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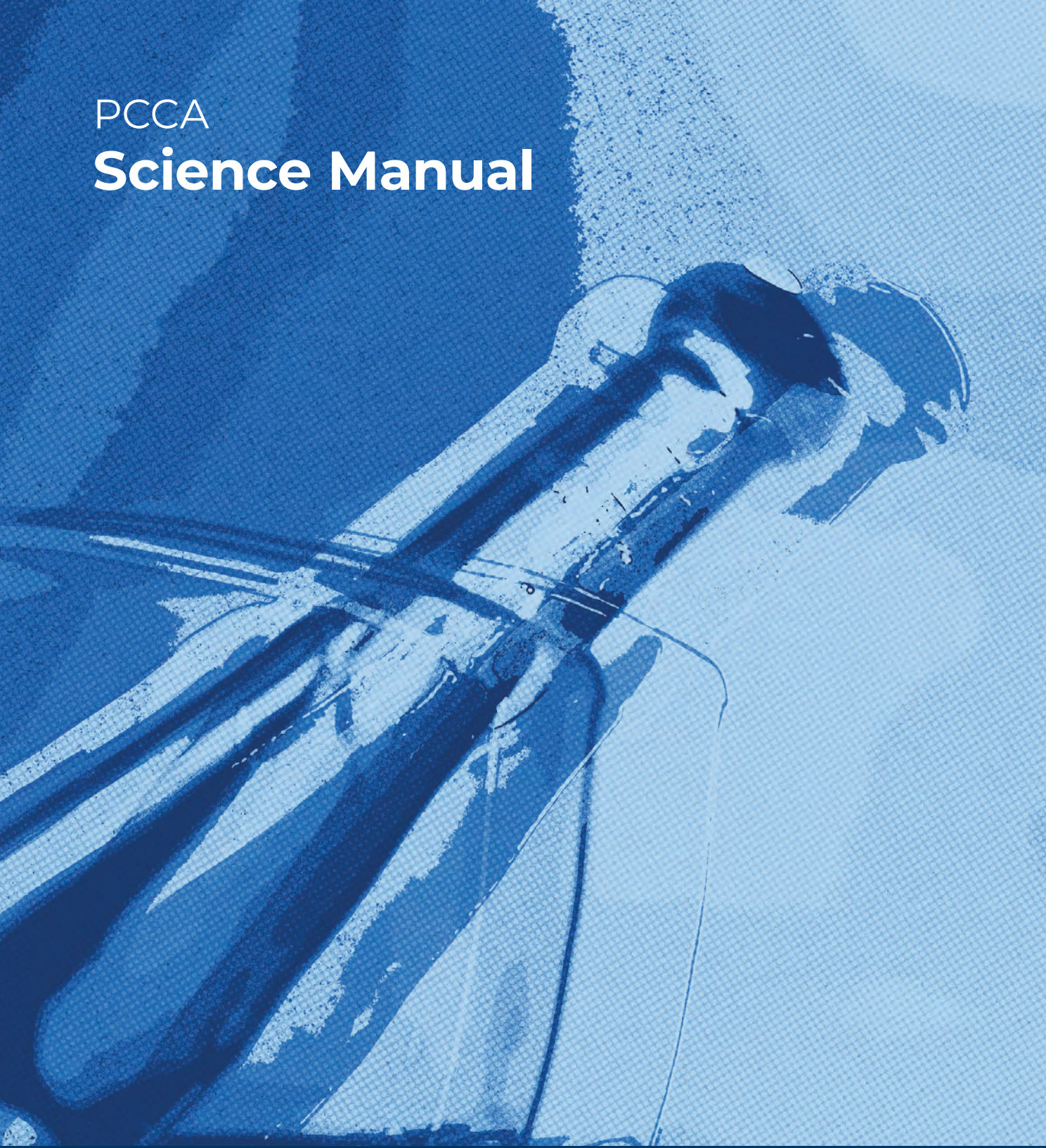

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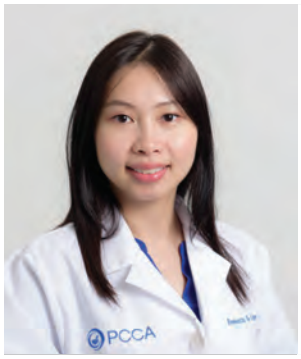

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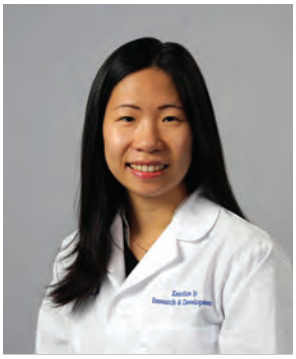

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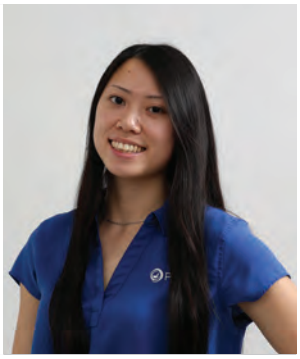

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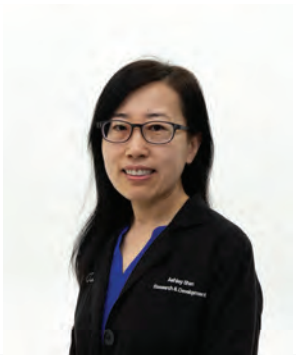

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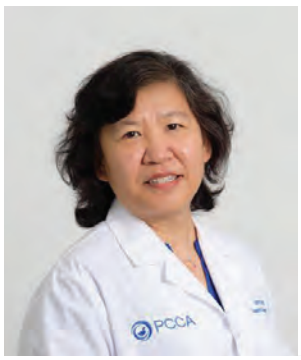

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Hormone Replacement Therapy

Anhydrous VersaBase® HRT
Atrevis Hydrogel®

Archives of Dermatological Research (2024) 316:291
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ORIGINAL PAPER

In vitro evaluation of the percutaneous absorption of progesterone in anhydrous permeation-enhancing base using the Franz skin finite dose model and mass spectrometry

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Abstract
Progesterone is used for hormone replacement therapy through various routes of administration. This study was conducted to (a) evaluate the stability of progesterone in a proprietary anhydrous permeation-enhancing base (APEB) and the efficiency of its skin permeation, and (b) determine the appropriateness of mass spectrometry as a method of analysis for permeated progesterone. Using a proven stability-indicating ultra-performance liquid chromatographic method, the compounded hormone (100 mg progesterone/g APEB gel) was determined to be physically and chemically stable at room temperature for six months. Skin permeation analysis using the Franz skin finite dose model and mass spectrometry imaging showed an optical density of 1699 for the permeated progesterone compounded in APEB and 550 for the permeated progesterone in a water containing VBC, which is a statistically significant different ($P = 0.029$). The study suggests that APEB can be used as a compounding base for effective skin permeation of progesterone, and mass spectrometry is a reliable method for visualization and quantitative analysis of permeated progesterone.

Keywords Anhydrous permeation-enhancing base · Hormone replacement therapy · Progesterone

Introduction

Progesterone is a steroid sex hormone that plays an important role in menstruation and early pregnancy. It thickens the uterine lining that creates an appropriate environment for fertilized egg implantation. Lower levels of progesterone cause the uterine lining to break down and commences the menstrual cycle.

The level of progesterone decreases during menopause and causes uncomfortable symptoms including hot flashes, night sweats, headaches, mood changes, depression, vaginal dryness, weight gain and discomfort during sex; hence, progesterone hormone therapy is commonly recommended [1]. Progesterone may be administered through various routes including oral, intranasal, transdermal, vaginal, rectal, intramuscular, subcutaneous, and intravenous injection. Its route of administration should be judiciously selected because it affects the pharmacokinetics and efficacy of progesterone. For example, the oral bioavailability of progesterone is less than 10% due to its poor gastrointestinal tract absorption and extensive first-pass metabolism by the liver [2, 3]. Vaginal administration is preferred because it provides local therapeutic actions, ease of use, rapid absorption, and higher bioavailability but side effects including irritation and bleeding have been observed [4, 5]. The convenience, safety and non-invasiveness of transdermal delivery make it more popular. However, it has low bioavailability due to skin barriers [6].

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This drawback is overcome by compounding progesterone with an appropriate base that improves its solubility and skin permeability. A prior study determined that liquid crystalline nanoparticles facilitated permeation of progesterone up to 65% of applied dose which was 6-fold better than the aqueous suspension [7], suggesting that a non-aqueous topical base for use in compounding with lipophilic drugs, such as progesterone, could provide for better delivery.

Additionally, a previous study showed that percutaneous absorption of progesterone in an anhydrous permeation-enhancing gel base (APEB, also called PCCA VersaBase® Anhydrous HRT), was significantly higher than absorption of progesterone in a water-containing VersaBase® Cream (VBC) [8]. However, the amount of progesterone distributed in various skin layers is unknown. The present study evaluates the skin distribution of progesterone, after application of an extemporaneously compounded progesterone gel and cream (APEB and VBC bases, respectively), using the Franz skin finite dose model and mass spectrometry. The method qualitatively and quantitatively analyzes the distribution of progesterone in human skin samples.

Materials and methods

Compounded topical formulation

The compounded formulations used in this study contained progesterone USP (PCCA Special Micronized) in either APEB or VBC from Professional Compounding Centers of America (Houston, TX). The ingredient's list for the APEB gel is as follows: cyclopentasiloxane, caprylyl methicone, PEG-16 macadamia glycerides, polysilicone-11, PEG-12 dimethicone/PPG-20 crosspolymer, 1,2-hexanediol, phosphatidylcholine, jojoba esters, isopropyl jojobate, jojoba alcohol, and tocopheryl acetate. In contrast, the ingredient's list for the aqueous VBC is as follows: water, emulsifying wax NF, ethylhexyl stearate, cyclopentasiloxane, sorbitol, aloe barbadensis leaf juice, glycerin, tocopheryl acetate, lactobacillus ferment, citric acid, disodium EDTA, sodium levulinate, sodium anisate, and sodium benzoate.

Evaluation of the stability of progesterone compounded in APEB

a) UPLC parameters The stability of progesterone was determined using Ultra-Performance Liquid Chromatography (UPLC) to separate progesterone from its degradation products. The mobile phase consisted of water with 0.1% trifluoroacetic acid (A), acetonitrile with 0.1% trifluoroacetic acid (B), acetonitrile (C), and 50% methanol in water (D). A 2.1 mm x 100 mm Waters UPLC Cortecs C18+ column with 1.6-µm particle size (Waters Corp., Milford, MA) maintained at 25°C was used to separate molecules with a flow rate of 0.35 mL/min. Molecules were detected at a 243 nm wavelength. Samples (1 µL) were injected into the Aquity UPLC separation module equipped with a photodiode array detector (Waters Corp.). Data were acquired and analyzed using Empower version 3 (Waters Corp.).

b) Validation of UPLC The UPLC method was validated in accordance with the ICH "Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology Q2(R1)" [9] and the USP General Chapter: <1225> Validation of Compendial Procedures [10] for system suitability, linearity, accuracy, precision, robustness, solution stability, and specificity. This validation was performed to demonstrate that the analytical procedure for progesterone 100 mg/g in APEB gel was a stability-indicating method, accurate and precise for the assay of progesterone. The specificity was assessed by subjecting the compounded formulation (0.4 g) to heat (80°C for 3 days), acid hydrolysis (0.2 N HCl at 40°C for 3 days), base hydrolysis (0.2 N NaOH at 40°C for 3 days), and oxidation (6% H_2O_2 at 40°C for 3 days).

To prepare the stock solutions for UPLC analysis, a precise quantity of progesterone was dissolved in HPLC grade methanol. The stock solution was then diluted with methanol to various concentrations as standard solutions.

Sample concentrations with weight corrections were calculated as previously described [11] and shown below.

Measured concentration:

$$c_x = ((A_r - b)/m) \cdot w_x/w_1 \cdot a \cdot [1 - l] \cdot (d_x \cdot v_x)/w_2$$

where c_x = measured concentration of progesterone in sample.

$w_{x\,=}$ target weight of progesterone topical gel in APEB sample.

w_1 = actual weight of progesterone topical gel in APEB sample.

a = assay of progesterone standard.

l = loss of drying content in progesterone standard.

d_x = specific gravity of solvent.

v_x = volume of second dilution sample.

w_2 = actual weight of second dilution sample.

c) Chemical stability of progesterone in solution The stability of progesterone standard and sample solution in HPLC vials was evaluated for 1 day at 10 °C. Fresh solutions were prepared on day 0 and analyzed against a freshly prepared working standard solution according to the test methods. The solutions were re-analyzed against a freshly prepared

working standard solution according to the test method at each time interval.

d) Stability of progesterone in APEB A Beyond-Use Date (BUD) study for progesterone 100 mg/g APEB gel was performed using the validated sample preparation and instrumental conditions. All formulations were prepared and stored at room temperature (20°C – 25°C). The progesterone potency was analyzed after 14, 90, 120, 150 and 180 days. The recovery percentage of progesterone at the indicated time point was used to assess the stability of progesterone. The results from the stability study were reported as mean ± standard deviation from the two samplings. A retention of 90–110% of the initial concentration was considered stable.

