (F)	13,741.43	13,741.43	13,741.43
Limits	LOD (μg/mL)	0.07	0.07	0.03
	LOQ (μg/mL)	0.21	0.21	0.08
Precision	Repeatability (CV, %)	0.87	0.23	3.89
	Intermediate Precision (CV, %)	0.64	1.88	3.55
Specificity	Discrepancy (%)	|0.15|	|1.82|	|0.69|
Accuracy	Recovery (%)	100.24	100.24	100.24

Acceptance criteria were as follows: r > 0.99; F (significance of regression) >> 4.67; discrepancy < 2%; repeatability and intermediate precision < 5%; and recovery = 100% ± 2%. All analytical ranges (μg/mL) were considered adequate to analyze the concentrations used. API = active pharmaceutical ingredient; CV = coefficient of variation; LOD = limit of detection; LOQ = limit of quantification (20 μL injections).

2.4. Stability Study at 1.0% and 7.0% Concentration

2.4.1. Preparation of Samples of Hair Solutions and Foams

The hair solutions and foams were prepared according to the following composition and preparation procedures:

Minoxidil hair and foam samples

Minoxidil.....1.0% or 7.0% (*w*/*v*)
Sodium benzoate.....0.1%
TrichoSol™ or TrichoFoam™.....q.s. 100.0%

(1) Calculate and weigh all ingredients of the formulation.
(2) Pour 2/3 of the total amount of the vehicle of choice (TrichoSol™ for a solution or TrichoFoam™ for a foam) into volumetric glassware.
(3) Grind the minoxidil and sodium benzoate in a mortar.
(4) Add Step 3 to Step 2, mixing well for about 5 min until a clear and homogeneous solution is formed.
(5) Add the vehicle to volume and mix well.
(6) Package in low-actinic, light-resistant glass bottles with a pipette dropper (for hair solution) or plastic airless foamer (for hair foam) and label.
(7) Immediately assay the samples at T = 0 and then store them at room temperature (15–30 °C) for the duration of the study.

2.4.2. Stability Study

The API samples were assayed using HPLC at pre-determined time points to verify the stability of the API in TrichoSol™. Before analysis, the bottles were shaken until a visual inspection confirmed the uniform dispersal of the API. Aliquots for quantification were withdrawn and diluted to obtain working solutions. Sampling times were: 0 days (T = 0), 7 days (T = 7), 14 days (T = 14), 30 days (T = 30), 60 days (T = 60), 90 days (T = 90), 120 days (T = 120), 150 days (T = 150), and 180 days (T = 180). Minoxidil hair solution was also measured at 380 days (T = 380). The pH was also measured at all sampling times, and a visual inspection was performed to check for separation, sedimentation, and discoloration phases.

All solutions were assayed six times, and the results are expressed as means from six independent measurements. For this purpose, samples were diluted, sonicated for 10 min, and then filtered in 15 mm regenerated cellulose syringe filters with 0.45 µm pore size before injection into the HPLC system. The evaluation parameter was the percent recovery with respect to T = 0, measured using the HPLC method (results given as percentage ± standard deviation).

2.4.3. Antimicrobial Effectiveness Testing

The samples were analyzed via antimicrobial effectiveness testing (AET) at 0 and 180 days after compounding, following the general USP <51> chapter [28]. This was performed to verify that no microbiological instability or contamination was present (which could potentially interfere with the color of the samples). The aliquots were withdrawn from the initial product and diluted to obtain working solutions. The microorganisms used in the AET were as follows: *Candida albicans*, ATCC 10231; *Aspergillus brasiliensis*, ATCC 16404; *Escherichia coli*, ATCC 8739; *Pseudomonas aeruginosa*, ATCC 9027; *Staphylococcus aureus*, ATCC 6538.

A suspension of microorganisms was prepared and standardized on an optical scale at a concentration equivalent to 10^8 colony-forming units (CFU)/mL. Afterward, the suspension was inoculated in the sample respecting the range of 0.5% and 1.0% in relation to the weight of the total product.

A neutralizing agent (Polysorbate and Lecithin) was added to the sample prepared for plating dilution. The depth plating method determined the number of CFUs in the sample at the initial time (0 h) and at each required time interval (14 and 28 days). The analyses were performed at T = 0 and T = 180 of the physical–chemical study.

