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Compatibility of Personalized Formulations in Cleoderm™, A Skin Rebalancing Cream Base for Oily and Sensitive Skin

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Abstract: Inflammatory skin conditions are prevalent in the general population and are a source of much concern for those who suffer from them. Acne is an extremely common condition and can significantly impact the quality of life of affected patients. Rosacea is another common dermatological disorder that often affects the face and can present with flushing, irritated skin, and pimples. In addition to being key for acne and rosacea, inflammation can also play a role in prematurely aging skin and contributes to the formation of wrinkles. Given the prevalence and patient impact of dermatological conditions on the face, such as those previously described, there is a demand for personalized medicines to manage these conditions when commercially available options are unsuitable, unavailable, or insufficient to fully resolve the condition. When designing an appropriate personalized therapy for a patient, both the vehicle and the active pharmaceutical ingredient choices are key to the success of the treatment. Cleoderm™ is a topical cream designed for use as a vehicle for the preparation of dermatological treatments by compounding pharmacies. Its ingredient profile was specifically curated to be gentle on the skin, allowing its use as a vehicle for compounded preparations that may be applied to sensitive and affected skin. In this bracketed study, benzoyl peroxide, cyproterone acetate, estriol, metronidazole, niacinamide, progesterone, retinoic acid, spironolactone, and tranexamic acid were selected, due to their known applications for dermatological skin conditions. To evaluate the compatibility and stability of Cleoderm™ in these formulations, high-performance liquid chromatography, followed by antimicrobial effectiveness testing, were performed for 180 days. For most formulations, a beyond-use date of 180 days was observed when stored at room temperature, except for retinoic acid, which had a beyond-use date of 30 days. Through the outcomes of this study, we concluded that Cleoderm™ presents increased convenience for both the compounding pharmacist and the patient, suggesting that it is an adequate candidate vehicle for compounding different dermatological formulations with adequate stability, presenting itself as a good alternative to commercially available treatments that cannot be personalized.

Keywords: acne; rosacea; sensitive skin; oily skin; affected skin; personalized medicine; cream vehicle; dermatological compounding; beyond-use date

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1. Introduction

Inflammatory skin conditions are prevalent in the general population and are a source of much concern for those suffering from them. One such extremely common condition is acne: one study reported lifetime prevalence as 85%, with most of these cases presenting during adolescence, though adult onset can occur as well [1,2]. Acne can significantly impact the quality of life of affected patients, and management of this condition can be important for self-esteem [2]. Rosacea is another common dermatological disorder; it affects approximately 10% of the population, with a disproportionate number of those patients being of the female sex. It is a chronic inflammatory disorder that often affects the face and can present with flushing, irritated skin, and pimples [3,4]. In addition to being key for acne and rosacea, inflammation can also play a role in prematurely aging skin and

contributes to the formation of wrinkles [5]. While aging skin is, in part, due to intrinsic chronological aging and is inevitable, inflammatory processes can contribute to photoaging and the premature aging of the skin [6].

Given the prevalence and patient impact of dermatological conditions on the face, such as acne, rosacea, and prematurely aging skin, there is a demand for personalized medicines to help manage these conditions when commercially available options are unsuitable, unavailable, or insufficient to fully resolve the presentation of the condition. When designing an appropriate compounded therapy for a patient, both the vehicle and active pharmaceutical ingredient (API) choices are key to the success of the treatment.

Cleoderm™ is a topical cream designed for use as a vehicle for the compounding of personalized dermatological treatments. Its ingredient profile was specifically curated to be gentle on the skin to allow for its use as a vehicle for compounded preparations that may be applied to the face. Cleoderm™ has a positive role in decreasing sebum production, lipid peroxidation, and reactive oxygen species; inhibition of *Cutibacterium acnes* proliferation; and control of inflammation [7]. It contains hyaluronic acid, a glycosaminoglycan that binds and retains water molecules, commonly a component of antiaging creams intended to keep the skin appropriately hydrated [8]. Another ingredient of note is *Cleome gynandra*, a plant extract with anti-inflammatory and antioxidant activities [7]. In addition, Cleoderm™ also contains palmitoyl tripeptide-8 and bisabolol, ingredients also noted to reduce pro-inflammatory markers and oxidative stress [7,9]; functional oils (*Persea gratissima*, *Simmondsia chinensis*, *Rosa canina*, *Cocos nucifera*, *Lavandula angustifolia*, *Melaleuca alternifolia*, *Rosmarinus officinalis*, *Vitellaria paradoxa*, and tocopheryl acetate); a natural oil-in-water emulsifier that is polyethylene glycol-free, non-ionic, preservative-free, and biodegradable; and, finally, an acrylamide-free thickener. The ingredient profile of Cleoderm™ makes it a good choice as a vehicle for topical use for inflammatory conditions or conditions in which inflammation can worsen its severity. Given the product's frequent use as the vehicle for compounded treatments for dermatological conditions such as acne, rosacea, and prematurely aging skin, the aim of this study was to establish the stability and compatibility of APIs commonly used for these treatments in Cleoderm™ to cement its utility as a vehicle.

In this study, benzoyl peroxide, cyproterone acetate, estriol, metronidazole, niacinamide, progesterone, retinoic acid, spironolactone, and tranexamic acid were selected and their compatibility with Cleoderm™ was evaluated. Benzoyl peroxide or a topical retinoid such as retinoic acid are recommended by the American Academy of Dermatology working group as first-line options for treating mild acne, and combinations of these APIs with other ingredients are suitable for moderate or severe acne [10]. Spironolactone for topical use has also been studied and found efficacious for mild to moderate acne in placebo-controlled trials, though no commercially available topical product currently exists on the market [11]. Cyproterone acetate has also been studied and demonstrated efficacy in placebo-controlled trials for the management of moderate to severe acne [12]. Niacinamide, in addition to demonstrating its utility for acne, has also been found efficacious for other inflammatory skin conditions, such as prematurely aging skin [13,14]. Metronidazole was chosen to highlight Cleoderm™'s utility for rosacea, and estriol, progesterone, and tranexamic acid were chosen to highlight its utility for aging skin. Hormones such as estriol and progesterone have been demonstrated in comparative and placebo-controlled trials to improve skin firmness, elasticity, moisture, and wrinkle depth [15,16]. Similarly, tranexamic acid has also been demonstrated to improve the appearance of dark spots on the skin and post-inflammatory hyperpigmentation [17]. These APIs for various dermatological conditions were selected for compatibility testing to further increase the utility of Cleoderm™ as a vehicle for these APIs and to demonstrate that it is a suitable vehicle for dermatological preparations for inflammatory skin conditions, with adequate stability.