In vitro permeation test of progesterone compounded in APEB

a) Preparation of skin samples Donated human cadaver abdomen skin tissues from one donor were purchased from BioIVT (Westbury, NY, USA). They were stored at − 20 °C in tightly sealed water-impermeable plastic bags until use. The skin samples were thawed at room temperature prior to use and soaked in a diffusion medium at room temperature for at least 30 min. The skin samples were then examined for signs of diseases and physical damage. To avoid skin cell death due to repeated freeze-thaw, no skin tissue was re-frozen.

b) Franz skin finite dose model The skin samples were fitted into the Franz diffusion system (surface area of 1.77 cm^2) as previously described [12]. Briefly, the recipient chamber of the Franz system was filled with 12 mL of a receptor solution (Phosphate-Buffered isotonic Saline (PBS), pH 7.4 ± 0.1 plus 0.5% of 2-hydroxypropyl-β-cyclodextrin and 50 µg/mL of gentamycin), and the chamber chimney left open to ambient laboratory conditions. All Franz diffusion chambers were connected to a circulating water bath and the skin surface temperature was maintained at 32.0 °C ± 1.0 °C. The receptor medium contained within each diffusion cell was mixed at approximately 600 RPM using a magnetic stirring device to ensure appropriate homogenization of the release drug in the receptor phase throughout the experiment.

A Precision LCR meter set at low voltage alternating current was used to determine the integrity of the skin samples. An electrical resistance of 4 kΩ was used as a cut-off value; any skin with resistance less than 4 kΩ was rejected [13] as this value corresponds to a tritiated water permeability coefficient of 4.5×10^{-3} cm/h [14]. A positive displacement pipette was used to apply ~ 10 mg/cm^2 of the compounded formulation (progesterone in either APEB or VBC) on each skin sample, and a pellet pestle was used to spread the product across the skin surface. The amount of progesterone in the skin sample was determined after 4 h.

Quantification of permeated progesterone

The skin surface was cleaned twice with Kimwipes, and skin samples were snap-frozen by direct immersion in liquid nitrogen. The frozen samples were trimmed to the center and sectioned at 12 µm thickness using a Thermo NX50 cryostat (Thermo Scientific, San Jose, CA) and sections collected onto a standard microscope slide. Serial sections were collected of each sample for H&E staining using standard protocols [15]. Sections were dried in a desiccator for 15 min before application of the matrix.

Fiducial points were placed on the slide with the skin samples and an optical image was acquired at 4800 dpi using an Epson V600 flatbed scanner. A solution of 40 mg/mL 2,5-dihydroxybenzoic acid in 90% methanol, 0.1% trifluoroacetic acid was sprayed over the surface of the tissue section using HTX M5 Robotic Reagent Sprayer (HTX Technologies, Chapel Hill, NC). Matrix was applied over 8 passes with a solvent flow rate of 0.100 mL/min, a track speed of 1200 mm/min, a track spacing of 2 mm, a criss-cross track pattern, a nitrogen gas pressure of 10 psi, a nozzle height of 40 mm, and a nozzle temperature of 75 °C.

Mass spectrometry images were collected on a Bruker timsTOF fleX QTOF mass spectrometer (Bruker Daltonics, Billerica, MA) in positive ion mode at 40 µm spatial resolution over the m/z range 100–1000. Voltages were optimized for the detection of progesterone as follows: Funnel 1 RF of 250.0 Vpp, Funnel 2 RF of 300.0 Vpp, Multipole RF of 350.0 Vpp, Collision Energy of 5.0 eV, Collision RF of 1300.0 Vpp, Quadrupole Ion Energy of 5.0 eV, Focus Pre TOF Transfer Time of 60 µs, and a Pre Pulse Storage Time of 6.0 µs. The laser was operated at 10,000 Hz and 1000 laser shots were summed up per pixel. FlexImaging 7.0 was used for data acquisition and SCiLS Lab Pro 2023c was used for data visualization and analysis.

Microscopy images of the stained sections were acquired using a Hamamatsu NanoZoomerSQ Digital Slide Scanner (Hamamatsu, Bridgewater, NJ).

Statistical analysis

A t-test was used to determine statistical differences among mean values of progesterone contributed from the two different delivery bases. *P* values less than 0.05 were considered statistically significant. All results are expressed as mean ± SD of treatments in triplicates.

Results

Validation of the UPLC method

A stability-indicating assay method is critical for the determination of a BUD [16]. The UPLC method used to analyze the chemical stability of progesterone in APEB was therefore evaluated. The methods, results, and acceptance criteria for each parameter are shown in Table 1.

Results from the determination of system suitability, linearity, accuracy, precision, robustness, and solution stability are all valid within the acceptable criteria. The specificity was performed to determine if the UPLC method could separate and detect progesterone from its degradation products under stressed conditions. Figure 1 shows the chemical stability of progesterone in APEB at room temperature (untreated); the elution time (8.30 min) of progesterone is almost the same as the standard progesterone (elution time = 8.32 min). Progesterone preparations exposed to heat at 60°C for 14 days or subjected to oxidation with 20% H_2O_2 for 7 days showed similar profile as the untreated preparation (Fig. 1).

Stress conditions with acid and base, however, showed minor degradation products which did not interfere with the progesterone chromatogram (Fig. 1; Table 2). All these results suggest that the UPLC method met all the necessary criteria and was considered stability-indicating for the detection and quantitation of progesterone in APEB samples.

Stability of progesterone in APEB at room temperature

After the UPLC method was validated, it was used to determine the stability of progesterone in APEB at room temperature for 180 days. Based on the UPLC analysis, the concentration of progesterone, a reflection of its potency, ranged between 96 and 106% relative to its initial concentration (Fig. 2). The results are within the acceptable limits of 90–110%.

Table 1 Assay method validation parameters, corresponding methods, acceptance criteria, and results. The method was demonstrated to be linear, precise, accurate, robust, and suitable as well as stability-indicating

Parameter	Methods	Acceptance Criteria	Results
System suitability	Six replicate injections of progesterone standard solution at 100% of the target concentration were analyzed.	RSD ≤ 2.0% Tailing factor ≤ 2.0 Column Efficiency ≥ 2000	RSD = 0.224% Tailing factor = 1.22 Column Efficiency = 29,740
Linearity	Progesterone standard solutions were prepared at 5 concentrations (50%, 75%, 100%, 125%, and 150% of assay level). Each solution was injected in triplicate to generate a calibration curve.	$R^2 \geq 0.998$	Regression line: y = 20600x – 10,800 $R^2 = 0.999973$
Accuracy	Spiked placebo at 50%, 100% and 150% of the assay level was prepared in triplicate. Each solution was injected and quantitated against a 5-point calibration curve.	95.0% ≤ Recovery ≤ 105.0% RSD ≤ 5.0%	Recovery at 50% = 98.3%; RSD = 0.400% Recovery at 100% = 99.2%; RSD = 0.242% Recovery at 150% = 99.7%; RSD = 0.105%
Precision (Repeatability)	A reference standard solution of progesterone at 100% assay level was prepared and analyzed 6 times.	RSD ≤ 2.0%	RSD = 0.224%
Precision (Intermediate)	Prepared triplicate spiked samples at 3 concentrations (50%, 100%, and 150% of target concentration). Samples were assayed on two different UPLC systems and on the same UPLC on two different days.	RSD ≤ 2.0%	RSD from day 1, UPLC 1 at 50% = 0.65%; at 100% = 0.53%; at 150% = 0.26% RSD from day 2, UPLC 1 at 50% = 0.40%; at 100% = 0.24%; at 150% = 0.11% RSD from day 1, UPLC 2 at 50% = 0.60%; at 100% = 0.53%; at 150% = 0.17%
Robustness	Determined with variations in column temperature, organic mobile phase content, and flow rate using spiked placebo	Resolution ≥ 1.5	Column temperature ± 2 °C Organic mobile phase ± 2% Flow rate ± 3%
Solution stability	Inter-day prepared standard solution and sample solution were analyzed.	% Difference Relative to Day 0 ≤ 2.0%	Stability (days) = 1 at 10 °C % Difference from standard = 1.0% % Difference from sample = 0.1%
Specificity	Placebo and sample were analyzed to detect degradation under stressed conditions (forced degradation studies)	No chromatogram interference 5–30% degradation in at least one stressed condition Resolution ≥ 1.5 Purity flag: No	Conditions: Thermal, oxidation, acid, and base Degradation: Yes (Thermal, acid, and base) Interference: No Resolution: ≥ 1.5 Purity flag: No

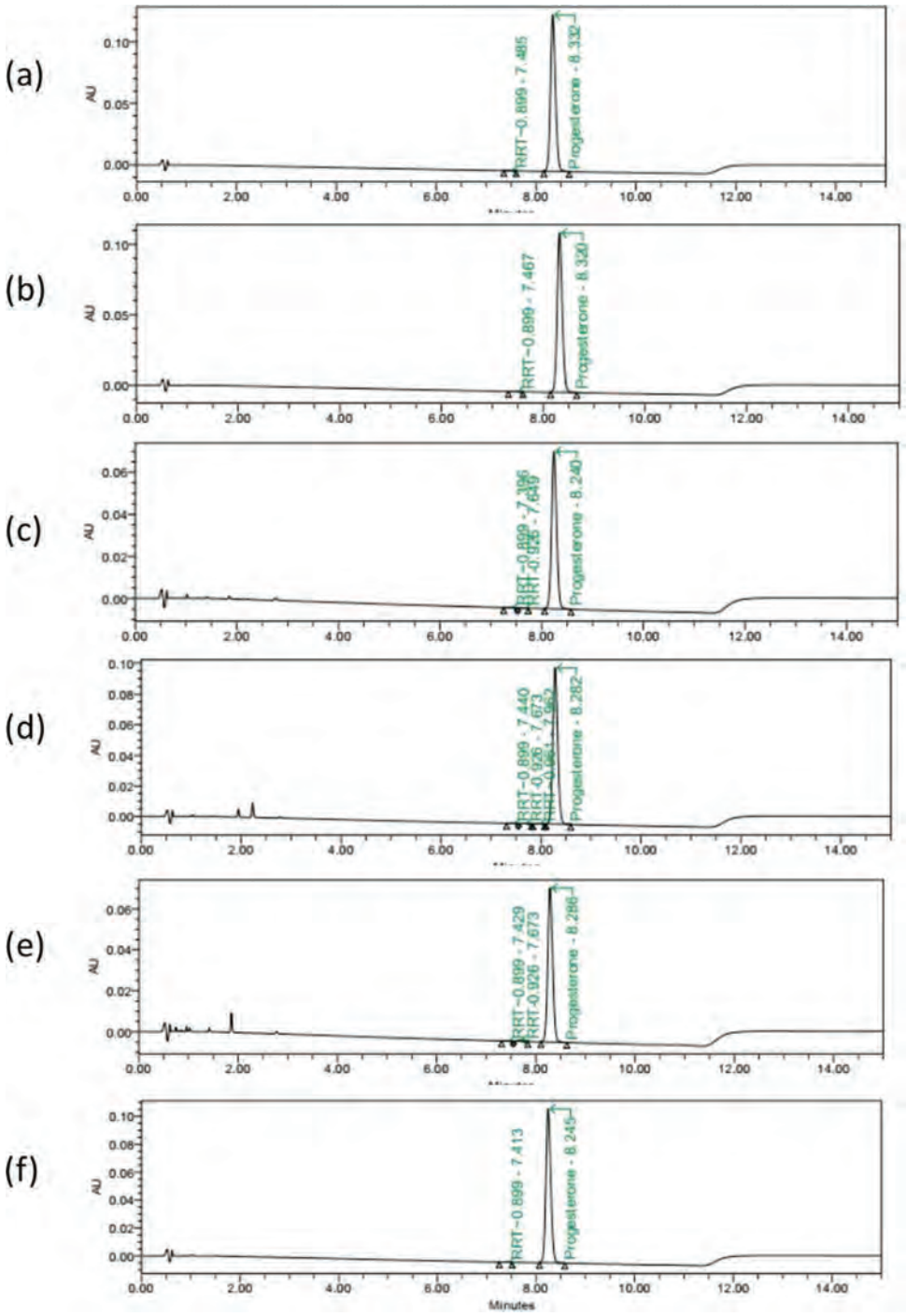

Fig. 1 UPLC chromatograms of progesterone in APEB subjected under different conditions. Progesterone was prepared as (**a**) standard or extracted from compounded progesterone 100 mg/Gm APEB (**b**) at room temperature, after (**c**) thermal (60oC for 14 d), (**d**) acidic (0.1 N HCl for 7 d at room temperature), (**e**) basic (0.1 N NaOH for 7 d), and (**f**) oxidation (20% H2O2 for 7 d) degradation

Table 2 Degradation of progesterone topical gel under stressed conditions

Degradation Condition	Degradation (%)	Resolution	Purity Flag
Thermal	34.84%	8.58	No
Acid	11.13%	1.81	No
Base	34.29%	3.39	No
Oxidation	3.90%	4.85	No

Skin permeation of progesterone in APEB compared with progesterone in VBC using mass spectrometry imaging

The percutaneous absorption of progesterone in APEB and VBC formulations were compared using mass spectrometry imaging. Three skin samples for each formulation were analyzed. Figure 3A, B and C show the

