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Chemical Stability of Progesterone in Compounded Topical Preparations using PLO Transdermal Cream™ and HRT Cream™ Base over a 90-Day Period at Two Controlled Temperatures

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Abstract

Topical preparations containing progesterone are increasingly compounded in pharmacies, and when done so, have expiration dates of 6 months post preparation or the stated expiration date of the API, whichever comes first. Using an HPLC method developed in our lab for the separation and quantitation of progesterone, we determined the room temperature (25°C) and refrigerated (4°C) stability of compounded progesterone in proprietary PLO Transdermal Cream™ and HRT Cream™ both manufactured by Medisca Inc. over a 90 day period. Area under the curve (AUC) measurements for the 30 and 60 day analysis reveal both compounded preparations retain >95% of the stated initial potency regardless of temperature storage conditions. However, a significant decrease in stability is noted during the last 30 day study period regardless of storage temperature for both preparations (71 and 73% for HRT™ at 25°C and 4°C respectively and 69 and 71% for PLO Transdermal Cream™ 25°C and 4°C respectively). Our data suggests that compounded progesterone preparations maintain chemical stability per USP standards (± 10%) for up to 60 days in each of the tested bases, regardless of storage temperature. Due to the significant loss in chemical stability, usage beyond 60 days post-preparation is not recommended.

Introduction

Progesterone (Figure 1), is classified as a sex hormone and is currently used for varied indications including prevention of endometrial hyperplasia in postmenopausal women, assisted reproductive technology (ART), and amenorrhea [1,2]. For the aforementioned indications, progesterone can be administered as an oral dosage form, an intramuscular injection, or as an intravaginal gel. Intravaginal tablets are also available for ART. Given these dosage forms a study conducted by Leonetti et al. [3] revealed that topical progesterone is preferred to oral administration when treating menopausal women. Topical progesterone has also been recently investigated for the treatment of genital lichen sclerosus, bone density stabilization in postmenopausal women, and for halting the progression of atherosclerotic plaques in postmenopausal women however results from randomized controlled trials suggest no benefit for progesterone in any of these diseases [4-6]. Repurposing progesterone for uses other than those listed above is expected to continue as is the need for compounded progesterone preparations.

The base or vehicle used in compounding may have an impact on the amount of progesterone delivered to the patient. Moreover, the vehicle itself may have an impact on the chemical stability of progesterone. To ensure stated potency of a product is afforded to the patient, The United States Pharmacopeia-National Formulary (USP-NF) has suggested guidelines regarding the beyond use date for such compounded formulations. In general, the USP recommends beyond use dating of six months for topical preparations, or the stated expiration date of the API [1].

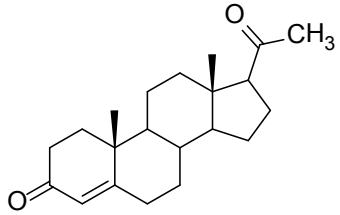

Figure 1: Chemical Structure of Progesterone.

Progesterone stability is vastly dependent on storage conditions and the microenvironment the molecule is exposed to. The solution stability of progesterone can vary from 46% of the initial content at room temperature in absence of polymeric additives to 70% after six months in the presence of polymeric additives in an aqueous two-phase system [7]. Most topical progesterone preparations, including those investigated in this study, are commonly compounded in pharmacies using vehicles formulated with appreciable water and polymeric additives. Our hypothesis is that progesterone retains appropriate stability per USP standards in such aqueous, polymeric, two-phase vehicles for prolonged periods of time.

The purpose of this study was to determine the stability of progesterone when compounded in PLO Transdermal Cream™ and HRT Cream™ Base, proprietary vehicles produced by Medisca Inc. with water contents that approach 51% and 75-85% respectively. The study was conducted over time points of 30, 60 and 90 days. Samples from both arms were analyzed after incubation at both room temperature (25°C), and under refrigeration (4°C).

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Materials and Methods

Progesterone USP was provided by Medisca Pharmaceutique Inc. Quebec, Canada. Analytical grade acetonitrile (ACN) was purchased from Fisher Scientific. Deionized water (18 Ω) was available in our lab. All other reagents used were of analytical grade.

Instruments

A Hewlett Packard 1050 HPLC system consisting of a quaternary pump (Model 79852A), an autosampler (Model 79855A), a degasser (Model G1303A), a diode-array-detector (Model HP1046A), a solvent tray and a desktop computer loaded with ChemStation software was used for our analysis. A Mettler AL204 electronic balance (Mettler-Toledo, Columbus, OH) and an unguator (Cito Unguator 014, Zella-Mehlis, Germany) were used for standard and sample preparation.