2. Materials and Methods

2.1. Reagents, Reference Standards, and Materials

All APIs (raw powders) and Cleoderm™ were obtained from Fagron (Saint Paul, MN, USA). The concentrations and intended uses are listed in Table 1. High-performance liquid chromatography (HPLC)-grade reagents (Panreac, Barcelona, Spain) were used. Ultrapure water obtained from AquaMax-Ultra 370 Series (Young Lin, Anyang, Korea) (18.2 MΩ-cm resistivity at 25 °C) was used throughout the experiments. The reference standards used were obtained from the primary United States Pharmacopoeia (USP) (Rockville, MD, USA) reference materials. Mobile phases and the receptor media were filtered through a 0.45-µm filter membrane (RC-45/15 MS; Chromafil, Düren, Germany) and degassed immediately before use with an ultrasonic apparatus (Model 1600A; Unique, Indaiatuba, Brazil) for 30 min. All volumetric glassware and the analytical balance used were calibrated.

Table 1. APIs tested for their compatibility with Cleoderm™ in the bracketed study.

Active Pharmaceutical Ingredient	Concentrations Tested (mg/g)	Pharmaceutical Indication *
Benzoyl peroxide	2.5% and 10.0%	Antibacterial drug, commonly used to treat acne
Cyproterone acetate	0.5% and 2.0%	Steroid hormone used (in combination or not with other substances) to treat women with severe acne and symptoms of androgenization
Estriol	0.1% and 1.0%	Estrogenic hormone used to improve general skin condition
Metronidazole	0.75% and 5.0%	Antibiotic drug, used to treat a wide variety of skin infections
Niacinamide	1.0% and 5.0%	Form of Vitamin B3, which can improve general skin condition and hydration when used topically
Progesterone	0.5% and 2.0%	A steroid hormone used to improve general skin condition
Retinoic acid	0.025% and 0.1%	Morphogen derived from retinol (Vitamin A), commonly used for treating severe acne
Spironolactone	1.0% and 5.0%	Anti-androgen drug, used topically for treating hormonal acne
Tranexamic acid	1.0% and 5.0%	A synthetic amino acid lysine derivate, which can act as a brightening agent to reduce dark spots and improve hyperpigmentation

* Reference [18]. API, active pharmaceutical ingredient.

2.2. Equipment

The HPLC analyses were performed in a qualified and calibrated Agilent (Santa Clara, CA, USA) chromatography system composed of the following: a binary pump (1260 Infinity), UV detector (1260 Infinity), an automatic injector (1290 Infinity), a column compartment (1260 Infinity), and a software controller (OpenLab).

2.3. Chromatographic Determinations

The chromatographic conditions used for each API are listed in Table 2. Each column was connected to a pre-column with the same packing (4.0 × 3.0 mm, 5 µm), from the same manufacturer.

Table 2. Chromatographic conditions used in the compatibility study of different APIs with Cleoderm™.

Active Pharmaceutical Ingredient	Mobile Phase Composition	Working Concentration (µg/mL)	Column	Flux (mL/min)	Ultraviolet Detection Wavelength (nm)
Benzoyl peroxide	Acetonitrile and water (750:250, v/v)	250.0, in acetonitrile; 20 µL injection	C18(L1), 4.6 mm × 250 mm; at 45 °C	1.0	254
Cyproterone acetate	Water, methanol, and acetonitrile (40:40:20, v/v/v)	100.0, in methanol; 20 µL injection	C18(L1), 4.6 mm × 125 mm; at 40 °C	1.5	282
Estriol	Ethanol and water (60:40, v/v)	40.0, in ethanol; 10 µL injection	C18(L1), 4.6 mm × 250 mm; at 30 °C	0.3	205
Metronidazole	Acetonitrile and Solution A (glacial acetic acid and water, 40:60, v/v) (40:60, v/v)	20.0, in methanol; 10 µL injection	C18(L1), 4.6 mm × 250 mm; at 30 °C	1.0	316

Table 2. *Cont.*

Active Pharmaceutical Ingredient	Mobile Phase Composition	Working Concentration (µg/mL)	Column	Flux (mL/min)	Ultraviolet Detection Wavelength (nm)
Niacinamide	Methanol, acetic acid, and sulfonate buffer (27:1:73, v/v/v)	100.0, in water; 20 µL injection	C18(L1), 4.6 mm × 150 mm; at 25 °C	1.0	280
Progesterone	Ethanol and water (65:35, v/v)	100.0, in ethanol; 20 µL injection	C18(L1), 4.6 mm × 250 mm; at 45 °C	1.2	254
Retinoic acid	Methanol, water, and glacial acetic acid (80:20:0.5, v/v/v)	10.0, in methanol; 50 µL injection	C18(L1), 4.6 mm × 150 mm; at 25 °C	2.0	353
Spironolactone	Water, phosphoric acid, methanol, and acetonitrile (435:2.7:50:515, v/v/v/v)	100.0, in water; 20 µL injection	C18(L1), 4.6 mm × 250 mm; at 25 °C	1.0	254
Tranexamic acid	Phosphate buffer pH 2.5 and methanol (60:40, v/v)	100.0, in ultra-purified water; 100 µL injection	C18(L1), 4.6 mm × 250 mm; at 35 °C	1.0	220

API, active pharmaceutical ingredient; v = volume.