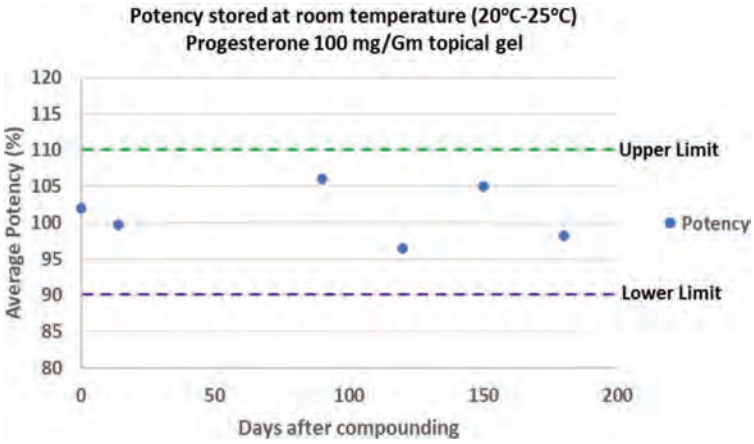

Fig. 2 Stability of progesterone in APEB. The compounded formulation was stored at room temperature for 180 days and analyzed for the level of progesterone by UPLC. The dashed lines represent the specific range of 90.0–110.0% acceptable limits. The values are an average of four UPLC injections from two extractions

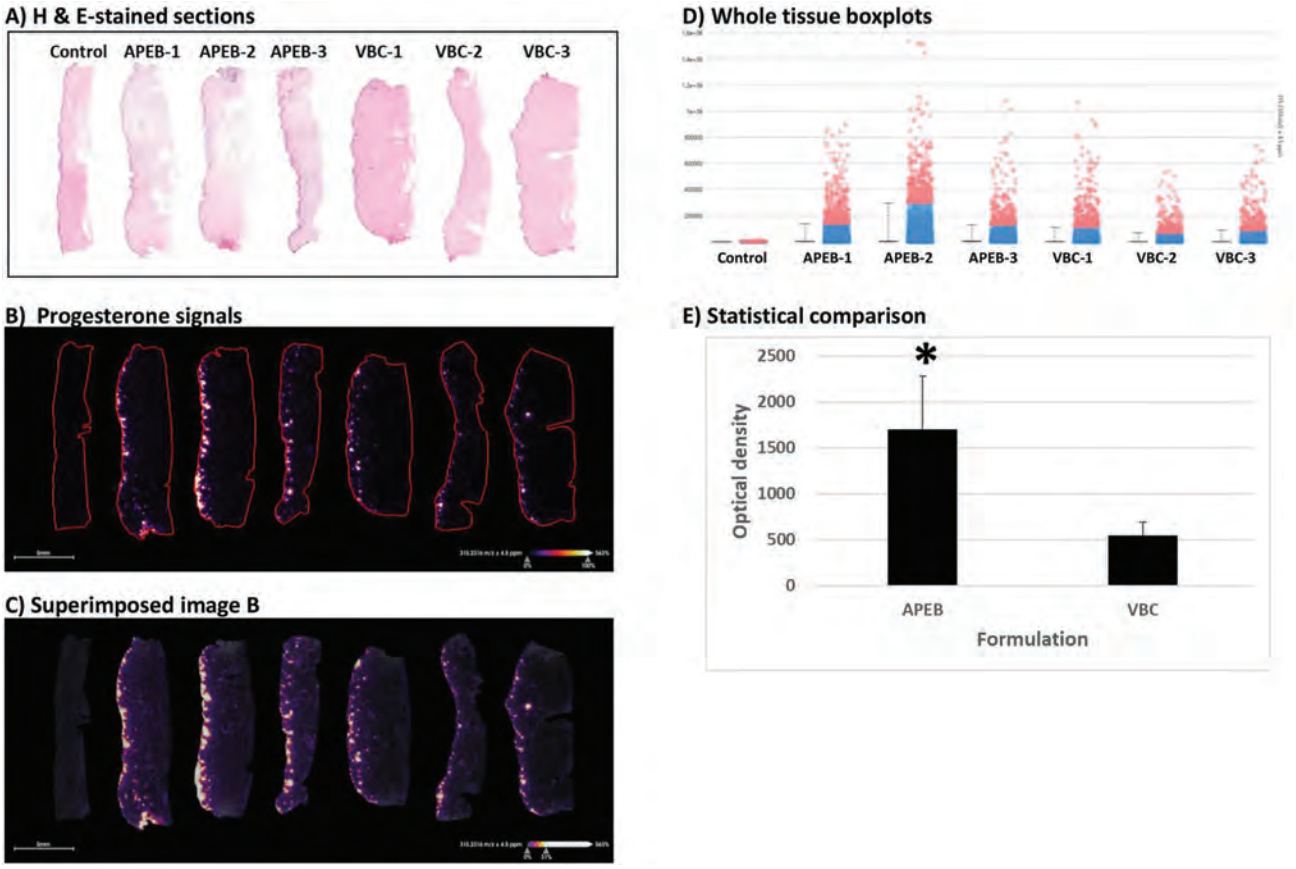

Fig. 3 Mass spectrometric analysis of the skin permeated progesterone. Progesterone compounded in either APEB or VBC was allowed to penetrate the skin using the Franz diffusion system. Skin sections were stained (**A**), and mass spectrometry images were collected (**B**). Panel (**C**) shows the superimposed images in **B**. All spectrum signals were quantitated and presented as bar graphs in (**D**). The average optical densities of the progesterone signals were compared using the two compounding bases (**E**)

H&E-stained tissues, progesterone signals and superimposed images. The bright signals in Fig. 3B represent progesterone which were measured quantitatively as shown in Fig. 3D. Each dot in the boxplot represents a single spectrum of progesterone from the tissue. The blue dots are spectra that are within the second and third quartile of the data and the pink dots are outside the second and third quartile of the data. Progesterone in APEB showed an average optical density of 1699 compared with progesterone in VBC which had an average optical density of 550. Statistical analysis shows a significant difference ($P = 0.029$) in the skin permeation of progesterone compounded in APEB or VBC (Fig. 3E). These results suggest that anhydrous APEB is potentially a better base to

compound progesterone compared with water containing VBC.

Discussion

The chemical composition of the compounding base is critical for the skin permeation of APIs. The methods used to quantitatively analyze the API are equally important. The present study shows that the anhydrous APEB is potentially better than the water containing VBC in facilitating the percutaneous permeation of progesterone, which was analyzed with mass spectrometry methods. A stability-indicating UPLC assay method was employed to evaluate the stability of progesterone in APEB. The stability of progesterone in APEB within 180 days at room temperature suggests a reasonable BUD for this formulation (Fig. 2). The mass spectrometry imaging method showed better penetration of progesterone through the skin when compounded in APEB, whereas VBC was relatively less superior (Fig. 3).

The better efficacy of APEB may be attributed to its chemical composition. APEB contains phosphatidylcholine and jojoba esters, which may have contributed to its permeation-enhancing property. Phosphatidylcholine, for example, was found to significantly enhance the permeation of caffeine [17] and jojoba esters, which are similar to the sebaceous lipids of the skin [18], facilitated the skin permeation of lipophilic molecules [19]. Phosphatidylcholine and jojoba esters could have made the lipid barrier of the skin more fluid, and the nonpolar nature of progesterone could have made it easier to blend with these oily components, thereby facilitating its passive permeation through the skin. The anhydrous property of APEB also provides for better solubility of the nonpolar progesterone and unfavorable conditions for growth of microorganisms, without compromising the efficacy of the skin permeation.

Although three donated skin samples were enough to show statistical differences between APEB and VBC, better statistical power and more significant comparisons could have been obtained if greater numbers were used. Moreover, additional compounding bases available in the market could have been compared with APEB in facilitating skin permeation of progesterone. The age of the donor and the organ source of the skin are important factors that may affect the skin permeation of progesterone in APEB and therefore should have been considered. In spite of these limitations, this study shows that APEB efficiently facilitates the skin permeation of progesterone and the results from this preclinical in vitro evaluation can be used as basis for a warranted clinical trial in using APEB as a base for compounded topical progesterone in hormone replacement therapy.

Conclusions

APEB is a promising base for delivery of progesterone through the skin. The compounded formulation tested is stable at room temperature with a BUD of six months, an extended period that underscores the benefits and convenience of using this formulation. Moreover, mass spectrometry imaging is an effective method for the quantitative analysis of progesterone that permeated through the skin. The results from this study suggest that APEB is a reliable option for compounding pharmacists in the preparation of compounded progesterone for hormone replacement therapy.

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Author contributions "D.B. and A.S.B. provided conceptualization and supervision. G.S., K.I., E.H.S., S.T.L., I.B. and G.F. were in charge of the formal analysis, investigation and validation. B.C.V. wrote the main manuscript text. All authors reviewed the manuscript."

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors DB, GS, KI, IB and ASB are affiliated with PCCA, the manufacturer of the proprietary anhydrous permeation-enhancing base (APEB), and water-containing VersaBase® Cream (VBC) discussed in the manuscript.

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Testosterone Therapy for Late-Onset Hypogonadism: A Clinical, Biological, and Analytical Approach Using Compounded Testosterone 0.5–20% Topical Gels

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Abstract: Testosterone is integral to men's sexual and overall health, but there is a gradual decline in the ageing male. The topical administration of testosterone is a valuable option as a supplement (replacement) therapy to alleviate hypogonadal symptoms. The clinical efficacy of a compounded testosterone 5% topical gel was assessed retrospectively in a male patient in his seventies by evaluating the laboratory testing of the serum total testosterone and the results of a validated androgen deficiency questionnaire. After treatment, the patient's hypogonadal symptoms improved and the serum total testosterone level achieved was considered clinically optimal. The skin permeation of the testosterone topical gel (biological testing) was evaluated in vitro using the Franz finite dose model and human cadaver skin, and it is shown that testosterone can penetrate into and through ex vivo human skin. Testosterone therapy is often prescribed for extended periods, and consequently, it is crucial to determine the beyond-use date of the compounded formulations. The analytical testing involved a valid, stability-indicating assay method for compounded testosterone 0.5% and 20% topical gels. This multidisciplinary study shows evidence supporting topically applied testosterone's clinical efficacy and the compounded formulations' extended stability. Personalized, topical testosterone therapy is a promising alternative in current therapeutics for hypogonadal patients.

Keywords: hormone supplement (replacement) therapy; testosterone; late-onset hypogonadism; androgen deficiency; case study; skin permeation; physicochemical stability; personalized preparations; pharmacy compounding

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1. Introduction

Male hypogonadism is a broad term that refers to androgen deficiency or low testosterone in men. Andropause is a common form of hypogonadism that refers to the gradual testosterone decline in the ageing male. This decline is predominantly due to a failure in the function of the hypothalamic–pituitary unit, and thus, it is also referred to as late-onset hypogonadism [1]. The prevalence of andropause is difficult to estimate due to the heterogeneity of the populations and methodologies used in the studies, as well as a lack of consistency of reference biochemical values [2]. However, it is suggested that it is a widespread condition that currently affects over one-quarter of men in the United States. Studies indicate that the prevalence of andropause is likely to increase due to the ageing population and co-morbidities such as obesity, diabetes, dyslipidemia, hypertension, metabolic syndrome, and chronic obstructive pulmonary disease (COPD) [3–5].

Testosterone plays an integral role in men's sexual and overall health. As such, androgen deficiency is linked to a complexity of symptoms, such as erectile dysfunction, loss of libido, depressed mood, lethargy, osteoporosis, and declining muscle tone. It is essential to consider testosterone supplement (replacement) therapy to improve the quality of life and long-term health of late-onset hypogonadal patients. There are several options for testosterone supplementation, such as implantable pellets, parenteral dosing, sublingual or buccal administration, and topical application. Orally administered testosterone suffers

an extensive first-pass effect and it is associated with liver toxicity. Pellets are implanted at the doctor's office; it is an invasive procedure that lacks the flexibility of dosing adjustments. Parenteral dosing via intramuscular (IM) injection is a common form of administering testosterone, but there are difficulties in achieving a steady-state release upon dosing. Subcutaneous administration is preferable, better tolerated, and more convenient than IM. Sublingual or buccal administration, although non-invasive and effective, requires multiple dosing throughout the day, which often leads to poor compliance. Currently, topical application appears to be the most physiologic route of dosing as it allows for a continual, steady-state release of testosterone. Topical gels, in particular, at concentrations of testosterone from 1% to 5% have proven to be effective and user-friendly. Topical application is a simple procedure and attention must only be paid to the potential transfer of testosterone to a child or female. Furthermore, compounded topical gels may be customized throughout the treatment to the meet the patient's variable dosing needs [6,7].

This multidisciplinary study aims to test compounded testosterone gels' clinical efficacy, skin permeation, and physicochemical stability. The clinical efficacy is tested in a patient case study, whereas the skin permeation is tested in vitro in a biological laboratory. The physicochemical stability testing is performed in an analytical laboratory. The purpose of this study is to generate clinical and scientific evidence to support testosterone topical therapy in hypogonadal patients.