Chromatographic conditions

The mobile phase consisted of acetonitrile 70% and deionized water (18 Ω) 30%. The stationary phase consisted of a Zorbax Eclipse Plus Column (C18, 4.6 mm ID × 150 mm, 3.5 µm particle size, 95 Å pore size, pH range 2-9) (Agilent Technologies, Santa Clara, CA) and a compatible Zorbax pre-column. The column temperature was maintained at 30°C while the flow rate was maintained at 1 mL min^{-1} for a run time of 10 minutes. The injection volume for each sample was 10 µL and the detection wavelength was 220 nm. Under the described chromatographic conditions the retention time of progesterone was 2.5 minutes.

General procedure for preparation of standards and samples

Primary standard: Ten milligrams (0.01 gram) of progesterone and one gram of each base (PLO Transdermal Cream and HRT Cream) was weighed with a margin of plus or minus (0.0005 gram). Each component (progesterone plus base) was placed in a volumetric flask and the volume was brought to 100 mL with ACN. The resulting solution afforded a final concentration of 100 µg mL^{-1} for the primary standard (PS).

Secondary standards: Using the 100 µg mL^{-1} primary standard, dilutions were prepared to afford concentrations of 25, 50, and 75 µg mL^{-1} which were subsequently used in generation of the standard curve.

Standard curve for progesterone in PLO transdermal cream

Sample vials were labeled and filled with approximately 2 mL of primary standard (100 µg mL^{-1}) and 2 mL of each secondary standard (25, 50, 75 µg mL^{-1}). Standards were placed in the autosampler and separation was initiated with an injection volume of 10 µL using the method previously described under Chromatographic Conditions. Each secondary standard was run in quintuplicate fashion to generate area under the curve (AUC) data.

Plots of AUC versus concentration were generated for visual inspection. Linear regression analysis was performed to establish the concentration (µg mL^{-1}) versus area under the curve relationship in quantitative terms. The relationship is expressed by the following equation:

$y = 1.6624 x - 0.3$

Where x = concentration of progesterone and y= AUC (area under the curve for progesterone) (dimensionless number). The correlation coefficient (R^2= 0.9998) value indicates a near perfect linear fit of the data (five replicates). The standard curve is shown in Figure 2a.

Standard curve for progesterone in HRT cream

Sample vials were labeled and filled with approximately 2 mL of primary standard (100 µg mL^{-1}) and 2 mL of each secondary standard (25, 50, 75 µg mL^{-1}). Standards were placed in the autosampler and separation was initiated with an injection volume of 10 µL using the method previously described under Chromatographic Conditions. Each secondary standard was run in quintuplicate fashion to generate area under the curve (AUC) data.

Plots of AUC versus concentration were generated for visual inspection. Linear regression analysis was performed to establish the concentration (µg mL^{-1}) versus area under the curve relationship in quantitative terms. The relationship is expressed by the following equation:

$y = 8.379x - 6.341$

Where x = concentration of progesterone and y= AUC (area under the curve for progesterone) (dimensionless number). The correlation coefficient (R^2= 0.989) value indicates a near perfect linear fit of the data (five replicates). The standard curve is shown in Figure 2b.

Results and Discussion

Beyond use dating (BUD) stipulated by the United States Pharmacopeia is meant to ensure the integrity of compounded products. Adhering to BUD ensures the end user of a given product

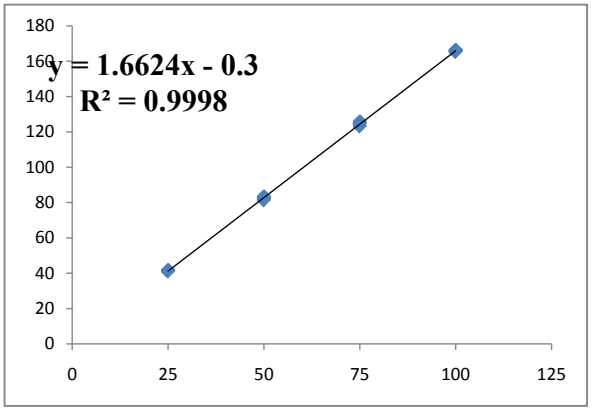

Figure 2a: The AUC vs. concentration (µg/mL) curve for progesterone in PLO Transdermal Cream.

