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Research Article

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

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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 estriol and estrone-containing topical preparations is lacking. Using a HPLC method developed in our lab for the separation and quantitation of estriol, estrone, and estradiol, 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, estriol, estrone, chemical stability, HPLC assay.

INTRODUCTION

Estriol and estrone are less well known estrogens that have well defined therapeutic uses[1-5]. 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 symptoms [6, 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 cancer [9, 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 estriol 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 estriol, 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 estradiol, to activate estrogen receptors, yet both are used therapeutically [2, 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.

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 10.01min 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

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.

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.

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.

Secondary Standards: By appropriately diluting the 200 µg mL^{-1} primary standards from above, secondary standards for each estrogen were prepared to afford concentrations of 10, 20, 30, 40, 50, and 100 µg mL^{-1}. The secondary standards were subsequently used to generate a standard curve.

Standard Curve**:** Sample vials were labeled and filled with approximately 2 mL of each secondary standard (10, 20, 30, 40, 50, and 100 µg mL^{-1}). Secondary 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 analyzed five times to generate area under the curve (AUC) data.

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

Estriol: $y = 2.5269x + 4.7887$; $R^2 = 0.9858$

Estradiol: $y = 2.1074x + 5.339$; $R^2 = 0.9952$

Estrone: $y = 2.1176x + 4.3981$; $R^2 = 0.9815$

Where, x = concentration of the respective estrogen and y= AUC (dimensionless number). The correlation coefficient (R^2) for each sample indicates an acceptable linear fit of the data (five replicates). Accuracy and precision data for our method used to construct the standard curve for estriol, estradiol and estrone is shown in Table 1. The assay methods are found to be repeatable and robust in the range of 20-100 µg/ml for estriol, 10-100 µg/ml for estradiol and 30-100 µg/ml for estrone. The accuracy (measured by the mean as percentage of the theoretical concentration) of the method is within 10% for all the three estrogens. The data shown in table 2 reflects the mean (expressed as percent of original potency retained) and standard deviation of four separate analyses (each in quadruplet) of each estrogen in the preparations. As evident from the data (table 2), the standard error for all three of the compounds are very low. The precision, expressed as coefficient of variation (standard deviation as % of the mean value) is within 5% of the mean for all the three compounds for all data points.

A topical preparation containing estradiol, estriol, and estrone (0.025%, 0.2%, 0.025% respectively) prepared in HRT Cream Base was incubated at RT and 4°C for a total of 90 days. Samples from both preparations were taken at each of the following time points during the 90 day period: 30 days, 60 days and 90 days. At the end of each incubation period, sample was accurately weighed, solubilized, and further diluted with ACN followed by separation using the chromatographic conditions described previously.

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.

API[a]	Calibration level (µg/ml)	Assay Mean[b] (µg/ml)	Accuracy %
Estriol	20	18.22	91.11
	30	28.09	93.65
	40	43.14	107.87
	50	54.5	108.14
	100	97.32	93.32
Estradiol	10	10.32	103.18
	20	21.31	106.55
	30	28.8	96
	40	39.1	97.74
	50	50.1	100.2
	100	100.38	100.38
Estrone	30	27.99	93.29
	40	43.45	108.62
	50	55.22	110.44
	100	97.2	97.2

Table 2. Stability data of estrogens (estradiol 0.025%, estriol 0.2%, and estrone 0.025%) at room temperature (25°C) and refrigeration (4°C) over 30, 60, and 90 days. Coefficient of variation as percentage of the mean are calculated for the actual samples and signifies the precision of the HPLC assay method

				RT	RT	RT	4°C	4°C	4°C
API			Time 0	Day 30	Day 60	Day 90	Day 30	Day 60	Day 90
Estradiol 0.025%	Mean API (% of labeled amount)		99.36	95.99	96.32	90.5	101.31	99.78	95.76
	Standard deviation		2.32	1.86	4.36	4.17	3.35	3.13	3.26
	Coefficient of Variation (% of mean)		2.33	1.94	4.53	4.61	3.31	3.14	3.4
Estriol 0.2%	Mean API (% of labeled amount)		100.99	99.14	99.24	94.1	96.86	98.42	95.56
	Standard deviation		2.27	1.88	3.96	3.5	1.92	2.2	2.68
	Coefficient of Variation (% of mean)		2.25	1.87	4	3.72	1.98	2.24	2.78
Estrone 0.025%	Mean API (% of labeled amount)		101.65	101.66	98.2	92.9	102.57	98.8	94.92
	Standard deviation		2.06	3.46	2.7	3.86	2.09	3.57	6.09
	Coefficient of Variation (% of mean)		2.03	3.4	2.75	4.16	2.04	3.61	4.62

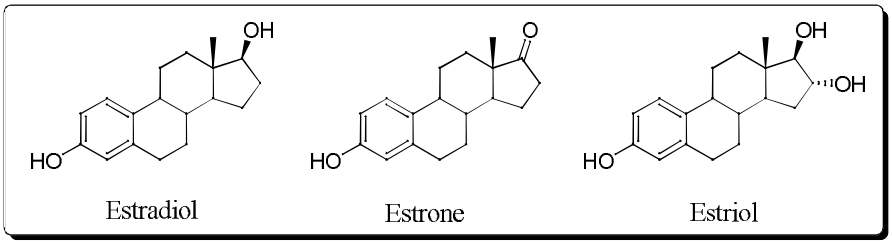

Figure 1. Chemical structure of the estrogens used in the topical preparation.

RESULTS AND DISCUSSION

Beyond-use-dating is intended to ensure that the end user of a given product will experience the maximum benefit of a compounded product provided the use does not extend past the stated date. A curiosity is the stability of a given active pharmaceutical ingredient(s) (API) in various vehicles for topical administration when stored at controlled room temperature or under refrigeration (4°C). An additional curiosity is the influence of higher temperatures (> 25°C) on API stability in compounded preparations. Should an API be heat-labile, does refrigeration alone abrogate the temperature-induced decomposition, or is it possible principles in the vehicle contribute to the degradation of the API? To address such curiosities, we performed stability studies on a compounded product containing a combination of estrogens (estradiol, estriol, and estrone) prepared in proprietary HRT Cream Base manufactured by Medisca Inc. Our study specifically examined the stability of each API at both RT and 4°C at three time points. Using the aforementioned HPLC method, separation was adequately achieved for all three estrogens. Our analysis of the preparation suggests that all three API's maintained adequate stability at both temperatures over all three time intervals (30 day, 60 day, and 90 day). USP-795 stipulates that a threshold of ± 10% the stated potency must be maintained. When comparing mean data to stated potency (0.025% w/w, 0.2% w/w, and 0.025% w/w for estradiol, estriol, and estrone respectively), all samples retain greater than 90% stated potency (Table 2). Noteworthy for our study is the influence of refrigeration on the stability of estradiol and estrone. The 90 day RT sample of estradiol has retained 90.5% of its stated potency while the analogous refrigerated sample retains 95.76% of stated potency.

CONCLUSION

From this data, we can conclude that although temperature seems to have an effect on the stability of all three API's, estradiol appears to be most sensitive to storage at room temperature as the relative potency retained at RT is 90.5% versus 95% for the 90 day refrigerated sample. We have no 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 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.

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