The case study concerns a male in his seventies with persistent late-onset hypogonadal symptoms. A physician prescribed compounded testosterone 5% topical gel in Atrevis Hydrogel® (PCCA, Houston, TX, USA), a proprietary topical base designed to deliver testosterone through the skin in male patients [8]. The compounded testosterone gel is dispensed in a 75 mL MegaPump® (PCCA), which delivers approximately 0.5 mL of gel per pump. The formula, method of preparation, and storage instructions for the topical gel are displayed in Table 1. The patient applied two pumps of the gel every morning on the inner forearm, thus approximately 50 mg of testosterone daily for six months, as directed by his physician. The patient provided a written consent for the publication of the results. The purpose of this case study is to discuss the management of hypogonadal symptoms with topical testosterone.

Table 1. Formula, method of preparation, and storage instructions for a testosterone 5% topical gel (100 g).

Formula for 100 g	
Testosterone USP Micronized CIII (Soy)	5 g
Propylene Glycol USP	10 g
Base, Atrevis Hydrogel®	85 g
Method of Preparation	
1. Calculate the required quantity of each ingredient for the total amount to be prepared. 2. Accurately weight each ingredient. 3. Prepare a paste of testosterone in propylene glycol. 4. Add the topical base and mix well until a homogeneous, slightly-off-white gel is obtained.	
Storage Instructions	
1. Store in an air-tight, light-resistant container. 2. Protect from light. 3. Store at a controlled room temperature of 20–25 °C.	

The biological testing consisted of evaluating the permeation of a compounded testosterone 10% topical gel over 30 h from a single application on human cadaver skin. The in vitro permeation test (IVPT) was the model used applying the finite dose technique and Franz diffusion cells to dose and culture the skin samples. Data defining the total absorption, absorption rate, and skin content can be accurately determined using the IVPT.

This model is a valuable tool in predicting the in vivo percutaneous absorption kinetics of topically applied drugs [9,10].

Testosterone therapy is often prescribed for extended periods, and consequently, it is essential to determine the beyond-use date (BUD) of the compounded topical gels. The analytical testing consisted of a valid, stability-indicating assay method developed for compounded testosterone 0.5% and 20% topical gels. The physicochemical stability was tested for six months.

2. Materials and Methods

2.1. Case Study

The clinical efficacy of the compounded testosterone gel was assessed retrospectively by inviting the patient to complete the Androgen Deficiency in the Ageing Male (ADAM) questionnaire, a non-invasive screening test to detect low testosterone in males over 40 years of age. It is a self-reported questionnaire with ten 'Yes/No' questions, from libido to work performance. The questionnaire was validated in the general population and was found to have 88% sensitivity and 60% specificity for detecting androgen deficiency. According to Morley et al. [1], the patient is likely to have low testosterone if he answers 'Yes' to question numbers 1 or 7 or if he answers 'Yes' to more than three questions. This primary outcome measure aims to report the change from baseline in the self-reported symptoms of androgen deficiency. As such, the patient was invited to complete the ADAM questionnaire twice by referring to his late-onset hypogonadal symptoms before treatment (baseline) and after treatment.

The secondary outcome measure of this case study was the laboratory testing of the patient's serum total testosterone by the end of the six months of compounded testosterone therapy. The patient was tested in the fasting state on a morning sample. The laboratory testing aims to evaluate the patient's hormone levels, which, together with the clinical assessment, aid in diagnosing and managing the condition. The serum total testosterone is the standard evaluation of androgen status, routinely used in clinical practice [11].

2.2. Biological Testing

The permeation of testosterone was evaluated in vitro by Diteba (Toronto, ON, Canada) according to an internal protocol (DTM-179-R00), which details the method of analysis, skin preparation, dose administration, and sample collection of the study. The IVPT model consists of human torso skin mounted on modified Franz diffusion chambers that maintain the skin at a temperature and humidity that match typical in vivo conditions. A finite formulation dose is applied to the skin's outer surface, and drug absorption is measured by monitoring its appearance rate in the receptor solution bathing the inner surface of the skin (transdermal flux) [9,10]. The drug distribution is measured by analyzing the skin content of the samples by ultra-performance liquid chromatography coupled with ultraviolet detection (UPLC-UV).

The dermatomed cryopreserved skin samples were obtained from three donors (Science Care, Phoenix, AZ, USA), all serologically tested and free of infectious diseases. The frozen skin was thawed at room temperature, cut into small sections, and soaked in a diffusion medium for at least 30 min. The samples were mounted on modified Franz cells with the stratum corneum facing upward. All diffusion cells were mounted in a diffusion apparatus and the diffusion medium (phosphate-buffered saline containing 0.1% Tween 20 and 0.008% gentamicin sulfate) was added to the receptor compartment. Before dose administration, the skin integrity test (transcutaneous electrical resistance) was performed using the Precision LCR meter and the tissues with electrical resistance 3 times higher than the reading of the diffusion medium were used.

Ten formulations of testosterone 10% in Atrevis hydrogel (PCCA, lot 20170518) were accurately weighed (8.8 mg ± 20%) and applied to a total of nine skin sections (three replicates per donor). The diffusion medium in the receptor compartment was stirred magnetically at approximately ~600 revolutions per minute (rpm) and the temperature was

maintained at 32 °C ± 0.5 °C. The diffusion medium samples were withdrawn at 2, 4, 6, 8, 12, 24, and 30 h and replaced with 0.5 mL of fresh diffusion medium. The samples were mixed with an internal standard solution, hexane, and vortexed for 1 min. The organic layer was separated using a dry-ice–acetone bath and then evaporated to dryness under airflow. Following reconstitution, the samples were transferred to a UPLC-UV vial for the analysis of the total absorption (sum of all diffusion medium samples) as well as the rate of absorption (transdermal flux). The ACQUITY UPLC® system (Waters Corporation, Milford, MA, USA) included a UPLC column Acquity UPLC BEH C18 (100 mm × 2.1 mm, 1.7 µm).

The distribution of testosterone within the skin samples was determined at the end of the 30 h diffusion process. The skin samples were washed three times with an internal standard solution, and the resulting washed sample solutions were diluted with 50% ethanol to fit within the range of the calibration curve. The skin was then tape-stripped five times to remove the stratum corneum using 3M Transpore® surgical tape. The tapes of each striped skin were collected into 20 mL of internal standard solution, vortexed for 30 min, and diluted 20 times. Following centrifugation, the resulting clear solutions were transferred to a UPLC-UV vial to analyze the skin content (stratum corneum).

The stripped skin samples were divided into two layers: epidermis and dermis. Each layer was cut into small pieces and transferred to separate test tubes. To each tube was added 10 mL of the internal standard solution; then, the tubes were vortexed for 30 min and centrifuged at 14,000 rpm for 10 min. The resulting clear solutions were transferred to a UPLC-UV vial to analyze the skin content (epidermis and dermis).

2.3. Analytical Testing

The topical gels were formulated in Atrevis hydrogel, which is considered a preserved aqueous vehicle. The USP General Chapter <795> "Pharmaceutical Compounding—Nonsterile Preparations" defaults the BUD of preserved aqueous dosage forms to be 35 days in the absence of a USP-NF compounded preparation monograph, or compounded nonsterile preparation-specific stability information [12]. Data from a stability study using a stability-indicating analytical method would allow the BUD to be extended to the timeframe indicated by the study. To establish a longer BUD on the topical gel formulations, a stability study was conducted.

The sample preparation consisted of setting two batches of 300 g for testosterone 0.5% topical gel (lots 07272017-01 and 07272017-02) and two batches of 300 g for testosterone 20% topical gel (lots 07272017-03 and 07272017-04). The method of preparation is detailed in Table 1. Testosterone USP Micronized CIII (Soy) (lot C181188), Propylene Glycol USP (lot C179804), and the topical base Atrevis hydrogel (lot 20170518) were all obtained from PCCA (Houston, TX, USA). Following preparation, the topical gels were evenly distributed into ten 30 g plastic pumps. According to USP General Chapter <795> "Pharmaceutical Compounding—Nonsterile Preparations", the suggested storage temperature for preserved aqueous dosage forms is controlled room temperature or refrigerator [12]. To assess the stability of testosterone in both storage conditions, one batch of each strength was stored in an environmentally controlled chamber (Thermo-Scientific, Waltham, MA, USA, model number 3940) at a relative humidity of 60% ± 5% and a temperature of 25 °C ± 2 °C (also referred to as controlled room temperature), and the other batch of each strength was stored at a temperature of 5 °C ± 3 °C in another environmentally controlled chamber (Thermo-Scientific, model number 3940). The temperature and humidity were monitored and registered daily.

For the physicochemical stability testing of the topical gels, one test pump was withdrawn from each storage condition (refrigerated temperature and controlled room temperature) at pre-determined time points, as follows: days 0, 7, 14, 28, 42, 60, 90, 123, and 182. Sampling was performed by pumping/dispensing directly from the pumping device.

1. Physical characteristics: The physical characteristics of the topical gels were evaluated by visually inspecting the samples for any changes in color and appearance, observing

for odor change, and testing for pH and viscosity. The observations and values determined on day 0 were used as the basis for any significant change. To assess the appearance and odor change, each sample was evenly spread on a watch glass for visual inspection. To determine the color, each sample was compared against a Munsell color reference chart (Sigma-Aldrich, St. Louis, MO, USA) under an artificial daylight lamp. The pH measurements were made with a Horiba LaquaTwin pH meter (Kyoto, Japan), which was calibrated with certified pH 4.0 and 7.0 buffer solutions before each use. The viscosity of the topical gels was obtained using a RheoSense portable viscometer (microVISC, San Ramon, CA, USA). A microVISC pipette was used to draw about 1 mL of sample. The pipette was inserted into the viscometer, and the measurement was run on the automatic setting.

2. Chemical characteristics: An UPLC method was validated to quantitate the testosterone in the topical gels. The assay determined on day 0 was used as the basis for any significant change. The stability-indicating UPLC method utilized a certified Waters Acquity H-class UPLC system (Milford, MA, USA) equipped with a separation module, a column heater/cooler, an auto-sampler, and an ultraviolet photo-diode array (PDA) detector. The chromatographic method employed a Waters Acquity CSH Phenyl-Hexyl C18 1.7 μm 2.1 mm × 100 mm column (part number 186005407; lots 01093516916627 and 01123628618226). The chromatographic data were acquired and processed using the Waters Empower 3 software.

The UPLC assay testing was designed by the ICH "Harmonised Triparty Guideline: Stability Testing of New Drug Substances and Products Q1A(R2)" [13]. It consists of a reverse phase, gradient chromatographic method with two different mobile phases (A:B) at a ratio of 70:30. The mobile phase A was 0.1% trifluoroacetic acid (lot 55267541; EMD Chemicals Inc., Gibbstown, NJ, USA) in water, whereas the mobile phase B was 0.1% trifluoroacetic acid in acetonitrile (part 34998-4L; Sigma-Aldrich, St. Louis, MO, USA). The method ran for 5.5 min with a flow rate of 0.5 mL/min. The column was heated to 50 °C, while the sample vials were stored at 6 °C in the autosampler. The standard solutions and sample solutions were injected into the separation module at a volume of 1 μL. Chromatographic data were acquired in 3-dimensions from 190 to 400 nanometers. The detection and quantitation of testosterone were performed at 245 nanometers.

The samples for the UPLC were prepared by weighing approximately 0.5 g of each topical gel (testosterone 0.5% and 20%) into a 50 mL centrifuge tube and diluting the gels with 39.5 mL of methanol. Following vortex-mixing and sonication, the solution was centrifuged for 10 min at 6000 rpm. The supernatant was pipetted into a 2 mL tube and micro-centrifuged at 14,000 rpm for 10 min. Finally, the supernatant was transferred to a UPLC vial for assay testing.

3. Method validation: The UPLC assay testing method was validated by analyzing the following parameters: system suitability, linearity, accuracy, precision (repeatability and intermediate), robustness, solution stability, and specificity. The results obtained met the established acceptance criteria for all the parameters. As such, the UPLC assay testing method indicates stability for the compounded testosterone gels. A summary of the parameters, acceptance criteria, and results for validating the UPLC assay testing method is displayed in the Supplementary Materials.

3. Results and Discussion

3.1. Case Study

The patient answered all questions of the ADAM questionnaire twice by referring to his late-onset hypogonadal symptoms before and after treatment with the compounded testosterone gel. Before treatment, a positive ADAM score was obtained since the patient answered 'Yes' to questions 1 and 7, which refer to the self-reported decreased libido and decreased strength of erections. According to this screening test, the patient suffered from male late-onset hypogonadism at baseline. After treatment, a negative ADAM score was

obtained since the patient answered 'No' to all 10 questions. As such, it is assumed that the patient's androgen deficiency improved with the testosterone therapy.

Regarding treatment safety, the patient did not report any side effects. In addition, the patient's PSA (prostate-specific antigen) did not increase while on testosterone supplementation. The patient reported performing the PSA test annually at the routine urologist visit since his sixties. Nevertheless, according to the latest guidelines, the American Urological Association (AUA) recommends against the routine PSA screening of men older than 70 years [14].

The laboratory result obtained after treatment for the patient's serum total testosterone was 1076 ηg/dL. The reference range used by the testing laboratory for testosterone is 250–1100 ηg/dL. According to this range, the level achieved is clinically optimal. However, there is a significant variation in the reference ranges for total testosterone among testing laboratories [11]. There is also conflicting information regarding the ideal method to evaluate the patient's hormone levels (serum, saliva, or urine testing) [15]. According to Livingston et al. [11], significant evidence supports that healthy reference ranges based on population distributions should not be used. Hormone levels are patient-specific, and the results obtained should be correlated with the clinical assessment.

