NINETY DAY CHEMICAL STABILITY OF COMPOUNDED ESTRADIOL, ESTRONE, AND ESTRIOL COMBINATION AND BEYOND-USE-DATE

Arindam Basu Sarkar1, Akshitha Kandimalla2, Roshini Durga Paruchuri1, Richard Dudley1
1College of Pharmacy, The University of Findlay, 1000 North Main Street, Findlay Ohio 45840, USA
2College of Business, The University of Findlay, 1000 North Main Street, Findlay Ohio 45840, USA
* Corresponding author E-mail: basusarkar@findlay.edu

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ABSTRACT
Hormone replacement therapy utilizing estrogens formulated in topical preparations is routinely used. While most commercially available estrogen-containing topical preparations contain estradiol, a therapeutic use for other estrogens including estriol and estrone has been realized. However, the commercial availability of estranol and estrone-containing topical preparations is lacking. Using a HPLC method developed in our lab for the separation and quantitation of estradiol, estrone, and estriol, we determined the 30, 60, and 90 day stability of compounded estrogens in Medisca Inc.’s proprietary HRT Cream Base at room temperature and 4°C. Area under the curve (AUC) measurements for all three time points (30, 60, and 90 day analysis) reveal that the compounded preparations retain > 90% of the stated initial potency regardless of temperature storage conditions.

Keywords: Estradiol, estrone, estriol, chemical stability, HPLC assay.

INTRODUCTION
Estriol and estrone are less well known estrogens that have well-defined therapeutic uses1–5. Estrogens, including estradiol, estrone, and estriol (Figure 1), have traditionally been used for hormone replacement therapy in menopausal and post-menopausal women to treat vasomotor symptoms6, 7. Estrogens find other therapeutic uses as well including vaginal atrophy associated with menopause, primary ovarian failure, and in males for maintaining sexual interest during androgen deprivation for the treatment of prostate cancer9, 10. Oral products containing estradiol and conjugated estrogens obtained from mare urine have been available for years. Additionally, estrone is available in an oral dosage form as the conjugated sulfate piperazine salt (Ogen®) and various other trade-name products as well. In addition to oral administration for replacement therapy, other routes of administration can be used. Patches have been employed as well as vaginal creams, both containing estradiol. To our knowledge, no commercially available preparations contain estranol as the sole ingredient, or as a part of a combination of active pharmaceutical ingredients (API).

The United States Pharmacopeia-National Formulary (USP-NF) stipulates beyond-use-dating (BUD) for compounded topical preparations which contain active pharmaceutical ingredients (API)8. When estradiol, estrone, and/or estradiol topical preparations are compounded as nonaqueous liquids and solid formulations, the product is assigned an expiration date of 6 months post preparation or 25% of the remaining expiration date of the product, whichever comes first. For water containing formulations (prepared from ingredients in solid form) the BUD is not later than 14 days for liquid preparations when stored in cold temperature between 2°C and 8°C. There remains confusion whether topical cream preparations should be considered in the category of solid formulations or water containing formulations. An area of uncertainty is the stability of any or all of the aforementioned hormones in proprietary HRT Cream Base manufactured by Medisca Inc. when prepared in the form of a cream (with the cream base containing water). Establishment of the beyond-use-date of these preparations is thus necessary to optimize their use.

Estriol and estrone have a decreased ability, relative to estriol, to activate estrogen receptors, yet both are used therapeutically2, 4, 5, 11. Since an estriol cream or topical preparation is not commercially available, physicians may prescribe compounded topical preparations containing estriol alone, or in combination with estrone and estradiol. The United States Pharmacopeia-National Formulary (USP-NF) has guidelines regarding the beyond-use-date for such compounded formulations. The purpose of this study was to determine the stability of each estrogen component (estradiol, estrone, and estriol) when compounded in HRT Cream Base, a proprietary vehicle with a water content that exceeds 75 percent. The study was conducted over time points of 30, 60, and 90 days where samples were incubated at room temperature RT, 25°C, and under refrigeration (4°C).

MATERIALS AND METHODS
Estradiol, estrone, and estriol USP were provided by Medisca Inc., Plattsburgh, NY. Optima 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. Chemical separation was achieved using a stationary phase consisting of a Zorbax Eclipse Plus Column (C18, 4.6mm ID x 150 mm, 3.5 µm particle size, 95Å pore size, pH range 2-9) (Agilent Technologies, Santa Clara, CA) and a compatible Zorbax pre-column.