2.4. Forced Degradation Studies: Characteristics Indicating Stability

API samples were submitted to the following stressing 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 (0.1 M HCl);
2. Dilution in base (0.1 M NaOH);
3. Dilution in hydrogen peroxide (H_2O_2);
4. Exposure to ultraviolet light at 365 nm (for 24 h);
5. Heating at 70 °C (for 24 h).

After the study, all solutions were assayed by HPLC. Any extraneous peaks found in the chromatograms were labeled. The resolution was determined between the degradant and the API, and a resolution of 1.5 between peaks was considered as a full separation.

For this study, we followed the recommendations of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline Q1A(R2)—Stability Testing of New Drug Substances and Products. These conditions are part of the stress testing: "it should include the effect of temperatures (…), humidity (…) where appropriate, oxidation, and photolysis on the drug substance. The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension. Photostability testing should be an integral part of stress testing".

2.5. Validation of the HPLC Methods

Validations of the HPLC methods listed in this study were conducted according to in-house protocols, previously published [19–22]. These protocols followed the USP guidelines, and the ICH [23].

The specificity of each method was determined according to the conditions listed in Section 2.3, in comparison with HPLC analyses of a standard solution, a blank Cleoderm™ solution, and a blank solution of the mobile phase/diluents, with and without the matrix. The acceptance criterion was defined as a percentage of the discrepancy between peak areas lower than 2%. All analyses were performed in triplicate. To ensure precision, the test was designed to assess the dispersion degree among the measurements obtained by the same analyst (repeatability) and between two analysts on 2 days (within-lab variations, intermediate precision) for solutions of the API at working concentration. The repeatability was determined by the consecutive analysis of six replicates by one analyst in 1 day. The intermediate precision was also tested in six replicates, but on two different days by different analysts. An injection precision of <5% in relation to the coefficient of variation was considered to be appropriate.

The same analyst performed the accuracy measurements by injecting the chromatographic samples to which the matrix was added (at the same concentration levels used for the linearity test; n = 3 for each concentration level). The results were expressed as the 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 3, to assess the relationship between the concentration of the analyte and the obtained areas. For this purpose, the data for each concentration range of the curve, after fitting by the ordinary least squares method, were evaluated by analysis of variance (ANOVA) and subjected to the least-squares method to determine the correlation coefficient of the calibration curve.

Table 3. Summary of the validation results of the HPLC methods.

Active Pharmaceutical Ingredient	Linearity						Specificity	Precision		Accuracy
	Range (µg/mL)	Analytical Curve	R^2	ANOVA Significance of Regression (F)	LOD (µg/mL)	LOQ (µg/mL)	Discrepancy (%)	Repeatability (CV, %)	Intermediate Precision (CV, %)	Recovery (%)
Benzoyl peroxide	180.60–335.40	y = 369,637.54x − 2,191,586.24	0.9988	5507.09	0.01	0.02	1.82	1.76	1.69	99.75
Cyproterone acetate	72.80–135.20	y = 481,222.28x + 5,470,613.44	0.9917	774.07	0.01	0.02	0.86	0.99	0.98	98.04
Estriol	28.28–52.52	y = 3,451,831.25x − 8,500,128.91	0.9951	1315.26	0.01	0.03	1.78	0.65	2.08	99.91
Metronidazole	14.01–26.03	y = 95.83x − 29.50	0.9904	660.16	0.06	0.17	0.60	3.32	4.84	100.85
Niacinamide	72.24–134.16	y = 58,009.28x + 197,874.53	0.9988	5547.81	0.03	0.09	1.17	0.15	0.59	99.52
Progesterone	70.56–131.04	y = 27.36x + 40.39	0.9983	3845.49	0.97	0.32	0.85	0.56	0.81	99.74
Retinoic acid	7.49–13.91	y = 330.11x + 191.91	0.9961	1641.15	0.12	0.37	1.81	1.17	2.57	101.09
Spironolactone	70.14–130.26	y = 41.21x − 35.69	0.9993	9420.07	0.13	0.39	0.44	0.72	1.38	99.50
Tranexamic acid	145.60–270.40	y = 31,315.34x − 82,589.51	0.9988	5499.49	20.01	6.60	1.52	1.87	2.91	99.85

HPLC, high-performance liquid chromatography; ANOVA, Analysis of variance; CV, coefficient of variation; LOD, limit of detection; LOQ, limit of quantification. The acceptance criteria were: $R^2 > 0.99$; F (significance of regression) >> 4.67; discrepancy < 2%; repeatability and intermediate precision < 5%; and recovery = 100% ± 2%. All analytical ranges were considered adequate to analyze the concentrations used.

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

$$LOD = \frac{3.3 \times \sigma}{IC} \quad (1)$$

$$LOQ = \frac{10 \times \sigma}{IC} \quad (2)$$

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

2.6. Preparation of API Cream Samples for the Compatibility Study

The creams were prepared using the following general protocol:

1. The required quantity of each ingredient for the total amount to be prepared was calculated.
2. Each ingredient was accurately weighed.
3. The API was placed in an adequate EMP jar, and combined with a small amount of Levigant, according to each API's properties.
4. The Cleoderm™ was further added into the mixture, and the formulation was mixed using an electronic mixing device (FagronLab™ EMP, Scheßlitz, Germany) for 4 min at a medium mixing speed.
5. The product was then passed through a roll mill (FagronLab™ TRM Ointment Mill, Saint Paul, MN, USA) thrice.
6. The final product was packaged in airless precise-dose, light-resistant bottles and labeled.
7. The creams were then immediately assayed at T = 0 and stored at room temperature (15–30 °C) for the duration of the study.