A limitation to consider in this case study is the single data point of serum total testosterone. Ideally, more data points should have been obtained, such as baseline and at 3-months post-treatment, but the retrospective methodology limited the study to the data that had been previously collected.

In this case study, the patient and the physician stated that the results obtained with the testosterone therapy were beyond their expectations.

3.2. Biological Testing

The rate of percutaneous absorption and the distribution of testosterone across the skin layers were determined by the UPLC-UV quantitative method. The rate of percutaneous absorption is presented as a flux, a time-averaged value determined across the sampling period and reported at the mid-point of sample collection (1, 3, 5, 7, 10, 18, and 27 h). Figure 1 illustrates the mean flux (ug/cm^2/hour) plotted as the amount of testosterone absorbed through the skin over time. Upon dose application, it is observed that there is a rise in flux to a peak of testosterone at approximately 8–12 h across all samples, followed by a slow decline. The testosterone penetration profiles were essentially similar for the majority of samples, though differing in magnitude. Sample 2 showed the most significant penetration (11.53 μg/30 h) through the skin, whereas sample 9 showed the least penetration (4.03 μg/30 h).

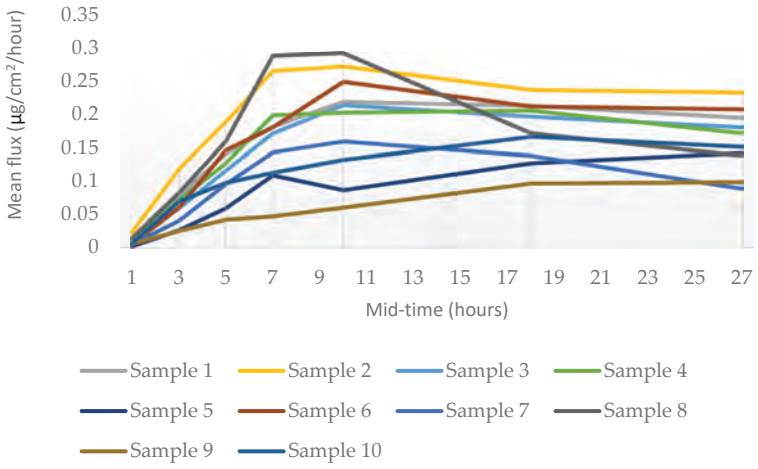

Figure 1. Rate of the percutaneous absorption (mean flux) of testosterone through ex vivo human skin over 30 h from a single application (mean, n = 3 donors).

The data obtained from the in vitro Franz finite dose model indicated that testosterone in all the ten cream formulations can penetrate into and through ex vivo human skin.

The distribution of testosterone following a dose exposure of 30 h to ex vivo human torso skin is presented as mass recovered per dose area. It is observed that the dose applied to the skin's outer surface permeates into and through the stratum corneum, followed by the epidermis and then the dermis, to reach the receptor medium at last. As shown in Figure 2, most of the applied dose was retained in the skin's stratum corneum, accounting for 64–88% across all formulations. The receptor medium content was in the range of 0.4–1.1%, whereas the dermal content was in the range of 0.5–1.6%, and the epidermis content in the range of 1.6–9.4%. The overall mass balance was outstanding, in the range of 99–104% of the applied dose across all ten formulations.

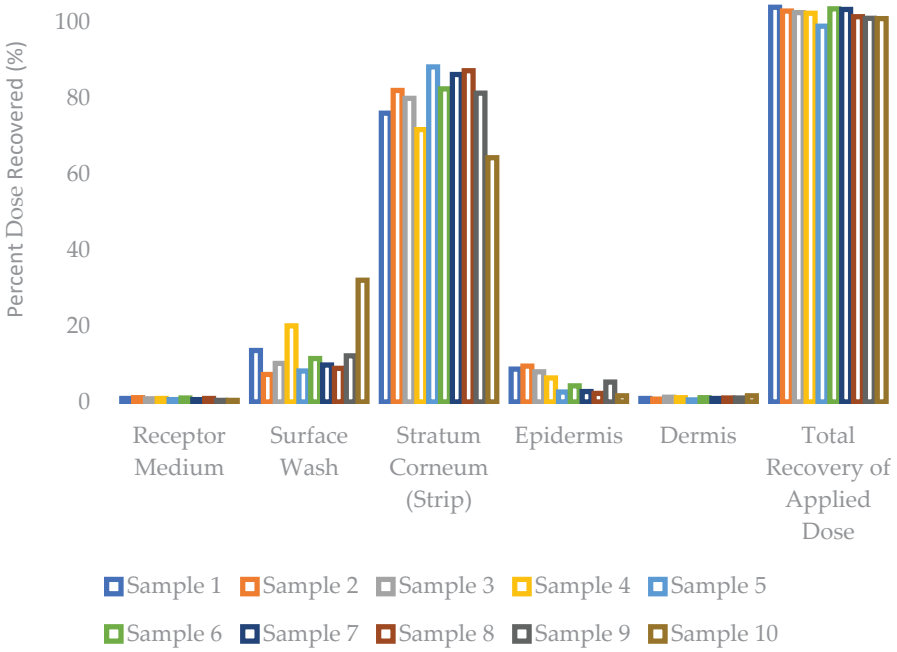

Figure 2. Distribution of testosterone into and through ex vivo human skin over 30 h from a single application (mean, n = 3 donors).

A limitation to consider in biological testing is the in vitro methodology of the study. The ideal setting to evaluate drug permeation is an in vivo human, hence the relevance of the case study described in this multidisciplinary study. Nevertheless, skin models are increasingly used for the testing of transdermal drugs, including those using ex vivo human skin [16]. These studies may only be considered a prediction of the in vivo skin permeation and, although a correlation exists, there are differences to consider when drugs permeate vascularized skin.

3.3. Analytical Testing

The physical characteristics of the testosterone gels were all within the specifications. The testosterone 0.5% topical gel exhibited a faint beige color, whereas the testosterone 20% topical gel exhibited a white color. Both gels presented a smooth, homogeneous appearance and a characteristic odor. A slight separation was noted on the top of the container for the testosterone 0.5% topical gel, stored at a refrigerated temperature from day 123 onwards, but it was considered insignificant. The ranges of viscosity and pH for the testosterone 0.5% topical gel were as follows: 1044.7–1146.0 mPa.s and 5.69–6.00 (room temperature) and 1025.2–1168.0 mPa.s and 5.54–5.89 (refrigerated conditions), respectively. The ranges of viscosity and pH for the testosterone 20% topical gel were as follows: 3452.0–6595.0 mPa.s and 5.67–6.05 (room temperature) and 3933.7–6595.0 mPa.s and 5.74–6.05 (refrigerated conditions), respectively.

The chemical characterization of the topical gels was conducted using UPLC assay testing, which measured the central chromatographic peak, as shown in Figure 3. It provided the mean strength of testosterone per time point. Both the testosterone 0.5% and 20% topical gels, stored at room temperature and refrigerated conditions, remained within the United States Pharmacopeia-National Formulary (USP-NF) specifications of ±10% variation in limits (90–110%) for the duration of the study [12]. The strength of the testosterone 0.5% gel, at room temperature and refrigerated conditions, varied in the range of 98.23–101.70% and 97.83–102.09%, respectively (Figure 4). Likewise, the strength of the testosterone 20% gel, at room temperature and refrigerated conditions, varied in the range of 98.57–102.61% and 98.90–102.00%, respectively (Figure 5).

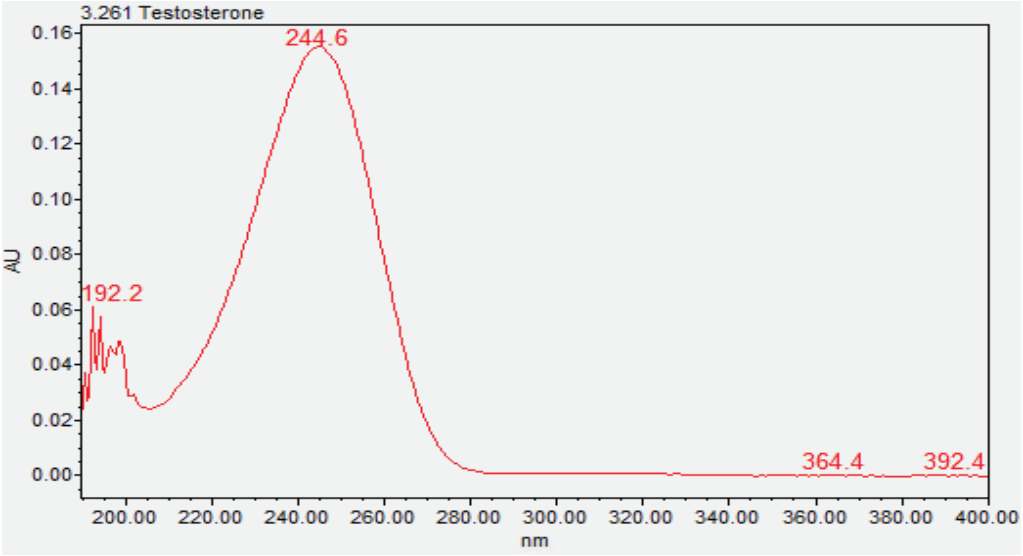

Figure 3. Ultraviolet spectrum of testosterone (3.261, Waters Acquity PDA Detector).

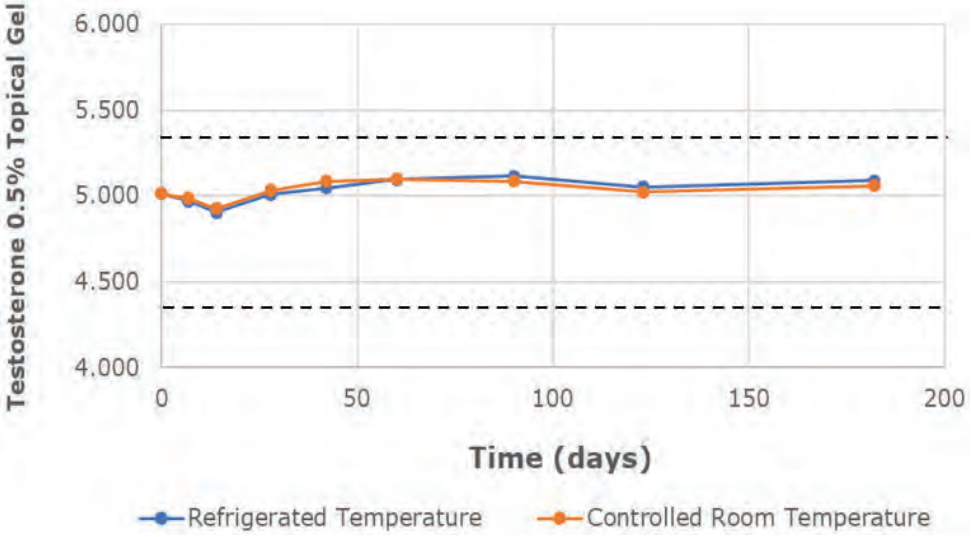

Figure 4. The mean testosterone concentration was 0.5% in the topical gel, stored at refrigerated and controlled room temperatures, over a study period of 6 months. Dashed lines represent the lower and upper limits, corresponding to 90% and 110% of the labeled concentration (5 mg/g), respectively.

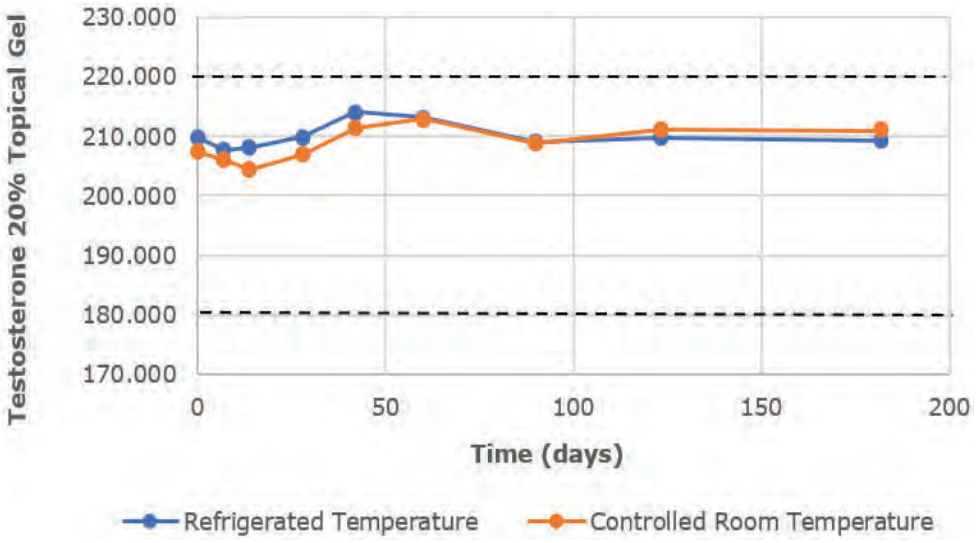

Figure 5. The mean testosterone concentration was 20% in the topical gel, stored at refrigerated and controlled room temperatures, over a study period of 6 months. Dashed lines represent the lower and upper limits, corresponding to 90% and 110% of the labeled concentration (200 mg/g), respectively.