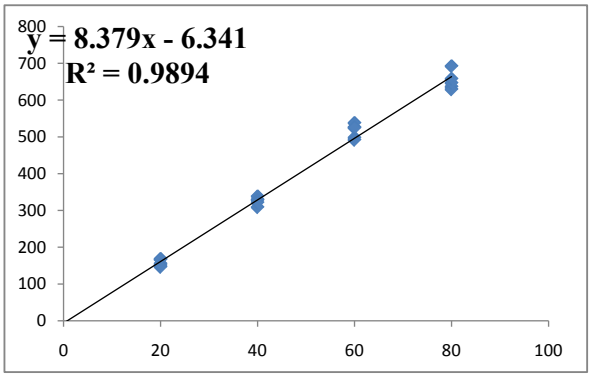

Figure 2b: The AUC vs. concentration (µg/mL) curve for progesterone in HRT Cream.

Preparation name		Day 0	Day 30 at 25°C	Day 30 at 4°C	Day 60 at 25°C	Day 60 at 4°C	Day 90 at 25°C	Day 90 at 4°C
Progesterone HRT Cream (10% w/w)	Mean API (% of Labeled Amount)	99.51	101.49	101.29	100.04	98.13	*73.72*	*71.24*
	Number of repetitions	4	4	4	4	4	4	4
	Standard Deviation	2.13	1.67	2.19	3.86	7.7	4.21	5.9
	Coefficient of Variation (% of Mean)	2.14	1.65	2.6	3.86	7.84	5.71	7.68
Progesterone PLO Transdermal Cream (10% w/w)	Mean API (% of Labeled Amount)	98.67	98.78	100.91	100.13	101.71	*69.66*	*71.24*
	Number of repetitions	4	4	4	4	4	4	4
	Standard Deviation	1.13	1.9	1.14	1.39	2.25	1.26	1.03
	Coefficient of Variation (% of Mean)	1.14	1.9	1.13	1.39	2.21	2.29	1.45

Table 1: Stability data of progesterone (10%) at 25°C and 4°C over a 90 day period in each base (PLO Transdermal Cream and HRT Cream).

will receive the maximum therapeutic benefit provided the use does not extend past the stated expiration date. A curiosity is the stability of a given active pharmaceutical ingredient (API) in various vehicles for topical administration. Moreover, does storage temperature influence chemical stability? To address such curiosities, we performed stability studies on a compounded progesterone 10% cream using proprietary PLO Transdermal Cream and HRT Cream, both manufactured by Medisca Inc. Our study specifically examined the stability of progesterone at both 25°C (room temperature) and 4°C over a 90 day interval. When analyzed using the aforementioned HPLC method (see chromatographic conditions), both preparations maintained robust stability regardless of temperature for the first 60 days of the study. As mean data is compared to stated potency (10% w/w), all samples retain greater than 95% stated potency through day 60 (Table 1). A drastic change in potency is noted for the 90 day analysis across both bases at all temperatures. As shown in Table 1, the 90 day stability data indicate a significant reduction in the progesterone content (potency reduced to 73 and 71% in the HRT Cream arm for 25°C and 4°C respectively and 69 and 71% for the PLO Transdermal Cream for 25°C and 4°C respectively).

Our data strongly suggest that the use of topical progesterone, when compounded in HRT Cream or PLO Transdermal Cream, should not extend beyond 60 days post-preparation. A new supply of the cream should be provided for continued use. While we are confident in this recommendation, we are unsure of the fate of progesterone during the last 30 days of the study. There is little research reporting the stability of progesterone in other topical vehicles. Additionally, we could not identify literature reporting on the degradation products of progesterone when compounded in creams or gels (both prescription only and over-the-counter). Since both bases used in this study contain significant amounts of water, it is possible that hydrolysis of one of the structural features of this steroid hormone may be occurring. Extremes of heat have been noted to cause hydrolysis of the D-ring in other studies involving other steroid-based molecules [8]. This seems to be unlikely given the ambient nature of the incubation temperatures. The major limitation to this study is the lack of identifying degradation products formed during the last 30 days of the incubation period. To address this, we plan to prepare fresh samples and incubate as discussed before. However, during the last 30 days of the 90 day study, we plan to sample preparations and analyze with mass spectrometry to identify formed degradation products. Such data will allow us to speculate on the degradation pathway involved and perhaps make recommendation on how to circumvent these factors. We plan to investigate the chemical stability of parenteral progesterone preparations and closely related hormones of clinical significance in the near future.

Acknowledgement

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