2.5. Stability Study at 5.0% and Investigation of Color Change in Minoxidil Products

2.5.1. Preparation of Samples of Hair Solutions and Foams

A minoxidil solution in TrichoSol™ and foam in TrichoFoam™ were prepared as previously described but at 5.0% (*w*/*v*). In addition, a hydroalcoholic minoxidil solution at the same concentration was also prepared as below:

Minoxidil hydroalcoholic hair solution sample
Minoxidil.....5.0% (*w*/*v*)
Propanediol.....35.0%
Ethanol 96 °GL.....40.5%
Sodium hyposulfite.....0.05%
Purified water.....q.s. 100.0%

(1) Calculate and weigh all ingredients in the formulation.
(2) Add minoxidil to volumetric glassware and slowly add propanediol, mixing well.
(3) Add the ethanol and mix well.
(4) Measure the required amount of water, solubilize the sodium hyposulfite, and add it to Step 3. Mix well.
(5) Bring to volume with water, if needed.
(6) Package in low-actinic, light-resistant glass bottles with a pipette dropper and label.
(7) Immediately assay the samples at T = 0 and then store them at room temperature (15–30 °C) for the duration of the study.

2.5.2. Color Change Investigation

The API samples were assayed using HPLC at pre-determined time points to verify the stability of the API in the vehicles. Before analysis, the bottles were shaken until a visual inspection confirmed the uniform dispersal of the API. Aliquots for quantification were withdrawn and diluted to obtain working solutions. Sampling times were as follows: 0 days (T = 0), 7 days (T = 7), 14 days (T = 14), 30 days (T = 30), 60 days (T = 60), and 90 days (T = 90). All solutions were assayed six times, and the results are expressed as the means from six independent measurements. For this purpose, samples were diluted,

sonicated for 10 min, and then filtered in 15 mm regenerated cellulose syringe filters with a 0.45 µm pore size before injection into the HPLC system. The evaluation parameter was the percent recovery with respect to T = 0, using the HPLC method (results given as percentage ± standard deviation).

Other parameters analyzed were as follows: (i) organoleptic characteristics: color, odor, and formation of precipitates; (ii) pH; and (iii) content of iron (Fe), manganese (Mn), and chromium (Cr) using ICP-MS. At the end of the study, a color chart was prepared by directly comparing the products with their respective RGB (red, green, blue) color codes. For this, we took photos and pasted them to the Microsoft PowerPoint software, created a color chart, and used the Eyedropper tool to match the colors of the photos with the chart. Then, the RGB codes were copied from the software.

3. Results and Discussion

3.1. Stability Study at 1.0% and 7.0% Concentrations

Validation studies were performed, and all results (Table 1) met the respective acceptance criteria. Stability-indicating studies were also conducted to determine if the methods were fully validated and adequate for identifying the decomposition of the API by chromatographic analysis. The decomposition profile of the API was similar in the three vehicles and particularly similar between TrichoSol™ and TrichoFoam™. All factors impacted the stability of the API, except for the exposure of minoxidil to an alkaline agent in the hydroalcoholic solution, showing that some protection is provided by this vehicle in this pH environment (Table 2). Once the API's forced-degradation profiles were determined, the API's vehicle stability was assessed.

Table 2. Summary of the stability-indicating study of active pharmaceutical ingredients.

Active Pharmaceutical Ingredients	HCl	NaOH	UV	Heat	H_2O_2
	%d	%d	%d	%d	(%d)
Minoxidil in TrichoSol™	**−17.932**	**80.48**	**−23.41**	**−12.30**	**−40.82**
Minoxidil in TrichoFoam™	**−24.11**	**88.70**	**−18.84**	**−9.87**	**−43.72**
Minoxidil in hydroalcoholic solution	**−8.99**	−1.63	**−2.88**	**−12.00**	**12.29**

Results are presented as the average of three replicates at the working concentration.%d = Percentage of the discrepancy between the active pharmaceutical ingredient peak without exposure to stress factors (negative control), and the peak of a sample subjected to one of the cited accelerated-degradation factors. Areas are given as mV. Maximum acceptable value = 2% (values above this are in bold). HCl = hydrochloride; NaOH = sodium hydroxide solution; UV = ultraviolet; H_2O_2 = Hydrogen peroxide.

At each sampling time, the visual appearance of the hair solutions and foams was evaluated to verify their homogeneity and physical stability. Throughout the study, no phenomena such as sedimentation, discoloration, or phase separation were observed when the drug content was within specifications.

Chemical stability results are shown in Table 3 as a relative percentage of recovery (initial sampling time = 100%) and Figure 1 as absolute amounts of the API. The relative percentage recovery should be between 90% and 110% for the hair solutions to be stable.

