In Vitro Drug Release of *Estriol 0.1% and Testosterone 0.1%* from VersaBase® Cream and PCCA Ellage™ Anhydrous Vaginal

SUMMARY: The *in vitro* drug release, a product performance test for topical drug products, is mainly used during the research and development phase of a new product to ensure its performance and comparability to a product of reference. This test has demonstrated that estriol 0.1% and testosterone 0.1% have comparable release profiles when incorporated in the well-established PCCA VersaBase Cream *versus* the newly-developed PCCA Ellage Anhydrous Vaginal.

Introduction:

Semisolid dosage forms, such as creams and gels, may be considered extended-release preparations and their drug release depends largely on the formulation. During the research and development phase, it is important to test the *in vitro* drug release of the new product to ensure its performance and comparability to a product of reference. This test is not intended though to predict *in vivo* performance, as opposed to the skin percutaneous absorption studies, since the primary factor that impacts bioavailability and clinical performance is skin permeation. However, this test can detect *in vitro* changes, as a result of formulation differences, that may correspond to altered *in vivo* performance of the dosage form. For this reason, its main purpose and use is comparison testing in which any difference in delivery rate is undesirable [1]. This test is required by the FDA to determine the acceptability of minor process and/or formulation changes in commercially-approved semisolid dosage forms [2]. The Unites States Pharmacopoeia (USP) recognizes different apparatus for the *in vitro* drug release test in the monograph <1724> Semisolid Drug Products – Performance Tests [1].

The aim of this study was to evaluate and compare the *in vitro* drug release of the following PCCA formulas:

- Estriol 0.1% and Testosterone 0.1% in PCCA VersaBase® Cream (PCCA Formula 13931)
- Estriol 0.1% and Testosterone 0.1% in PCCA Ellage™ Anhydrous Vaginal (PCCA Formula 13845)

VersaBase Cream is a well-established and referenced compounding base for topical and vaginal hormone replacement therapy, whereas Ellage is a newly-developed anhydrous vaginal base with superior mucoadhesive and self-emulsifying properties.

Methodology:

The *in vitro* drug release test was evaluated using the Franz Diffusion System (surface area 1.77 cm^2) for a group of 6 diffusion cells, which were mounted in a diffusion apparatus including Vaginal Fluid Simulant (VFS) as the receptor medium. The study methodology was adapted from the USP monograph <1724> Semisolid Drug Products – Performance Tests [1].

Initially, the VFS was prepared to model the fluid produced in the human vagina by healthy, nonpregnant premenopausal women. The composition of the fluid medium was based on the research by Owen and Katz and includes the following ingredients: NaCl 3.51 g, KOH 1.4 g, $Ca(OH)_2$ 0.222 g, BSA 0.018 g, lactic acid 2.0 g, acetic acid 1.0 g, glycerol 0.16 g, urea 0.4 g, glucose monohydrate 5.0 g, HCl qs pH 4.2-4.5 and H_2O qs 1,000 mL [3]. The VFS was degassed by filtering through a 0.2 µm membrane and maintaining a vacuum for 2 min; it was then warmed in a water bath at 37°C. The dialysis membranes (cut off 12-14 kD) were soaked in water overnight, followed by VFS for 30 min at 37°C. The dialysis membranes were then mounted on the Franz diffusion cells (no bubbles) and applied 100 µL of VFS, prior to dosing 200 mg of the test samples (PCCA Formulas 13845 and 13931). The receptor medium solution was stirred magnetically at approximately ~600 RPM with the water jacket temperature controlled to maintain at 37 ± 1.00°C. The receptor medium samples were collected at 1, 2, 3, 4, 5 and 6 hours by stopping the stirrer, withdrawing 1 mL of sample, and replacing the same volume with VFS. All receptor medium samples were filtered with a PVDF membrane prior to quantification of estriol / testosterone by the analytical method Ultra High Performance Liquid Chromatography (UPLC) with Ultraviolet Photodiode Array (PDA).

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It consisted of a reverse phase, gradient chromatographic method with two different mobile phases: deionized water (A) and acetonitrile (B). The chromatographic column used was an Acquity UPLC BEH C_{18} (1.7 µM) 2.1 mm x 100 mm. The injection volume was 10 µL and the flow rate was 0.5 mL/min with a run time of 5 minutes. The column temperature was maintained at 50°C and the sample tray at 6°C. The ultraviolet PDA detector was set to an acquisition wavelength of 190–400 nm, with a detection wavelength of 245 nm for testosterone and 280 nm for estriol.