As a result, the testosterone topical gels are physically and chemically stable at both storage conditions for 6 months. The physicochemical characterization of the testosterone 0.5% and 20% topical gels stored at room temperature and refrigerated conditions is reported in detail in the Supplementary Materials.

4. Conclusions

Testosterone therapy may be a key treatment option in andropause patients with laboratory evidence for low testosterone and self-reported symptoms of androgen deficiency. Although not a life-threatening condition, androgen deficiency may have a considerable negative impact on the quality of life of men. Pharmacists are uniquely positioned to work with these patients and their physicians to achieve better treatment outcomes and overall health benefits by providing personalized topical compounded medications. As shown in this case report, a compounded testosterone gel of 1–5% is presented as a promising topical treatment option for testosterone therapy to benefit the complexity of low testosterone symptoms (androgen deficiency questionnaire) and increase the total serum testosterone level (laboratory results). The clinical efficacy is consistent with the results obtained in the biological testing. It is shown in vitro that testosterone is able to penetrate into and through human skin, which correlates with the results obtained in clinical practice. The analytical testing assures pharmacists of a quality formulation with an extended stability of 6 months. As such, compounded testosterone 0.5–20% topical gels are deemed to be stable and effective when treating late-onset hypogonadism.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pharmaceutics16050621/s1. Table S1: Summary of the parameters, acceptance criteria, and results for the validation of the UPLC assay testing method; Table S2: Physical and chemical characterization of the testosterone 0.5% topical gel stored at room temperature for 180 days; Table S3: Physical and chemical characterization of the testosterone 0.5% topical gel stored at refrigerated conditions for 180 days; Table S4: Physical and chemical characterization of the testosterone 20% topical gel stored at room temperature for 180 days; Table S5: Physical and chemical characterization of the testosterone 20% topical gel stored at refrigerated conditions for 180 days; Figure S1: UPLC-PDA chromatograms (absorbance units vs. time) of testosterone in methanol: (A) testosterone 0.5% topical gel (B) and testosterone 20% topical gel (C).

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original draft preparation, M.C.; writing—review and editing, K.I., G.S. and F.B.; supervision, D.B.; project administration, D.B. and M.C. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors are affiliated with Professional Compounding Centers of America (PCCA), the manufacturer of the topical base Atrevis hydrogel discussed in the manuscript. DB, KI, AS, FB and GS are employed by PCCA. BB is a shareholder at PCCA. MC is a consultant for PCCA.

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Oral Mucosa

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ORIGINAL ARTICLE

Journal of Oral Pathology & Medicine WILEY

Dexamethasone solution and dexamethasone in Mucolox™ for the treatment of oral inflammatory ulcerative diseases: A phase II randomized clinical trial

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Abstract

Background: Mucolox™ is a mucosal drug delivery system that prolongs the contact time between the oral mucosa and topical corticosteroids, potentially reducing the need for multiple applications daily. This study aimed to assess the clinical efficacy and tolerability of dexamethasone 0.5 mg/5 mL solution in Mucolox™ for the management of oral inflammatory ulcerative diseases.

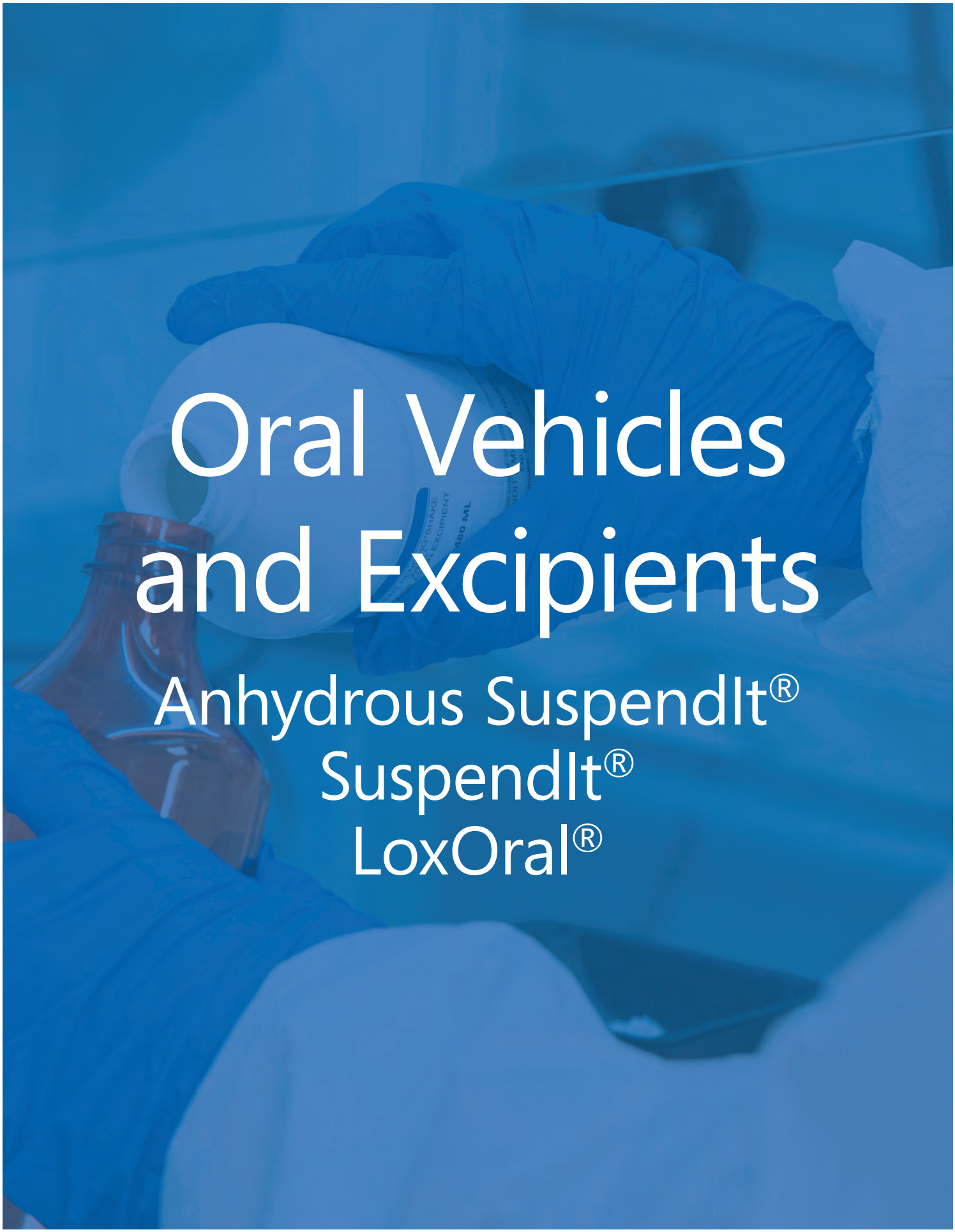
Methods: Participants were randomly assigned to receive dexamethasone 0.5 mg/5 mL in Mucolox™ (Mucolox™ arm) or dexamethasone 0.5 mg/5 mL solution (standard arm) and instructed to swish/gargle for 5 min three times daily. Changes from pre- to posttreatment patient's sensitivity score (0–10 on a visual analog scale), reticulation/erythema/ulceration score, and oral health-related quality of life were evaluated at baseline and at the end of the study period.

Results: Twenty nine patients (75% females) with a median age of 58 years (range 18–79) were enrolled and randomly allocated to the Mucolox™ or standard arm. One subject was excluded. Although statistically significant in both arms, the pre- to posttreatment sensitivity score reduction was higher in the Mucolox™ arm (6.3 vs. 4.4-point reduction). Both arms showed a decrease in the reticulation/erythema/ulceration score between the two visits (7.2 vs. 4.7 [Mucolox™ arm]; 8.0 vs. 4.8 [standard arm]; $p > 0.05$). Mucolox™ in dexamethasone 0.5 mg/5 mL solution was better tolerated when taste and level of comfort were considered.

Conclusions: Both treatments were effective in the management of oral inflammatory ulcerative diseases. Dexamethasone 0.5 mg/5 mL in Mucolox™ was better tolerated and was slightly better in controlling patients' oral sensitivity. Larger studies are needed to confirm these findings in oral inflammatory ulcerative diseases patients.

Trial Registration: ClinicalTrials.gov Identifier: NCT04540133.

Michele Lodolo and Burinrutt Thanasuwat contributed equally as co-first authors.



Oral Vehicles and Excipients

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pharmaceutics

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Article

Analysis of the Physical Characteristics of an Anhydrous Vehicle for Compounded Pediatric Oral Liquids

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Abstract: The paucity of suitable drug formulations for pediatric patients generates a need for customized, compounded medications. This research study was set out to comprehensively analyze the physical properties of the new, proprietary anhydrous oral vehicle SuspendIt® Anhydrous, which was designed for compounding pediatric oral liquids. A wide range of tests was used, including sedimentation volume, viscosity, droplet size after dispersion in simulated gastric fluid, microscopic examination and content uniformity measurements to evaluate the properties of the anhydrous vehicle. The results showed that the vehicle exhibited consistent physical properties under varying conditions and maintained stability over time. This can be attributed to the unique blend of excipients in its formulation, which not only maintain its viscosity but also confer thixotropic behavior. The unique combination of viscous, thixotropic and self-emulsifying properties allows for rapid redispersibility, sedimentation stability, accurate dosing, potential drug solubility, dispersion and promotion of enhanced gastrointestinal distribution and absorption. Furthermore, the vehicle demonstrated long-term sedimentation stability and content uniformity for a list of 13 anhydrous suspensions. These results suggest that the anhydrous oral vehicle could serve as a versatile base for pediatric formulation, potentially filling an important gap in pediatric drug delivery. Future studies can further investigate its compatibility, stability and performance with other drugs and in different clinical scenarios.

Keywords: pediatric drug delivery; extemporaneous preparations; innovative technologies; pharmaceutical development; anhydrous vehicle; sedimentation stability; thixotropic behavior; droplet size; self-emulsifying properties; content uniformity

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JPPT | Compounding and Stability Study

Physicochemical and Microbiological Stability of Ursodiol Oral Compounded Suspensions

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OBJECTIVE In the United States, ursodiol is commercially available as solid dosage forms, which represents a problem for children who cannot swallow capsules or tablets. There is a lack of an age-appropriate formulation for ursodiol, and the extemporaneous preparation of an oral suspension with an extended beyond-use-date (BUD) may represent a good therapeutic alternative for the pediatric population. However, all pharmacists need validated stability studies to prepare oral liquids with high quality and safety.

METHODS Oral compounded suspensions for ursodiol 20 to 60 mg/mL were prepared by adding the contents of ursodiol 300-mg commercial capsules (Actavis, KVK Tech, and Mylan) to a proprietary oral suspending vehicle. The BUD of the oral compounded suspensions was determined by using a valid, stability-indicating analytical method. The physical characterization consisted of observing all samples for appearance and color, and testing for pH. Microbiological stability testing followed the United States Pharmacopeia (USP) Chapter 51: Antimicrobial Effectiveness Testing.

RESULTS The ursodiol oral compounded suspensions exhibited a homogeneous white color and the pH did not change significantly. The potency of the oral suspensions remained within ±10% of the specifications. Considering the microbiological characterization, there was no growth of challenge microorganisms throughout the study for all samples.

CONCLUSION This study demonstrates that ursodiol (Actavis, KVK Tech, and Mylan) is physically, chemically, and microbiologically stable in the oral suspending vehicle at room temperature for up to 6 months.

ABBREVIATIONS APIs, active pharmaceutical ingredients; ATCC, American Type Culture Collection BUD, beyond-use-date; PCCA, Professional Compounding Centers of America; UPLC, ultra performance liquid chromatography; USP, United States Pharmacopeia

KEYWORDS extemporaneous preparations; oral vehicle; pharmaceutical compounding; ursodiol suspensions

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Introduction

Ursodiol (ursodeoxycholic acid) is a naturally occurring bile acid that promotes the dissolution of gallstones rich in cholesterol.[1] It is frequently prescribed in children with cholestasis and cystic fibrosis. Typical dosing in children ranges from 5 to 15 mg/kg, depending on therapeutic indication and age. A standard dose for cystic fibrosis in children is 10 to 15 mg/kg/day administered twice daily.[2] In the United States, ursodiol is commercially available as solid dosage forms (200–500 mg),[3] which represents a problem for children who cannot swallow capsules or tablets, and also for the caregivers who have to adjust the dosage strength to meet the individual patient needs. This can often result in cutting tablets or opening capsules to get the required dose.[4]

There is a lack of an age-appropriate formulation for ursodiol, and the extemporaneous preparation of an oral suspension with an extended beyond-use-date (BUD) may represent a good therapeutic alternative for the pediatric population. Oral suspensions may be compounded to include alternative excipients and active pharmaceutical ingredients (APIs) in dosage strengths specifically adapted for children. Despite its advantages, there are complex aspects in terms of formulation and stability of oral compounded suspensions that need to be accounted for, including solubility; uniformity; taste masking; and physical, chemical, and microbiological stability.[5] Oral compounded suspensions may be rapidly prepared by using ready-made vehicles, also adapted for children, which facilitate the extemporaneous preparation in the pharmacy and make the oral administration more pleasant. Ready-made vehicles are particularly important in a hospital setting, considering the particular and often critical health conditions of hospitalized patients who are likely

to need customized medications in a timely manner.[6] Hospital pharmacists require standard operating procedures and stability-indicating studies to rapidly prepare and dispense oral suspensions with the appropriate quality and safety.