The beyond-use dates (BUDs) found for the hair solutions tested here were: 380 days for minoxidil 7.0% in TrichoSol™, 180 days for minoxidil 7.0% in TrichoFoam™, 150 days for minoxidil 1.0% in TrichoSol™, and 120 days for minoxidil 1.0% in TrichoFoam™. Here, one point to mention is that, according to the United States Pharmacopeia (USP), the BUD for a nonsterile compounded preparation can be extended up to a maximum of 180 days [29], meaning that for the US market, the BUD for minoxidil 7.0% in TrichoSol™ would be 180 days and not 380 days.

Table 3. Stability of active pharmaceutical ingredients in compounded hair solutions using TrichoSol™ and TrichoFoam™ as vehicles.

Elapsed Time (Days)	% Recovery, with Respect to T = 0	pH	% Recovery, with Respect to T = 0	pH
	Minoxidil 1.0% in TrichoSol™		**Minoxidil 7.0% in TrichoSol™**	
T = 0	100.00 ± 0.26	2.62	100.00 ± 0.16	3.71
T = 7	99.36 ± 0.13	2.64	100.97 ± 0.14	3.74
T = 14	99.14 ± 0.26	2.64	98.18 ± 0.32	3.77
T = 30	98.03 ± 0.61	2.72	100.63 ± 0.13	3.78
T = 60	98.38 ± 0.37	2.76	100.57 ± 0.13	3.90
T = 90	95.95 ± 0.14	2.72	97.28 ± 0.17	3.67
T = 120	95.35 ± 0.15	2.67	97.11 ± 0.18	3.63
T = 150	95.41 ± 0.45	2.70	98.33 ± 0.29	3.69
T = 180	77.78 ±0.84	2.85	94.63 ± 1.36	3.74
T = 380	-	-	97.53 ± 0.39	3.79
	Minoxidil 1.0% in TrichoFoam™		**Minoxidil 7.0% in TrichoFoam™**	
T = 0	100.00 ± 0.25	3.71	100.00 ± 0.33	3.70
T = 7	100.72 ± 0.47	2.74	98.09 ± 0.48	3.75
T = 14	96.25 ± 0.96	3.77	98.20 ± 0.20	3.78
T = 30	99.92 ± 0.12	3.78	101.62 ± 0.57	3.79
T = 60	98.12 ± 0.10	3.90	101.10 ± 0.51	3.85
T = 90	96.90 ± 0.14	3.67	101.54 ± 0.17	3.64
T = 120	96.41 ± 0.14	3.63	100.55 ± 0.40	3.63
T = 150	85.48 ± 0.35	3.69	100.59 ± 0.34	3.68
T = 180	-	-	99.50 ± 1.30	3.72