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Chromatographic Conditions
The mobile phase consisted of Solvent A (acetonitrile, ACN) and Solvent B (deionized water). The following gradient elution method was used: 80% to 40% (v/v) of solvent B over 10 min returning to 80% (v/v) of solvent B from 10.01 min until the end of the run at 12 min. The column was kept at ambient room temperature while the flow rate was maintained at 1.0 mL min⁻¹. 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 estriol was 1.7 min, estradiol was 2.6 min., and estrone 3.2 min.

Preparation of standards and samples
Estradiol Primary Standard: Twenty milligrams (0.02 gram) of estradiol was weighed with a margin of plus or minus (0.0005 gram). This quantity was placed in a volumetric flask and the volume was brought to 100 mL with ACN. The resulting solution afforded a final concentration of 200 µg mL⁻¹ for the estradiol primary standard.

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Estrone Primary Standard: Twenty milligrams (0.02 gram) of estrone was weighed with a margin of plus or minus (0.0005 gram). This quantity was placed in a volumetric flask and the volume was brought to 100 mL with ACN. The resulting solution afforded a final concentration of 200 µg mL⁻¹ for the estrone primary standard.

Estriol Primary Standard: Twenty milligrams (0.02 gram) of estriol was weighed with a margin of plus or minus (0.0005 gram). This quantity was placed in a volumetric flask and the volume was brought to 100 mL with ACN. The resulting solution afforded a final concentration of 200 µg mL⁻¹ for the estriol primary standard.

Secondary Standards: By appropriately diluting the 2 Estradiol Primary Standard:

The following table shows the accuracy for the high pressure liquid chromatography (HPLC) method of determination of Estriol, Estradiol and Estrone concentration in HRT Cream Base. A = active Pharmaceutical Ingredient, B = N=5.

<table>
<thead>
<tr>
<th>API</th>
<th>Calibration level (µg/ml)</th>
<th>Assay Mean (µg/ml)</th>
<th>Accuracy %</th>
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<tbody>
<tr>
<td>Estradiol</td>
<td>20</td>
<td>18.22</td>
<td>91.11</td>
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<tr>
<td></td>
<td>30</td>
<td>28.09</td>
<td>93.65</td>
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<td>40</td>
<td>43.14</td>
<td>107.87</td>
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<td></td>
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<td>54.5</td>
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<td></td>
<td>100</td>
<td>97.32</td>
<td>93.32</td>
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<tr>
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<td>10</td>
<td>10.32</td>
<td>103.18</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>21.31</td>
<td>106.55</td>
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<tr>
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<td>100</td>
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<td>100.38</td>
</tr>
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</tr>
<tr>
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<tr>
<td></td>
<td>100</td>
<td>97.2</td>
<td>97.2</td>
</tr>
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</table>

Table 1. Accuracy for the high pressure liquid chromatography (HPLC) method of determination of Estriol, Estradiol and Estrone concentration in HRT Cream Base. A = active Pharmaceutical Ingredient, B = N=5.
Beyond RESULTS AND DISCUSSION

From this data, we can conclude that although temperature versus 95% for the 90 day refrigerated sample. We have no temperature as the relative potency retained at RT is estradiol appears to be most sensitive to st

seems to have an effect on the stability of all three API

s, ‘

potency.

analogous refrigerated sample retains 95.76% of stated potency. 90.5% of its stated p

samples retain greater than 9% w/w for estradiol, estriol, and

stated potency must be maintained. When comparing temperatu

s

should not be realized beyond 90 days. HRT Cream Base manufactured by Medisca Inc. does not appear to enhance the degradation of the estrogens used in the preparation despite the presence of water in the base.

ACKNOWLEDGEMENT

This research work has been carried out with the financial help of Medisca INC., 661 Route 3, Plattsburgh, NY, 12901.

REFERENCES


RESERVATION on advising patients to use the compounded preparation up to 90 days while stored at room temperature. However, the therapeutic effect of such a topical preparation may extend beyond 90 days. HRT Cream Base manufactured by Medisca Inc. does not appear to enhance the degradation of the estrogens used in the preparation despite the presence of water in the base.

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