The purpose of this study was to determine the physiochemical and microbiological stability of extemporaneously prepared ursodiol oral compounded suspensions 20 to 60 mg/mL in SuspendIt (PCCA [Professional Compounding Centers of America], Houston, TX), for a period of 6 months. SuspendIt is a ready-made, proprietary, oral suspending vehicle used in pediatrics that includes the following ingredients: water, amorphophallus konjac root powder, monk fruit extract (natural sweetener), xanthan gum, potassium sorbate, sodium benzoate, citric acid, and disodium EDTA. This suspending vehicle has special thixotropic properties; it thickens upon standing to minimize settling of particles and becomes fluid upon shaking to allow convenient administration. It is also sugar-free, paraben-free, dye-free and gluten-free, all of which are advantageous in pediatrics.[7] There are many stability-indicating studies in the literature demonstrating extended BUDs of APIs in this suspending vehicle, most recently azathioprine 10 to 50 mg/mL,[8] pyrimethamine 2 mg/mL,[9] and amitriptyline hydrochloride 1 to 5 mg/mL.[10] Pramar et al[11] previously studied the stability of ursodiol (powder) 50 to 100 mg/mL in this suspending vehicle and determined a BUD of 181 days for the oral compounded suspensions stored at both 5°C and 25°C. However, bulk powder APIs are not always readily available in all settings because of the sourcing complexities. For this reason, the sources of ursodiol chosen for this study were the commercial capsules of ursodiol 300 mg from the pharmaceutical companies Actavis (Parsippany, New Jersey), KVK Tech (Newtown, Pennsylvania), and Mylan (Canonsburg, Pennsylvania).

The aim of this study is to provide all pharmacists with a standardized formula for ursodiol, indicated in pediatrics, which can be rapidly prepared extemporaneously by using commercially available products, and that can be used for a prolonged time owing to an extended BUD.

Materials and Methods

Extemporaneous Preparation. Oral compounded suspensions for ursodiol 20 mg/mL and 60 mg/mL were prepared extemporaneously by adding the contents of ursodiol 300-mg commercial capsules (Actavis, KVK Tech, and Mylan) to the oral suspending vehicle according to the general standardized formula and method of preparation detailed in Table 1. A batch of 495 mL was prepared for each strength and for each commercial source of ursodiol, which resulted in a total of 6 different batches. The oral compounded suspensions were evenly distributed into 6 prescription oval amber plastic bottles and stored in an environmentally controlled chamber (model No. 3940, ThermoScientific, Waltham, Massachusetts at a relative humidity of 60% ± 5% and a temperature of 25°C ± 2°C (also referred to as controlled room temperature). The samples were stored for the study period of 6 months.

Physical and Chemical Stability. The physical and chemical stability of the samples were evaluated on days 0 (baseline), 14, 30, 59, 90, and 181 from the extemporaneous preparation. At each predetermined time point, a study sample of each strength and each commercial source (Actavis, KVK Tech, Mylan) was

Table 1. Standardized Formula and Method of Preparation for Ursodiol 20 mg/mL and Ursodiol 60 mg/mL Oral Compounded Suspensions

Rx		495 mL	
Ursodiol 20 mg/mL oral compounded suspension		Ursodiol 60 mg/mL oral compounded suspension	
Ursodiol 300 mg commercial capsules	33	Ursodiol 300 mg commercial capsules	99
Oral suspending vehicle (SuspendIt)	q.s. 495 mL	Oral suspending vehicle (SuspendIt)	q.s. 495 mL

1. Add a SpinBar to a glass beaker calibrated to 495 mL.
2. Empty the contents of the ursodiol capsules to a mortar and pestle.
3. Add approximately 50 mL of the oral vehicle to the mortar and pestle and mix well to make a smooth paste.
4. Add approximately 90 mL of the oral vehicle to the mortar and pestle in portions while mixing and transfer completely to the glass beaker.
5. Rinse the mortar and pestle with approximately 50 mL of the oral vehicle and add to the glass beaker.
6. Add the oral vehicle up to the final volume of 495 mL and mix well.
7. Store in an air-tight, light-resistant container.

withdrawn from the storage chamber at controlled room temperature, shaken vigorously, and tested for physiochemical and microbiological stability.

Physical Stability Testing. The physical characterization of the oral compounded suspensions consisted of visually inspecting the samples, withdrawn from the storage chamber at controlled room temperature, for appearance and color, and testing for pH. The procedure to assess the appearance and color of the samples was to transfer about 5 mL of sample to a clear test tube after vigorously shaking the bottle. Sample was visually inspected against a white background. Any visually detectable characteristics was added to the description of the appearance. The pH was measured with a Horiba LaquaTwin pH meter (Kyoto, Japan). The pH meter was calibrated at pH 4.0 and 7.0 with certified pH buffer solutions before each use.

Chemical Stability Testing. The chemical characterization of the oral compounded suspensions consisted of assay testing using a stability-indicating ultra performance liquid chromatography (UPLC) developed and validated specifically for the quantitation of ursodiol in this formula. A Waters (Milford, Massachusetts) Acquity UPLC H-class system was equipped with a quaternary solvent manager, a sample manager with a flow-through needle, a column heater, and a photodiode array detector. A Waters Acquity UPLC CSH C18 column (130Å, 1.7 µm, 2.1 mm × 75 mm) was heated to 45°C per the method used. Mobile phases consisted of 0.1% trifluoroacetic acid in purified water (A), acetonitrile (B), and purified water (C), with a flow rate of 0.5 mL/min. The elution of the mobile phases is displayed in the supplemental material (Supplemental Table S1). Each injection had an injection volume of 2 µL and a run time of 8 minutes. The wavelength was set at 210 nm.

At each predetermined time point (0, 14, 30, 59, 90, and 181 days from the extemporaneous preparation), 0.5 mL of each oral compounded suspension was withdrawn from the storage chamber at controlled room temperature and transferred to a 50-mL conical centrifuge tube used for sample extraction. A volume of 9.5 mL and 29.5 mL of diluent (methanol) was added to the conical tube for the ursodiol 20 mg/mL and 60 mg/mL oral compounded suspensions, respectively. Samples were well mixed with a vortex mixer for 30 seconds, sonicated for 2 minutes, and centrifuged at 6000 rpm for 10 minutes. The supernatant was transferred to an UPLC vial for injection.

Method Validation. The UPLC method was validated for linearity and range, accuracy, precision (repeatability and intermediate), solution stability, robustness, system suitability, and specificity. The characteristics and acceptance criteria of the method validation testing are displayed in Supplemental Tables S2 and S3.

A linear relationship was evaluated across the range of the analytical procedure. Ursodiol reference standard solutions were prepared at 5 concentrations as 50%, 75%, 100%, 125%, and 150% of 1-mg/mL target concentration.

Accuracy was carried out by spiking the analyte in blank matrices. Spiked samples were prepared in triplicate at 3 concentrations over a range of 80% to 200% of the target concentration. Spiked samples were analyzed by using the analytical method.

Intermediate precision (within-laboratory variation) was performed on 2 different UPLC systems with 2 different lots of columns and on the same instrument on different days. The assay results were evaluated at 3 concentration levels (50%, 100%, and 150%). Spiked samples at each concentration were prepared in triplicate and assayed. Repeatability was analyzed by 6 replicate injections of ursodiol reference standard at target concentration.

The stability of ursodiol reference standard and sample preparation solutions in UPLC vials at room temperature was evaluated for 1 day.

The robustness study was done by ensuring sufficient separation between the ursodiol peak and the adjacent peak after making small changes in flow rate (±3%), mobile phase composition (±2%), and temperature (±2%) to the optimized method parameters.

System suitability tests are an integral part of an analytical method. These tests are used to verify that the chromatographic system is adequate for the intended analysis. Ursodiol reference standard solution at 100% of target level was prepared and injected 6 times.

Stress tests were performed to determine the specificity of the UPLC method for detection of degradation products during storage of the ursodiol oral compounded suspensions. The effects of acidic and basic conditions, as well as heat and oxidation, on the stability of ursodiol were studied. Ursodiol suspension samples were treated with 0.2M hydrochloric acid and 0.2M sodium hydroxide and the resulting preparations were then incubated at 60°C for 3 days to forcibly degrade the ursodiol under acidic and basic conditions. Samples were treated with 6% hydrogen peroxide (H_2O_2) as oxidative agent then incubated at 40°C for 3 days. Samples were incubated at 80°C for 13 days for heat-stressed testing. These samples were analyzed in order to observe for any interference to the ursodiol peak from degradant peaks on chromatograms. Results were expressed in percentage of degradation for acid, base, oxidation, and heat conditions (Supplemental Table S2).

Microbiological Stability Testing. Non-sterile aqueous dosage forms may be exposed to the growth of microorganisms inadvertently introduced during or after the extemporaneous preparation. As such, it is common to add antimicrobial preservatives to inhibit the growth of potential microorganisms. The microbiological attributes of the non-sterile ursodiol oral compounded suspensions were established at baseline, as well

as on days 90 and 181. Microbiological stability testing followed the United States Pharmacopoeia (USP) Chapter 51: Antimicrobial Effectiveness Testing, which uses cultures of the following challenge microorganisms: *Aspergillus brasiliensis* (American Type Culture Collection, ATCC No. 16404), *Candida albicans* (ATCC No. 10231), *Escherichia coli* (ATCC No. 8739), *Pseudomonas aeruginosa* (ATCC No. 9027), and *Staphylococcus aureus* (ATCC No. 6538). For oral products other than antacids, made with aqueous bases or vehicles (category 3), the following criteria apply: bacteria, no less than 1.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days; yeasts and molds, no increase from the initial calculated count at 14 days and 28 days.[12]

Results

Considering the physical characterization, the ursodiol 20-mg/mL and 60-mg/mL oral compounded suspensions presented a homogeneous, white color at every time point of the 6-month study. The pH values of the suspensions did not change significantly throughout the study period, as follows: 5.00 to 5.26 (Actavis ursodiol capsules), 5.00 to 5.24 (KVK Tech ursodiol capsules), and 5.10 to 5.50 (Mylan ursodiol capsules) (Table 2).

With regard to the microbiological characterization, there was no growth of challenge microorganisms throughout the study for all samples, as shown in Table 3. These results complied with microbiological stability requirements established in USP Chapter 51: Antimicrobial Effectiveness Testing.

Considering the chemical characterization, the mean concentration of ursodiol in the oral compounded suspensions did not change significantly throughout the 6 months of the study. For the ursodiol 20-mg/mL oral compounded suspensions, the mean concentration values ranged from 18.7 to 20.2 mg/mL (Actavis ursodiol capsules), 19.8 to 20.3 mg/mL (KVK Tech ursodiol capsules), and 19.5 to 21.2 mg/mL (Mylan ursodiol capsules). Similarly, for the ursodiol 60-mg/mL oral compounded suspensions, the mean concentration values ranged from 57.3 to 60.1 mg/mL (Actavis ursodiol capsules), 57.2 to 59.9 mg/mL (KVK Tech ursodiol capsules), and 59.4 to 61.4 mg/mL (Mylan ursodiol capsules). As such, the potency of all the oral suspensions remained within ±10% of the specification throughout the study, as displayed in Figures 1 and 2. The UPLC method validation demonstrated that the assay testing is linear, precise, accurate, robust, and suitable, as well as stability indicating. The specificity study showed that there was no chromatographic interference to the ursodiol peak and no indication of the detected peak being impure. The results of the method validation testing are displayed in Supplemental Tables S1 and S2.