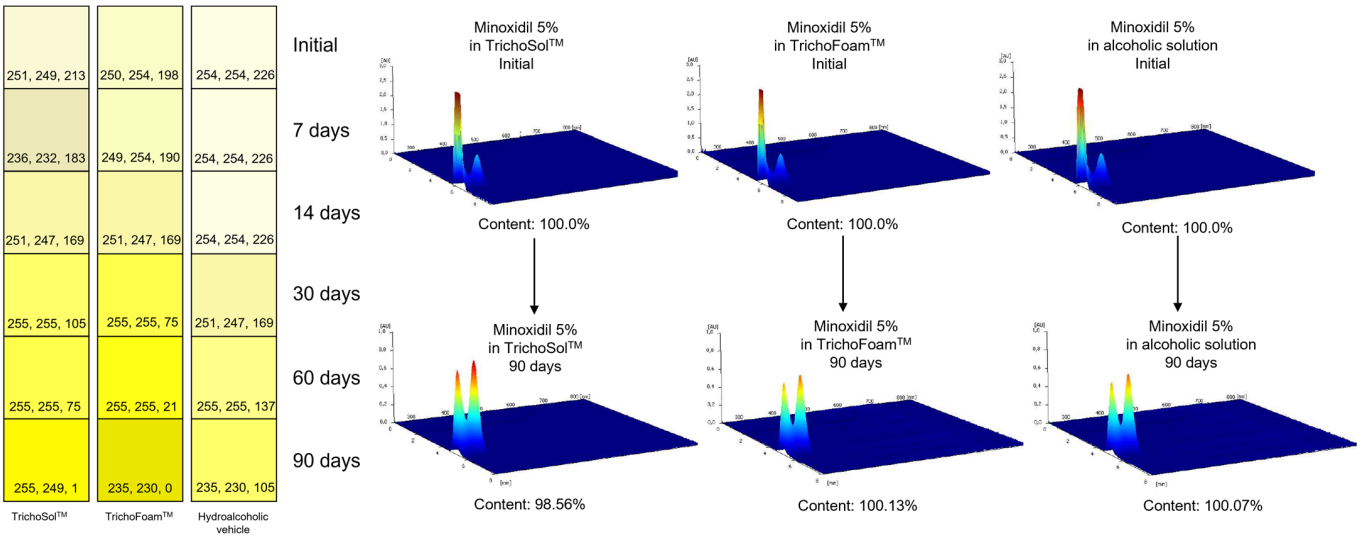

Figure 1. Indicative color chart of minoxidil stability in TrichoSol™ and TrichoFoam™, as well as hydroalcoholic vehicles (**left**) with respective RBG color codes. On the (**right**), representative chromatograms of the products at the beginning and end of the study.

As this is a bracketed study, it is expected that formulations with different concentrations within the tested range will have the same beyond-use date (BUD) as observed here for the low and high concentrations of API in the vehicle. For the hair solutions where stability is dependent on concentration, a conservative approach can be used, and then the shortest BUD can be applied as follows:

- Minoxidil 1.0% (including) to 7.0% (excluding) in TrichoSol™: 150 days (minoxidil 7.0%, specifically, has a BUD of up to 380 days, or 180 days in the USA);

- Minoxidil 1.0% (including) to 7.0% (excluding) in TrichoFoam™: 120 days (minoxidil 7.0%, specifically, has a BUD of up to 150 days).

The AET conducted at 180 days confirms adequate BUDs (Table 4). AET is an official test from the USP (chapter 51: "Antimicrobial effectiveness must be demonstrated for aqueous-based, multiple-dose topical and oral dosage forms and other dosage forms such as ophthalmic, otic, nasal, irrigation, and dialysis fluids"). This test measures the effectiveness of the preservative system of the formulations/product to show they provide microbiological stability throughout their physicochemical shelf life (minoxidil content). Additionally, chapter 795 from the United States Pharmacopeia (Pharmaceutical compounding—nonsterile preparations) requests that the test can be performed for any preserved aqueous dosage form with a beyond-use date longer than 35 days stored at controlled room temperature or refrigerator. This is the reason why it was used in this study. Finally, as the test is requested by the USP, which allows a maximum of 180 days BUD for this type of formulation, the AET was conducted at this sampling time.

Table 4. Antimicrobial effectiveness testing (AET) of TrichoSol™ and TrichoFoam™.

Elapsed Time * (Days)	Microorganism—Test in TrichoSol™ (Results as cfu/g; log Reduction)				
	C. albicans	*A. brasiliensis*	*E.coli*	*P. aeruginosa*	*S. aureus*
T = 0	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0
T = 14	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0
T = 28	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0
	Microorganism—Test in TrichoFoam™ (Results as cfu/g; log Reduction)				
	C. albicans	*A. brasiliensis*	*E.coli*	*P. aeruginosa*	*S. aureus*
T = 0	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0
T = 14	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0
T = 28	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0	<1.0 × 10/1.0

* Elapsed time of the AET testing after sampling for stability study (180 days).

The stability of minoxidil compounded topical formulations beyond 90 days is in accordance with the literature, which already shows that this API could be stable for up to 91 days in a water-based foam vehicle stored at room temperature [30].

Lower concentrations of formulations have a shorter BUD. This is also in accordance with the literature, reporting that minoxidil photodegradation occurs at a higher rate and lower concentration [31].

3.2. Color Change Investigation

Minoxidil products change color from transparent to pale yellow to dark yellow over time. Although the development of this yellow color was already reported not to impact its effectiveness [17], it can affect user experience, as the patient can feel uneasy about this change in color as a sign of lack of quality. Even oral minoxidil suspensions also show a color change after four weeks of compounding but with a low impact on API content, which remains in the acceptance range for 24 weeks under refrigeration [18].

Interestingly, this color change is not observed in the raw material of minoxidil, a white powder, even during transportation, storage, and manufacturing. However, minoxidil solutions experience this change, possibly due to the contact of this substance with solvents such as propylene glycol, ethanol (those two are not present in TrichoSol™ or TrichoFoam™), and purified water. Moreover, trace amounts of metal elements such as iron, chromium, and manganese are found in these solvents [32]. For example, it is reported that minoxidil preparations with an iron concentration of 60 ppb or less have a slow color development and slower progress of discoloration over time [32].

