Evaluation of the *in vitro* Human Skin Percutaneous Absorption of Estriol and Estradiol in PCCA VersaBase® Anhydrous HRT

SUMMARY: The human skin percutaneous absorption of PCCA Micronized Estriol and Estradiol in PCCA VersaBase Anhydrous HRT and PCCA VersaBase® Cream was evaluated *in vitro,* and it was shown that VersaBase Anhydrous HRT performs superior to our industry-leading VersaBase Cream in facilitating estrogen absorption. Since VersaBase Anhydrous HRT has a water activity below 0.6 (Aw <0.6), it may provide an excellent option for compounding pharmacists who rely on anhydrous topical formulations for extended default beyond-use dates (BUDs) without compromising on permeation performance.

Introduction:

Bio-identical hormone replacement therapy (BHRT) with opposed or unopposed estrogen is widely used for the management of postmenopausal symptoms. Low estrogen levels associate with vasomotor and vulvovaginal atrophy symptoms, dyspareunia and mood lability. All routes of estrogen administration can be effective, but their metabolic effects are different. Compared with the oral route, topical and transdermal application require lower doses of estrogen and appear to have lower risk of VTE, stroke and cancers [1, 2]. Estriol cream is currently not available in the U.S. and patients can only access it through a compounding pharmacy. VersaBase Cream is one of the suggested bases to deliver estrogens transdermally in women.

PCCA VersaBase Anhydrous HRT is a new proprietary base for topical delivery of female hormones into and through the skin with extended stability [3]. Compared with VersaBase Cream that contains water, VersaBase Anhydrous HRT has a water activity below 0.6 (Aw <0.6), classifying it as an anhydrous base. This allows extended default beyond-use dates (BUDs) for preparations that do not have stability studies.

The purpose of this study is to compare the percutaneous absorption of estriol and estradiol incorporated in PCCA VersaBase Anhydrous HRT and in VersaBase Cream (Table 1.) in an *in vitro* dermatomed skin model.

Estriol/Estradiol [50%/50%] 2 mg/Gm Topical Cream (VersaBase Anhydrous HRT)	
Estriol 10% / Estradiol 10% / Microcrystalline Cellulose (PH-105) Trituration	1%
Propylene Glycol USP	5%
Base, PCCA VersaBase Anhydrous HRT	94%

Estriol/Estradiol [50%/50%] 2 mg/Gm Topical Cream (VersaBase Cream)	
Estriol 10% / Estradiol 10% / Microcrystalline Cellulose (PH-105) Trituration	1%
Propylene Glycol USP	5%
[a]Base, PCCA VersaBase Cream	94%

Table 1. *Compounded formulas (PCCA Formula 13460) was used in the percutaneous absorption study.*

[a] *VersaBase Cream was used instead of VersaBase Anhydrous HRT in order to compare with F13460.*

Methodology:

Skin Preparation

The percutaneous absorption of estriol and estradiol were measured using human cadaver abdomen skin tissue from three Caucasian female donors. Dermatomed skin samples were purchased from BioIVT (Westbury, NY) and were cryopreserved and stored at -20°C in tightly sealed plastic bags. Prior to use, the skin samples were defrosted and then soaked in diffusion medium for at least 30 min at room temperature. The samples were visually checked for any significant damages, such as cuts or holes. Skin tissues from 3 donors and 3 replicates were used for each compounded formula.

Franz Cell Diffusion

The Franz diffusion system (surface area of 1.77 cm^2) was used in this study. The diffusion cells were mounted in the diffusion apparatus and the physiological diffusion medium was added to the receptor compartment. A skin integrity test was performed using a Precision LCR meter. Intact skin has transcutaneous electrical resistance at least 2 times greater than the diffusion medium. The finite dose, approximately 5 mg/cm^2 of the compounded formula, was applied on each skin sample using a positive displacement pipette and a pellet pestle to spread the product across the skin surface. The receptor solution (HBSS #14175-079, 25 mM HEPES, #15630-080 and 50 µg/mL Gentamicin, #15750-060, Gibco) was stirred magnetically at 600 rpm with the water jacket temperature maintained at 32±0.5°C. During the exposure period, samples of the receptor solutions (1 mL) were removed at predetermined time points: 2, 4, 6, 8, 12 and 24 hours after applying the compounded formula.

Estriol and Estradiol Quantification

The quantifications of estriol and estradiol in receptor solution were performed by ELISA (Cayman, Ann Arbor, MI) following manufacturer's instruction.

Results and Discussion:

Estrogens that were applied on the skin passed through the stratum corneum, epidermis and dermis layers of the skin model, and finally reached receptor solution. This process mimics estrogen penetrating through the skin and into systemic circulation *in vivo*. Percutaneous absorption of estrogens in this study refers to the amount of estriol and estradiol detected in the receptor solution and is shown in Figure 1. Estriol and estradiol delivered by both bases were detected across dermis as early as 2 hours after skin application, and the amount continued to increase. By the end of 24 hours, percutaneous absorption of estriol facilitated by VersaBase Anhydrous HRT was 55 ± 25 ng/cm^2 of skin surface, which is higher than 16 ± 5 ng/cm^2 by VersaBase Cream. The 24-hour absorption of estradiol is

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about 6 times more than the absorption of estriol. Total amount of 341± 122 ng/cm^2 estradiol was delivered by VersaBase Anhydrous HRT, and 157± 28 ng/cm^2 by VersaBase Cream.