2.7. Compatibility Study: Physico-Chemical Evaluation

Samples of the products were collected at 0, 15, 30, 60, 90, 120, 150, and 180 days after compounding. The samples were tested by HPLC for the API content at predetermined

time points to verify the API's stability in Cleoderm™. Aliquots were withdrawn from the initial creams and properly diluted to obtain working solutions at the concentrations described for the chromatographic conditions. All collections were tested 6 times. The evaluation parameter was the percentage of recovery in relation to T = 0, using the HPLC method (results given in percentage ± standard deviation). The pH was also evaluated using Hanna (Nieuwegein, Netherlands) equipment with direct reading of the sample. All samples were checked prior to the pH and HPLC tests for their general characteristics, namely: color, odor, phase separation, and possible changes in viscosity (visual); if any change was observed, the study was interrupted.

2.8. Compatibility Study: Microbiological Evaluation

The samples were analyzed for antimicrobial effectiveness testing (AET) at 0 and 180 days after compounding, following the general USP guidelines (Antimicrobial Effectiveness Testing) [24]. The aliquots were withdrawn from the initial product and diluted to obtain working solutions. The microorganisms used in the AET were: *Candida albicans*, ATCC 10231; *Aspergillus brasiliensis*, ATCC 16404; *Escherichia coli*, ATCC 8739; *Pseudomonas aeruginosa*, ATCC 9027; and *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; afterwards, the suspension was inoculated in the sample, respecting the range of 0.5% to 1.0% in relation to the weight of the total product.

A neutralizing agent (polysorbate and lecithin) was added to the dilution of the sample prepared for plating. 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 physico-chemical study.

3. Results

Studies indicating stability were conducted to determine if the methods used were fully validated and adequate for identifying the decomposition of the APIs by chromatographic analysis. The decomposition profiles of the APIs (Table 4) were similar under the different stress conditions. Acidic, alkaline, heat, and UV light stresses affected all APIs tested. Once the forced degradation profiles of the APIs were determined, the stability of the APIs in Cleoderm™ was assessed.

Table 4. Summary of the study indicating the stability of the APIs.

Active Pharmaceutical Ingredient	HCl (%d)	NaOH (%d)	UV (%d)	Heat (%d)	H_2O_2 (%d)
Benzoyl peroxide	29.99	−99.54	6.09	−93.40	22.10
Cyproterone acetate	−6.38	−99.34	8.11	6.61	5.58
Estriol	147.94	163.85	3.23	609.02	12.57
Metronidazole	11.57	−99.97	16.86	−6.67	12.29
Niacinamide	−92.17	−86.23	25.70	−6.11	−3.23
Progesterone	8.91	−68.25	−0.89	11.36	4.29
Retinoic acid	−31.76	0.99	−47.85	−27.78	−7.05
Spironolactone	−1.22	−96.74	−9.42	−2.79	6.98
Tranexamic acid	−5.54	−78.56	−8.78	−7.17	3.54

API, active pharmaceutical ingredient; HCl, hydrochloride acid; NaOH, sodium hydroxide solution; UV, ultraviolet; H_2O_2, peroxide. The results are presented as the average of six replicates at the working concentration. %d, percentage of discrepancy between the active pharmaceutical ingredient peak without stress factors (negative control) and the peak of a sample subjected to one of the accelerated degradation factors. A discrepancy of less than 2% indicates non-significant degradation of the API.

Validation of all methods of analysis was performed and all results (Table 3) met the respective acceptance criteria, confirming the methods' suitability for this work's objective.

To verify the physical stability and homogeneity, the visual appearance of the topical creams was also evaluated at each sampling time. When the drug content was within

the specifications, no phenomena such as phase separation, sedimentation, flocculation, coalescence, or creaming were observed throughout the entire period of the study. The chemical stability results are presented in Table 5, expressed as the relative percentage of recovery (initial sampling time = 100%), and the absolute amounts of the APIs are presented in Figure 1. The relative percentage of recovery should lie within 90% to 110% of the labeled amount for the creams to be considered stable.

Table 5. APIs' chemical stability in Cleoderm™, measured by HPLC.