In this study, we selected the most commonly (and historically) prescribed concentration of topical minoxidil (5.0%) to analyze such changes. As a first step, the stability of this API was assessed for 90 days in the different vehicles (Table 5). The color change

was remarkably noticeable, but apart from this parameter, no other quality attribute was affected throughout this time, including the minoxidil content, which presented only minor fluctuations. No precipitation was observed, and pH was relatively stable. The changes in reported concentrations of Fe, Mn, and Cr are possibly due to analytical variations, as chemical elements are not expected to be formed in situ. Another possible explanation is the leaching of such metals from the packaging, which would explain the gradual color change—as more iron is leached out from the packaging, more of this element is free to react with minoxidil, producing a yellow color. However, this is just a hypothesis, and more studies are needed to confirm this phenomenon.

Table 5. Stability and color change investigations of the active pharmaceutical ingredients in compounded hair solutions using TrichoSol™, TrichoFoam™, and hydroalcoholic solution as vehicles.

Elapsed Time (Days)	Organoleptic Characteristics	% Recovery, with Respect to T = 0	pH	Fe (mg/L)	Mn (mg/L)	Cr (mg/L)
Minoxidil 5.0% in TrichoSol™						
T = 0	Colorless liquid. No precipitates	100.00	3.61	0.060	0.007	0.018
T = 7	Slightly cloudy liquid. No precipitates	99.90	3.34	-	-	-
T = 14	Cloudy liquid. No precipitates	99.51	3.61	-	-	-
T = 30	Yellowish liquid. No precipitates	99.14	3.46	-	-	-
T = 60	Yellowish liquid. No precipitates	98.60	3.67	-	-	-
T = 90	Orange liquid. No precipitates	98.56	3.55	0.292	0.021	0.010
Minoxidil 5.0% in TrichoFoam™						
T = 0	Colorless liquid. No precipitates	100.00	3.38	0.088	0.075	0.024
T = 7	Slightly yellowish liquid. No precipitates	98.58	3.45	-	-	-
T = 14	Slightly yellowish liquid. No precipitates	100.47	3.38	-	-	-
T = 30	Yellowish liquid. No precipitates	99.83	3.38	-	-	-
T = 60	Yellowish liquid. No precipitates	99.93	3.56	-	-	-
T = 90	Orange liquid. No precipitates	100.13	3.45	0.281	0.019	0.012
Minoxidil 5.0% in hydroalcoholic solution						
T = 0	Colorless liquid. No precipitates	100.00	10.50	0.062	0.047	0.019
T = 7	Colorless liquid. No precipitates	98.28	10.30	-	-	-
T = 14	Slightly yellowish liquid. No precipitates	100.58	10.50	-	-	-
T = 30	Slightly yellowish liquid. No precipitates	97.36	9.40	-	-	-
T = 60	Slightly yellowish liquid. No precipitates	99.69	9.10	-	-	-
T = 90	Slightly yellowish liquid. No precipitates	100.07	8.81	0.271	0.007	0.008

One remark about color change was that the hydroalcoholic solution presented a much less significant change. Its pH was much more alkaline than the solution derived from TrichoSol™ or TrichoFoam™. Although the reported stability of pH is much closer to those last vehicles (around 4.5 [33]), all formulations presented a similar stability profile. Based on such results, Figure 1 was designed and presented an indicative color chart of the behavior of minoxidil in the vehicles studied. The color range shows acceptable colors of the hair solutions or foam (i.e., minoxidil decomposition was below 3%).

4. Conclusions

Based on the presented data, we conclude that the color changes observed during the stability study of minoxidil topical solutions or foams (using TrichoSol™ and TrichoFoam™, respectively) do not affect the API content. Therefore, they are suitable

for use. Additionally, the following beyond-use dates can be assigned for minoxidil in compounding vehicles:

- Minoxidil 1.0% (including) to 7.0% (excluding) in TrichoSol™: 150 days (minoxidil 7.0%, specifically, has a BUD of up to 380 days—or 180 days, in the USA);
- Minoxidil 1.0% (including) to 7.0% (excluding) in TrichoFoam™: 120 days (minoxidil 7.0%, specifically, has a BUD of up to 150 days).

We emphasize that this is important data for compounding pharmacy businesses worldwide, which may face questions about their personalized topical treatments provided for minoxidil and will now be able to present a science-based approach in this regard.

Author Contributions: Conceptualization, methodology, formal analysis, writing—original draft preparation, H.C.P.; writing—review and editing, C.C.V.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Fagron B.V.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors work for Fagron BV. The funders had no role in the study's design; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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