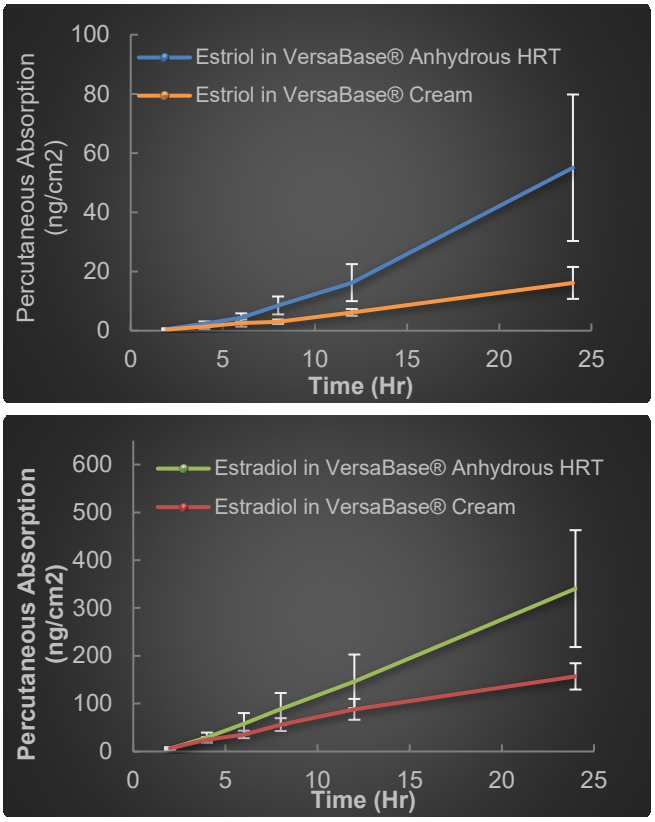

Figure 1. *Across donor summary: mean skin percutaneous absorption of estriol (upper panel) and estradiol (lower panel) (ng/cm^2 of skin) in two compounded formulations during 24 hours diffusion. Results were plotted as mean ± standard error.*

In order to understand the kinetics of estrogen absorption in the two compounded formulas, the rate of absorption, or flux rate, was determined in each skin donor and was summarized in Figure 2. The rate of percutaneous absorption shows a rapid penetration upon application and the maximum flux was achieved at approximately 6-8 hours post-application, followed by steady flux lasting through the 24 hours. The flux profiles generated from the two bases showed very similar trend, but VersaBase Anhydrous HRT delivers the estrogens at a rate 4 times higher than VersaBase Cream.

In summary, both VersaBase Anhydrous HRT and VersaBase Cream deliver estriol and estradiol percutaneously in a steady flux rate, however, the rate is higher by VersaBase Anhydrous HRT.

Conclusions:

This *in vitro* study performed in the dermatomed human skin model has demonstrated that the proprietary topical base VersaBase Anhydrous HRT facilitates the percutaneous absorption of estriol and estradiol across human cadaver skin. Both VersaBase Cream and VersaBase Anhydrous HRT produce a steady flux of estrogens for at least 24 hours without quick peaking or declining, with a higher rate by VersaBase Anhydrous HRT. The total amount of percutaneous absorption and the flux rate evaluated by Franz cell model in human skin in this study is consistent with previously published data [4, 5], supporting the reliability of the study. VersaBase Cream, as our industry-leading base, has already received satisfactory responses from patients. VersaBase Anhydrous HRT could provide another option to compounding pharmacists to extend default BUDs with the assurance of an excellent permeation performance.

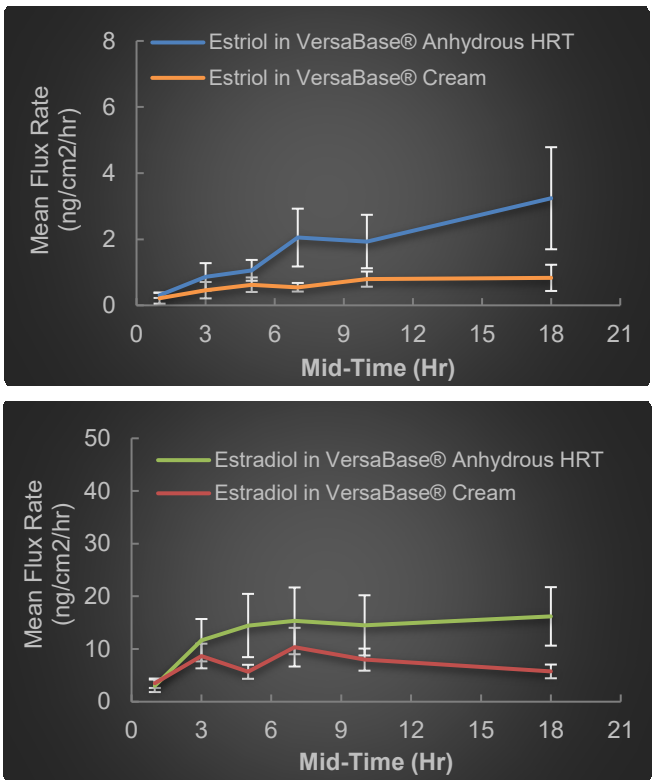

Figure 2. *Across donor summary: mean flux rate of estriol (upper panel) and estradiol (lower panel) (ng/cm^2/hr) in two compounded formulations during 24 hours diffusion. Absorption values were reported at midpoint of sample collection (e.g. mean flux rate at 18-hour time-point represents the rate of flux during 12 to 24 hours). Results were plotted as mean ± standard error.*

References:

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