Active Pharmaceutical Ingredient	Elapsed Time (Days)	% Recovery (Room Temperature, 15–30 °C)			
		Low Concentration	pH	High Concentration	pH
Benzoyl peroxide (2.5% and 10.0%)	T = 0	100.00 ± 1.00	4.82	100.00 ± 0.19	4.72
	T = 7	98.92 ± 0.20	4.64	100.22 ± 0.26	4.67
	T = 14	99.33 ± 0.17	4.63	99.90 ± 0.43	4.70
	T = 30	99.50 ± 0.28	4.65	100.00 ± 0.30	4.69
	T = 60	98.48 ± 0.37	4.68	99.36 ± 0.28	4.52
	T = 90	98.30 ± 0.94	4.67	99.88 ± 0.35	4.48
	T = 120	98.06 ± 0.74	4.63	99.03 ± 0.30	4.51
	T = 150	98.15 ± 0.30	4.66	98.72 ± 0.19	4.50
	T = 180	97.52 ± 0.46	4.65	99.23 ± 0.37	4.51
Cyproterone acetate (0.5% and 2.0%)	T = 0	100.00 ± 0.85	5.01	100.00 ± 0.22	5.01
	T = 7	99.60 ± 0.44	5.06	99.73 ± 0.44	5.03
	T = 14	99.06 ± 0.52	5.04	99.51 ± 0.36	5.01
	T = 30	99.11 ± 0.67	5.07	99.19 ± 0.24	5.06
	T = 60	99.65 ± 0.26	4.98	98.94 ± 0.07	4.92
	T = 90	98.95 ± 0.40	4.44	94.91 ± 0.52	4.94
	T = 120	99.69 ± 0.47	4.99	93.06 ± 0.41	4.94
	T = 150	99.09 ± 0.77	4.99	92.13 ± 1.13	4.94
	T = 180	99.10 ± 0.52	4.99	92.09 ± 0.90	4.94
Estriol (0.1% and 1.0%)	T = 0	100.00 ± 0.70	5.15	100.00 ± 0.53	5.13
	T = 7	100.63 ± 0.21	5.09	102.13 ± 1.09	5.05
	T = 14	101.53 ± 0.06	5.15	100.84 ± 0.44	5.20
	T = 30	102.74 ± 0.59	5.07	100.72 ± 1.18	5.11
	T = 60	102.94 ± 1.33	5.13	101.67 ± 0.94	5.17
	T = 90	103.31 ± 1.88	5.14	102.43 ± 1.73	5.15
	T = 120	104.32 ± 0.27	5.17	101.48 ± 0.40	5.09
	T = 150	104.30 ± 0.53	5.06	101.24 ± 0.49	5.06
	T = 180	103.23 ± 0.20	5.09	101.95 ± 0.43	5.05
Metronidazole (0.75% and 5.0%)	T = 0	100.00 ± 0.82	5.13	100.00 ± 0.41	5.14
	T = 7	98.07 ± 0.75	5.16	97.08 ± 1.26	5.15
	T = 14	98.57 ± 0.31	5.21	97.89 ± 0.49	5.23
	T = 30	98.25 ± 1.08	5.18	97.88 ± 1.24	5.20
	T = 60	98.00 ± 0.19	5.12	98.49 ± 0.92	5.11
	T = 90	98.06 ± 0.56	5.08	98.18 ± 0.37	5.11
	T = 120	98.00 ± 0.34	5.09	97.51 ± 0.14	5.11
	T = 150	97.80 ± 0.64	5.10	95.80 ± 0.56	5.13
	T = 180	97.62 ± 0.45	5.12	96.72 ± 0.76	5.12
Niacinamide (1.0% and 5.0%)	T = 0	100.00 ± 0.37	5.38	100.00 ± 0.21	5.39
	T = 7	98.99 ± 0.27	5.22	100.42 ± 0.53	5.50
	T = 14	98.95 ± 0.48	5.24	99.90 ± 0.39	5.51
	T = 30	99.57 ± 0.43	5.26	99.91 ± 0.42	5.52
	T = 60	99.55 ± 0.34	5.22	99.72 ± 0.12	5.42
	T = 90	99.76 ± 0.46	5.23	99.76 ± 0.32	5.50
	T = 120	99.26 ± 0.52	5.25	100.54 ± 0.21	5.51
	T = 150	99.94 ± 0.45	5.22	100.05 ± 0.67	5.53
	T = 180	99.72 ± 1.05	5.25	100.84 ± 0.57	5.50

Table 5. *Cont.*

Active Pharmaceutical Ingredient	Elapsed Time (Days)	% Recovery (Room Temperature, 15–30 °C)			
		Low Concentration	pH	High Concentration	pH
Progesterone (0.5% and 2.0%)	T = 0	100.00 ± 0.26	5.18	100.00 ± 0.32	4.98
	T = 7	100.05 ± 0.27	5.16	98.91 ± 0.30	4.97
	T = 14	99.58 ± 0.49	5.18	97.58 ± 0.19	4.97
	T = 30	99.64 ± 0.31	5.23	98.31 ± 0.37	4.55
	T = 60	99.48 ± 0.13	5.21	98.71 ± 0.11	4.94
	T = 90	99.22 ± 0.34	5.13	97.87 ± 0.64	4.95
	T = 120	98.73 ± 0.76	5.14	97.41 ± 0.31	4.91
	T = 150	98.82 ± 0.38	5.12	97.24 ± 0.32	4.93
	T = 180	99.18 ± 0.20	4.96	98.09 ± 0.06	5.06
Retinoic acid (0.025% and 0.1%)	T = 0	100.00 ± 1.11	5.09	100.00 ± 1.81	5.10
	T = 7	100.08 ± 0.44	4.94	99.64 ± 0.19	5.05
	T = 14	99.49 ± 0.52	4.91	100.16 ± 0.31	5.03
	T = 30	99.78 ± 0.24	4.90	99.70 ± 0.27	5.04
	T = 60	94.00 ± 0.36 *	5.01	92.35 ± 0.28 *	4.90
Spironolactone (1.0% and 5.0%)	T = 0	100.00 ± 0.28	5.12	100.00 ± 0.36	5.08
	T = 7	99.79 ± 0.29	5.15	99.60 ± 0.32	5.04
	T = 14	96.52 ± 0.26	5.12	98.63 ± 0.09	5.09
	T = 30	96.39 ± 0.13	5.13	98.02 ± 0.10	5.07
	T = 60	96.21 ± 0.31	5.06	97.34 ± 0.36	5.09
	T = 90	96.74 ± 0.10	5.07	97.39 ± 0.12	5.05
	T = 120	92.19 ± 0.35	5.09	95.39 ± 0.30	5.03
	T = 150	92.46 ± 0.12	5.07	95.13 ± 0.36	5.05
	T = 180	93.10 ± 0.06	5.09	94.96 ± 0.21	5.06
Tranexamic acid (1.0% and 5.0%)	T = 0	100.00 ± 0.87	5.91	100.00 ± 0.85	6.38
	T = 7	99.68 ± 0.77	5.83	100.31 ± 0.68	6.52
	T = 14	99.20 ± 0.38	5.79	100.45 ± 0.64	6.45
	T = 30	98.61 ± 0.76	5.69	101.11 ± 0.79	6.43
	T = 60	98.78 ± 0.64	5.59	100.75 ± 0.28	6.43
	T = 90	99.14 ± 1.38	5.51	100.83 ± 1.31	6.41
	T = 120	98.92 ± 0.97	5.53	100.82 ± 0.88	6.46
	T = 150	98.90 ± 0.65	5.49	101.05 ± 0.53	6.44
	T = 180	99.69 ± 2.25	5.55	100.87 ± 0.40	6.41

* Samples showed the formation of an undetermined peak impurity in the chromatographic profile. API, active pharmaceutical ingredient; HPLC, high-performance liquid chromatography; T, time (days).