Results and Discussion:

Estriol 0.1% exhibited a similar *in vitro* release profile from both Ellage and VersaBase Cream throughout the study period of 6 hours. The amount released from Ellage was higher at all time points in comparison to VersaBase Cream. By the end of the study, a total of 30.4 µg/cm^2 (27%) and 13.1 µg/cm^2 (11.5%) of estriol had been released from Ellage and VersaBase Cream, respectively (Figure 1).

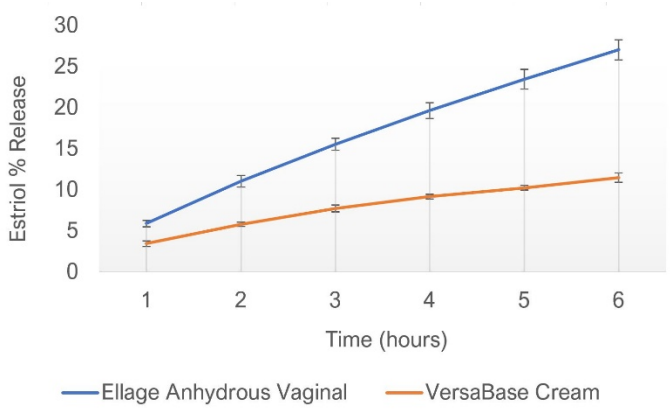

Figure 1. *In vitro percentage release of estriol from PCCA proprietary bases for 6 hours.*

Likewise, testosterone 0.1% exhibited a similar *in vitro* release profile from both Ellage and VersaBase Cream throughout the study period of 6 hours. However, the amount released from VersaBase Cream was higher at all time points in comparison to Ellage (Figure 2). By the end of the study, a total of 13.6 µg/cm2 (11.2%) and 7 µg/cm2 (6%) of estriol had been released from VersaBase Cream and Ellage, respectively (Figure 2).

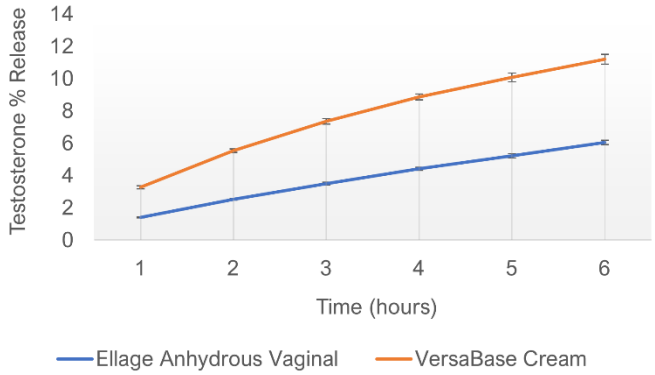

Figure 2. *In vitro percentage release of testosterone from PCCA proprietary bases for 6 hours.*

This comparative study was not designed to evaluate any statistical differences between the two PCCA proprietary bases. Instead, it is able to provide qualitative insights on the drug release performance of the bases.

Conclusions:

The *in vitro* drug release is a product performance test for topical drug products mainly used during the product research and development phase. According to the USP this test is not a measure of bioavailability but instead a demonstration of product comparability or compliance with FDA guidelines [1,2]. In the present study, the *in vitro* drug release test has demonstrated that estriol 0.1% and testosterone 0.1% have comparable release profiles when incorporated in the well-established PCCA VersaBase Cream *versus* the newly-developed PCCA Ellage Anhydrous Vaginal.

References:

1. The United States Pharmacopeial Convention (2014) 'General Information / <1724> Semisolid Drug Products – Performance Tests. *USP 37 -NF 32*. Rockville: USP, p. 1273-84.

2. FDA (1997) 'Guidance Document / SUPAC-SS: Nonsterile Semisolid Dosage Forms; Scale-Up and Post-Approval Changes: Chemistry, Manufacturing and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation'. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/supac-ss-nonsterile-semisolid-dosage-forms-scale-and-post-approval-changes-chemistry-manufacturing.

3. Owen, D.H., Katz, D.F. (1999) 'A vaginal fluid simulant'. *Contraception*, 59 (2), p. 91-5. doi:10.1016/s0010-7824(99)00010-4.