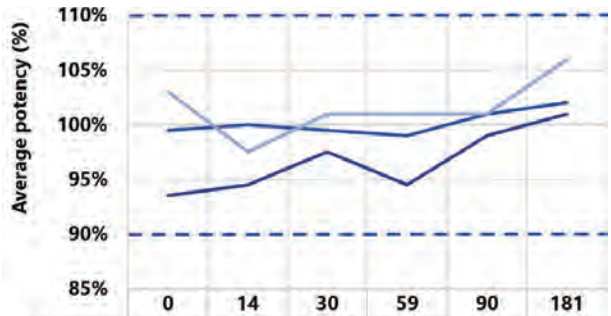
Table 2. pH Values for Ursodiol 20 mg/mL and 60 mg/mL Oral Compounded Suspensions (Actavis, KVK Tech, and Mylan) per Time Point

Time Points (days)	Ursodiol 20 mg/mL			Ursodiol 60 mg/mL		
	Actavis	KVK Tech	Mylan	Actavis	KVK Tech	Mylan
0	5.09	5.15	5.22	5.22	5.21	5.42
14	5.18	5.19	5.25	5.26	5.24	5.50
30	5.09	5.17	5.14	5.16	5.13	5.38
59	5.20	5.20	5.18	5.19	5.19	5.48
90	5.00	5.00	5.10	5.10	5.10	5.30
181	5.17	5.17	5.23	5.17	5.16	5.17

Table 3. Microbiological Stability Testing for Ursodiol 20 mg/mL and 60 mg/mL Oral Compounded Suspensions (Actavis, KVK Tech, and Mylan) at 0, 90, and 181 Days

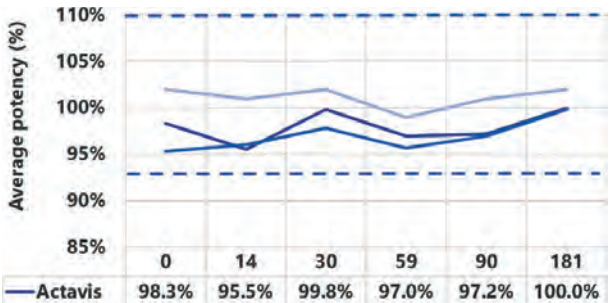
Challenge Microorganisms	Ursodiol 20 mg/mL			Ursodiol 60 mg/mL		
	Actavis	KVK Tech	Mylan	Actavis	KVK Tech	Mylan
Aspergillus brasiliensis	Pass	Pass	Pass	Pass	Pass	Pass
Candida albicans	Pass	Pass	Pass	Pass	Pass	Pass
Escherichia coli	Pass	Pass	Pass	Pass	Pass	Pass
Pseudomonas aeruginosa	Pass	Pass	Pass	Pass	Pass	Pass
Staphylococcus aureus	Pass	Pass	Pass	Pass	Pass	Pass

Figure 1. Average percent potency of the ursodiol oral compounded suspension 20 mg/mL over 6 months from extemporaneous preparation. Dashed lines represent the lower and upper limits, corresponding to 90% and 110% of the labelled concentration, respectively.



	0	14	30	59	90	181
Actavis	93.5%	94.5%	97.5%	94.5%	99.0%	101.0%
KVK Tech	99.5%	100.0%	99.5%	99.0%	101.0%	102.0%
Mylan	103.0%	97.5%	101.0%	101.0%	101.0%	106.0%

Figure 2. Average percent potency of the ursodiol oral compounded suspension 60 mg/mL over 6 months from extemporaneous preparation. Dashed lines represent the lower and upper limits, corresponding to 90% and 110% of the labelled concentration, respectively.



	0	14	30	59	90	181
Actavis	98.3%	95.5%	99.8%	97.0%	97.2%	100.0%
KVK Tech	95.3%	96.0%	97.8%	95.7%	97.0%	99.8%
Mylan	102.0%	101.0%	102.0%	99.0%	101.0%	102.0%

Discussion

It is hypothesized that the physical stability of the oral compounded suspensions was supported by the buffer system contained in the suspending vehicle, which may have contributed to a relatively constant pH. The pH value can affect the effectiveness of preservatives and the rate of growth of microorganisms. Microbial growth is optimal at a pH range of 6 to 8, and the growth rates of microorganisms are expected to decline outside this range. Therefore, the pH values of the ursodiol oral compounded suspensions were unfavorable for microorganism growth. It is also assumed that the preservative system in the suspending vehicle is likely to have protected the oral compounded suspensions from microbial contamination. The preservatives in the suspending vehicle are potassium sorbate and sodium benzoate, which are broad spectrum preservatives, potentially able to inhibit microorganism growth in the oral compounded suspensions.

The physical, chemical, and microbiological results obtained in this study[13] are consistent with the observations of Pramar et al,[11] who also attributed a BUD of 6 months to ursodiol (powder) 50 to 100 mg/mL in the suspending vehicle, stored at both 5°C and 25°C.

Conclusions

Oral compounded suspensions may be rapidly prepared, allow dosing flexibility, and are easy to administer. However, all pharmacists need validated stability studies to prepare oral liquids with high quality and safety. A palatable, sugar-free formula was developed for ursodiol 20 to 60 mg/mL in an oral suspending vehicle to facilitate the extemporaneous preparation in all settings. This study demonstrates that ursodiol (Actavis, KVK Tech, and Mylan) is physically, chemically, and microbiologically stable in the suspending vehicle at room temperature for up to 6 months. As such, all pharmacists may prepare ursodiol oral compounded suspensions in advance owing to its prolonged stability, ensuring that pediatric patients access an optimized formula in a timely manner.

Article Information

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Disclosure. The authors Kendice Ip, Courtaney Davis, A. J. Day, Craig Urwin, and Maria Carvalho are affiliated with Professional Compounding Centers of America (PCCA), the manufacturer of the proprietary oral suspending vehicle discussed in this study. The authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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CASE STUDY

Personalized Oral Low-Dose Naltrexone Titration for Pain Management

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Author(s): Rodriguez Franklin, Carvalho Maria, Banov Fabiana

Abstract: Naltrexone is a competitive opioid receptor antagonist indicated to treat opioid and alcohol dependence. In the U.S., naltrexone is commercially available as 50-mg tablets, and the adult dosage strength typically ranges between 50 mg once daily and 100 mg once daily. However, there is evidence to suggest that naltrexone prescribed in low doses, about 1/10th of the daily standard dosage, may be effective in managing a myriad of chronic conditions, including pain refractory to conventional pharmacological treatments. The U.S. Food and Drug Administration recently granted an orphan drug designation for low-dose naltrexone for the treatment of complex regional pain syndrome. This article provides a case study of a patient who was treated with a low dose of naltrexone for pain associated with the diagnosis of idiopathic hypereosinophilic syndrome.

Related Keywords: low-dose naltrexone, formulation, pain management, complex regional pain syndrome, case report, idiopathic hypereosinophilic syndrome, mepolizumab, analgesia, inflammation, analgesic agent, anti-inflammatory agent, Neuropathic Pain Symptom Inventory, NPSI, dose titration, dose increases, patient assessment, pain intensity

Related Categories: FORMULATIONS, PAIN MANAGEMENT, CASE REPORTS, PATIENT ASSESSMENT

CASE STUDY

Combination Therapy of Oral LDN and Topical Pentoxifylline, Rifampin, Clindamycin for Hidradenitis Suppurativa

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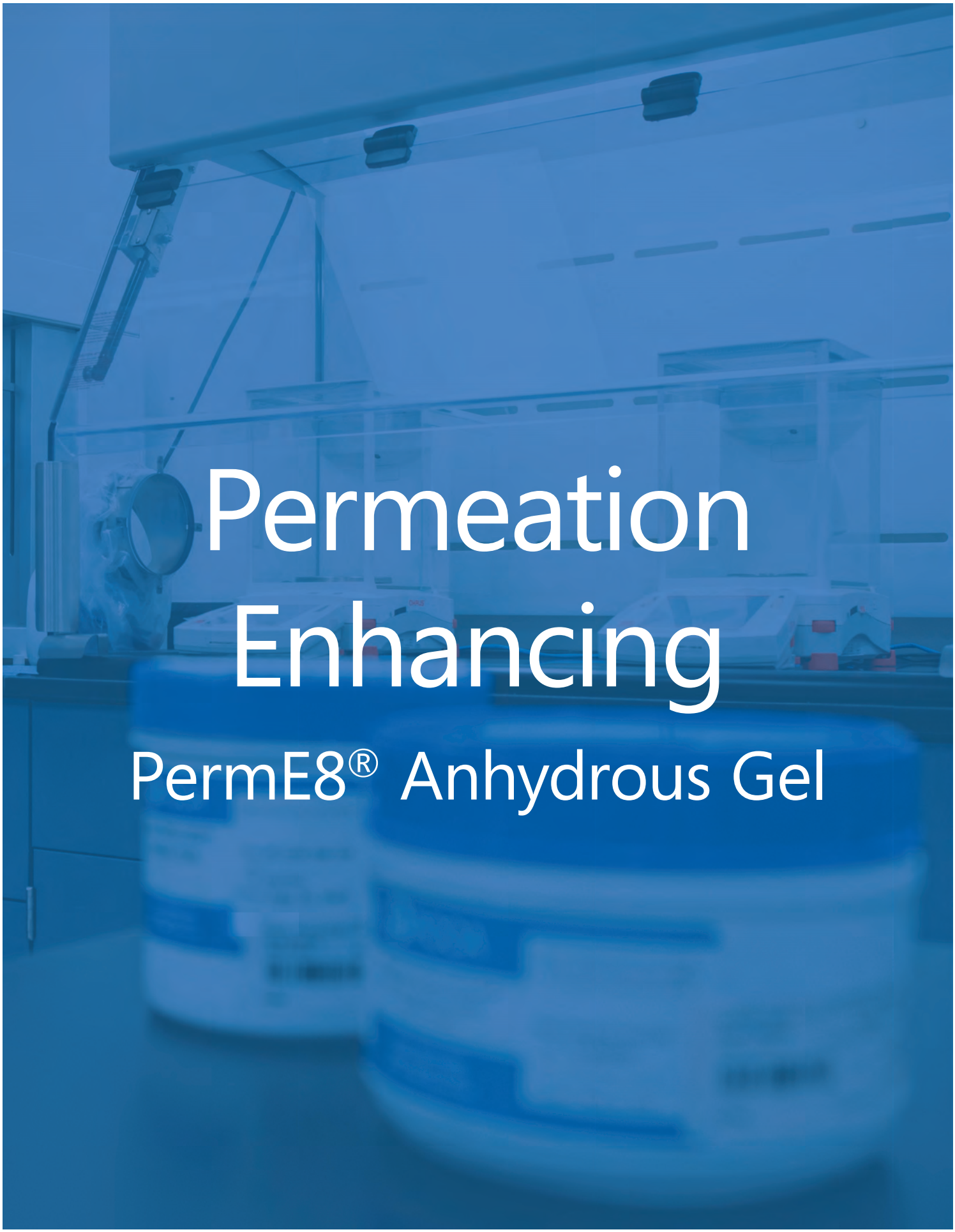
Author(s): Bertrand Joe, Bruno Matthew, Komuves Andrew, Jones Nat, Carper Kameryn, Banov Fabiana, Carvalho Maria

Abstract: Hidradenitis Suppurativa (HS) is a chronic inflammatory skin disease that may have profound effects on the patient's quality of life. A personalized HS combination therapy treatment was prescribed to a 54-year-old female suffering from multiple painful sores, as follows: naltrexone capsules titrated from 0.5 mg up to 4.5 mg; pentoxifylline 5%, rifampin 2%, clindamycin 1%, and glycolic acid topical cream. Clinical improvements were observed using two disease-specific outcome measures: Hurley Staging System and HS Score. The patient's HS improved from Stage II (moderate) to Stage I (mild), and the HS score decreased from 103 points with five anatomical regions reported, to 19 points with only three regions affected. Furthermore, the before and after treatment photographs showed a visible reduction in the number of boils/skin abscesses and an overall recovery. Improvements were also observed across all domains of the patient's self-reported quality of life (Hidradenitis Suppurativa Quality of Life Assessment). The patient did not experience any undesirable effects. Compounded medications may be customized to meet the patient's special needs and may be adjusted throughout the course of treatment to match the patient's individual progress. Although further studies are necessary, this personalized, combination therapy may be a key treatment option in HS.

Related Categories: DERMATOLOGY, WOUND CARE, CASE REPORTS



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ORIGINAL ARTICLE

WILEY

Evaluation of the in vitro human skin percutaneous absorption of ketoprofen in topical anhydrous and aqueous gels

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Abstract

Background: Ketoprofen is a nonsteroidal anti-inflammatory drug used for the treatment of acute and chronic pain associated with inflammatory conditions. This study aims to evaluate the in vitro percutaneous absorption of ketoprofen 10% formulated in proprietary anhydrous and aqueous gels using the Franz skin finite dose model.

Materials and Methods: The anhydrous gel was initially characterized for cytotoxicity using EpiDerm skin tissue model by cell proliferation assay and Western blot analysis. The Ultra Performance Liquid Chromatography method for measuring ketoprofen was validated and the stability of ketoprofen 10% in the anhydrous gel formulation was evaluated at 5°C and 25°C for 181 days. The percutaneous absorption of ketoprofen was determined using donated human skin. The tissue sections were mounted within Franz diffusion cells. A variable finite dose of each ketoprofen formulation in either anhydrous or aqueous gel was applied to the skin sections and receptor solutions were collected at various time points.

Results: Cell proliferation assay showed minimal cell death when EpiDerm skin tissue was exposed to the anhydrous gel for 24 h; the levels of protein markers of cell proliferation were not affected after 17-h exposure. Ketoprofen was stable in the anhydrous gel when stored at 5°C and 25°C. When compounded in the anhydrous and aqueous gels, ketoprofen had mean flux rate of 2.22 and 2.50 $\mu g/cm^2/h$, respectively, after 48 h. The drug was distributed to the epidermis and dermis sections of the skin. Both the anhydrous and aqueous gels facilitated the percutaneous absorption of ketoprofen without statistically significant differences.

Conclusion: The anhydrous gel can be used as a base to facilitate the transdermal delivery of ketoprofen. Although the anhydrous and aqueous gels can deliver a similar amount of ketoprofen, the anhydrous gel (water activity below 0.6) allows for extended default beyond-use-date of compounding preparations.

KEYWORDS
anhydrous gel, extemporaneously compounded formulations, permeation enhancers, skin, topical

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