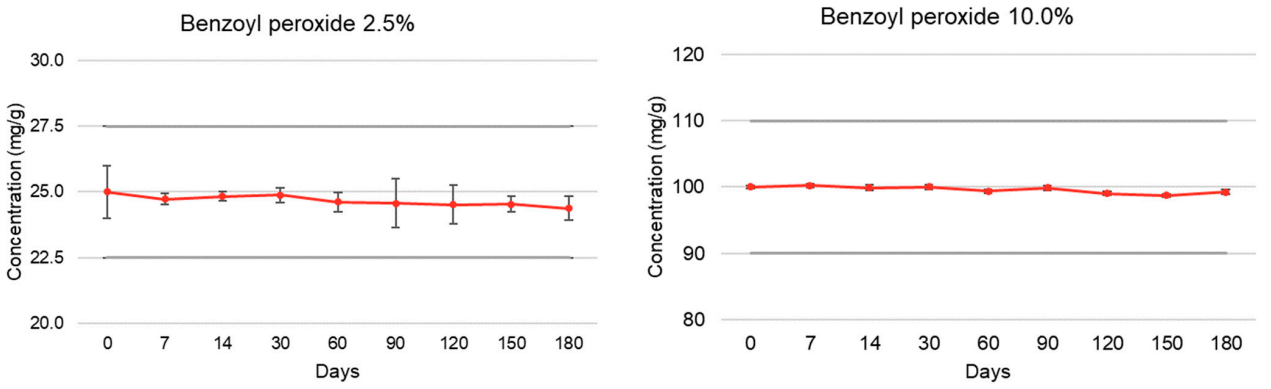

Figure 1. *Cont.*

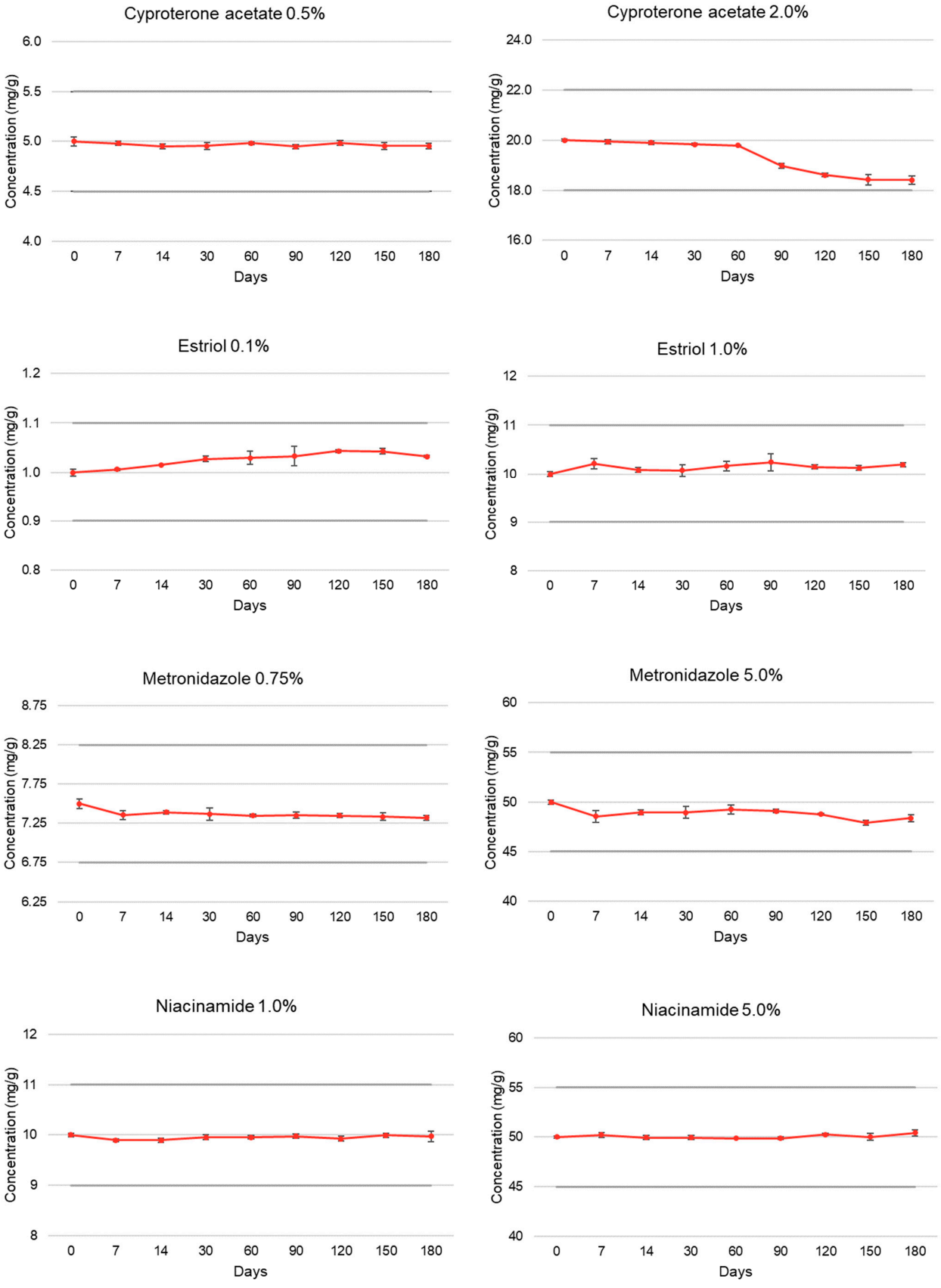

Figure 1. *Cont.*

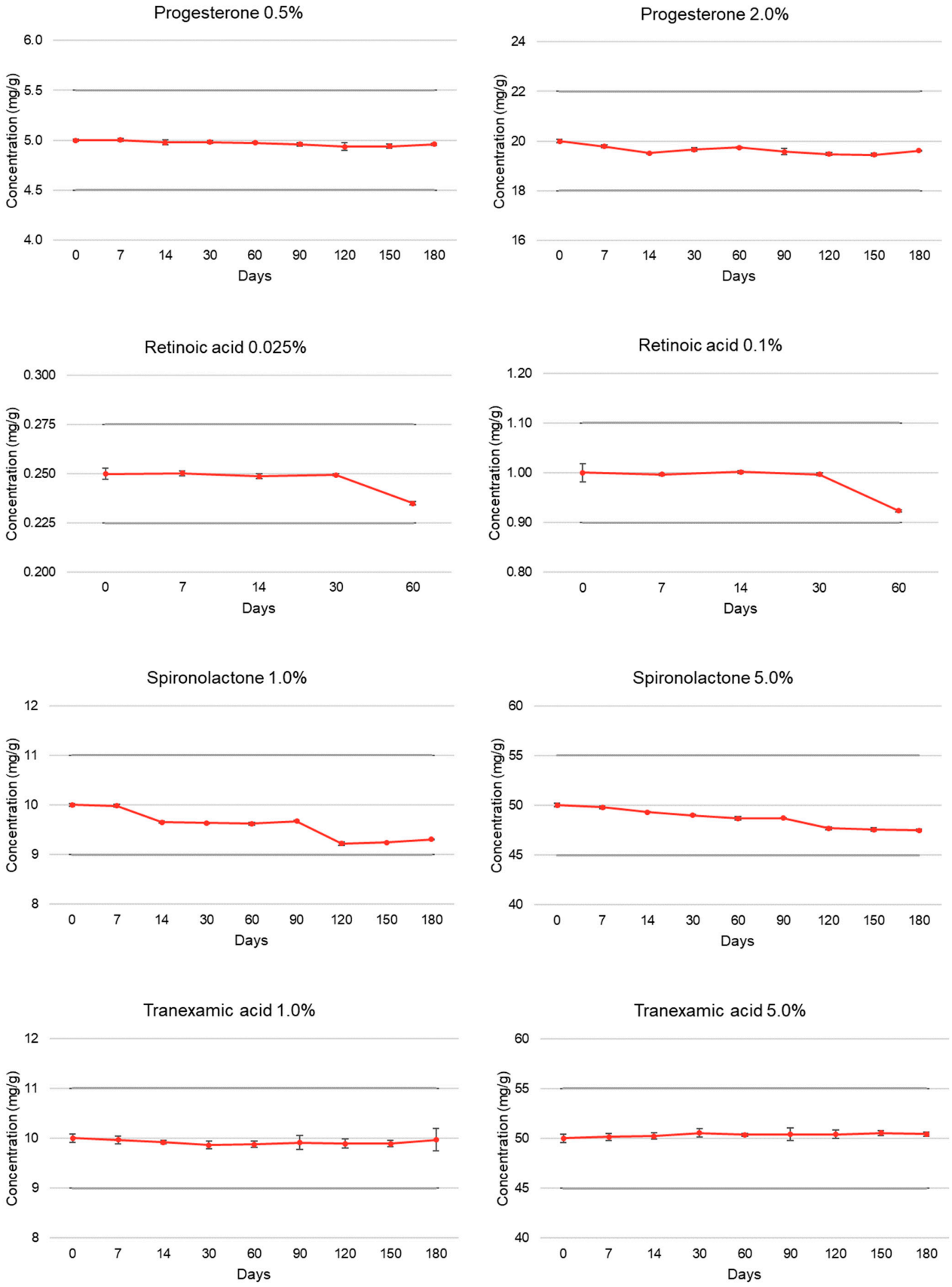

Figure 1. Active pharmaceutical ingredients" chemical stability in Cleoderm™, in absolute amounts, measured by high-performance liquid chromatography.

The microbiological stability of the formulations was tested to assess the safety and product quality during processing and storage. After 14 and 28 days, the number of microorganisms in all formulations was below the acceptance limit during the entire evaluation period (Table S1). There was no increase in the number of bacteria, with two or more log reductions in the number of initially inoculated CFUs.

After finalizing the microbiological and physico-chemical evaluation, our study observed a beyond-use date (BUD) of 180 days for most formulations stored at room temperature, except for retinoic acid, which had a BUD of 30 days. For this case, even though the API was still within the acceptance limit after 60 days, we observed the formation of an undetermined peak of impurity in the chromatographic profile (Figure 2), which was possible to identify due to the stability-indicating studies described in Section 2.4.

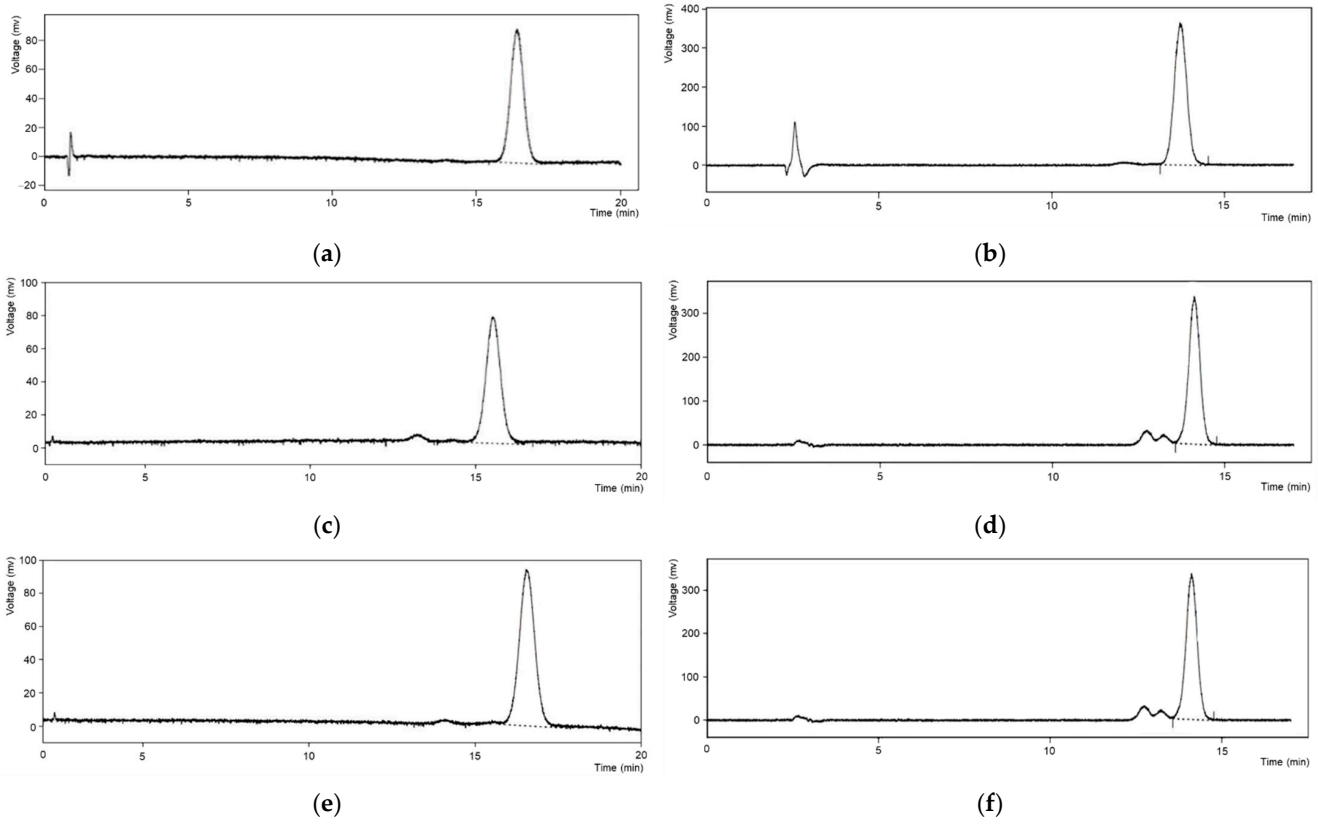

Figure 2. Chromatograms of retinoic acid in Cleoderm™. (**a**) Initial standard; (**b**) Standard at 60 days; (**c**) initial 0.025%; (**d**) 0.025% at 60 days; (**e**) initial 0.01%; (**f**) 0.01% at 60 days.

4. Discussion

In bracketed designs for stability studies such as this one, only the extreme design factors (i.e., strength, concentration, dosages, container size) are tested at each time point, allowing for the assumption that the stability of any intermediate condition will present the same behavior as the extremes tested [25,26]. In this study, all formulations were compounded using the same vehicle, making the bracketed design an advantageous method to evaluate the stability of different APIs, covering most of the prescription possibilities. The limitation of this study lies in the fact that the results apply only to the ranges of concentrations tested here and only the vehicle used (Cleoderm™); therefore, no extrapolation to lower or higher concentrations and other vehicles can be made.

The microbiological evaluation was performed according to the USP Standard 51. The AET was performed by spiking the formulation samples with a series of microorganisms, including Gram-positive, Gram-negative, and bacilli bacteria, as well as yeast and mold, each in their own separate sample [24]. After periods of 14 and 28 days, the samples were

monitored for the growth of the added organisms and had to meet a certain threshold for the preservative system to be deemed effective.

The USP Standard 795 requires a BUD of no longer than 30 days for water-containing topical/dermal formulations [27]. For this reason, the longer BUD of 180 days presented in this study offers increased convenience for both the compounding pharmacist and the patient.

Previous studies have shown the stability of benzoyl peroxide in commercially available products compared with extemporaneously made gel formulations [28] and in foaming emulsions [29], but no precise BUD has been defined. Metronidazole has previously shown a stability of up to 60 days in an emulsion and extemporaneous oral liquid compounds [30,31], and 180 days in a microemulsion [32]. The stability of niacinamide in commercially available creams and emulsions [33] and liposomes [34] has also been evaluated. No BUD was established. Progesterone has also been shown to remain stable in cream preparations for up to 60 days [35]. Spironolactone has been shown to remain stable for up to 30 days in emulsions [30] and up to 60 days in extemporaneous oral liquid compounds [31]. The stability of retinoids has been well studied in the literature, and it is well known that their stability is a common limiting issue in many formulations [36]. In most cases, either in solutions, cosmetic formulations, pharmaceuticals, or commercial products, there is a significant decline in retinoid concentrations at different time intervals [36,37]. To our best knowledge, no studies are available regarding the stability of cyproterone acetate or estriol in topical formulations.

5. Conclusions

Based on this study and according to the parameters established above, we concluded that formulations containing benzoyl peroxide, cyproterone acetate, estriol, metronidazole, niacinamide, progesterone, spironolactone, and tranexamic acid in Cleoderm™ remain stable for 180 days, and formulations containing retinoic acid remain stable for 30 days when stored at room temperature (15–30 °C), in relation to the parameters evaluated. As this is a bracketed study, it is expected that formulations compounded with different concentrations within the tested range will present the same BUD as the ones observed here for low and high concentrations of the API within the vehicle.

The longer BUD established in this study presents increased convenience for the compounding pharmacist and the patient, confirming that Cleoderm™ is a suitable candidate vehicle for compounding different dermatological formulations with adequate stability, presenting itself as a good alternative to commercially available treatments that cannot be personalized.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cosmetics9050092/s1. Table S1: Antimicrobial effectiveness testing of the APIs in Cleoderm™.

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