Final Summary of Hormone Stability/BUD Study

Purpose/Background:

The purpose of this study was to evaluate the physical, chemical and microbiological stability of six hormones (Progesterone- P4, Estrone-E1, Estradiol-E2, Estriol-E3, DHEA, and Testosterone) contained in HRT Heavy compounding base. The BUD/stability study is being conducted on High Concentrated Hormones (Humco's HCP), Low Concentrated Hormones, High Combo, and Low Combo formulations. These high/low/high combo/low combo formulations and the range of hormone concentrations cover majority of the prescription for the hormone therapy.

Table 1: Hormone Formulations and Theoretical Amount

Hormone	High Concentration	Low Concentration	High Combo	Low Combo
E3 - Estriol	100 mg/g	0.5 mg/g	5.25 mg/g	1.4 mg/g
E2 - Estradiol	100 mg/g	0.5 mg/g	1.5 mg/g	0.4 mg/g
E1 - Estrone	10 mg/g	0.1 mg/g	0.75 mg/g	0.2 mg/g
Testosterone	200 mg/g	0.5 mg/g	20 mg/g	0.5 mg/g
Progesterone	400 mg/g	10 mg/g	200 mg/g	10 mg/g
DHEA	50 mg/g	1 mg/g	50 mg/g	1 mg/g

Hormone Concentrates, low concentrations, high combo, and low combo were tested on comprehensive stability study which includes: physical (Description, Odor, pH, Weight Loss etc.), Chemical (Potency), and Microbiological. All the BUD samples were placed on stability chamber at Controlled Room Temperature (25°C/60%RH) and tested at day 0, 30 days, 60 days, 90 days, 120 days, 170 days and 180 days of storage. The potency at each time point was determined by the previously validated method RD-038: "Simultaneous Determination of Male and Female Hormones in HRT Heavy Compounding Base and Hormone Concentrates."

Analytical Testing Method:

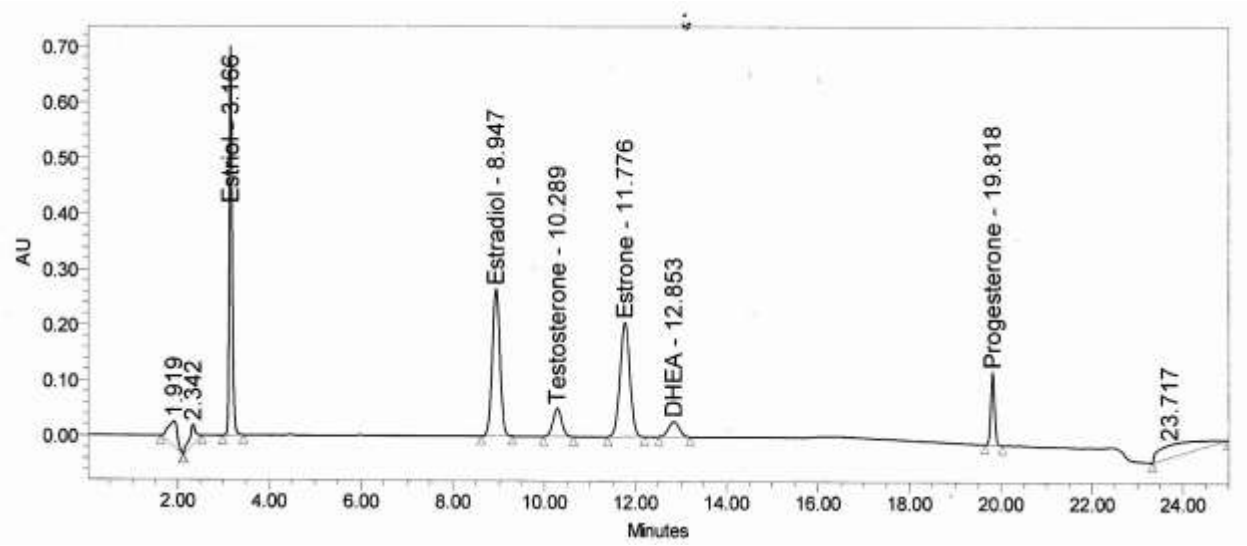
Above hormone formulations were tested using a Stability Indicating Validated method "Simultaneous Determination of Male and Female Hormones P4, E1, E2, E3, DHEA, and T in Hormone Formulations by RP-HPLC –DAD Instrument". Method was developed and validated in-house prior to use for the stability study testing. Method was validated based on the ICH guidelines Q2 (R1) Validation of Analytical Procedures. The method validations parameters used were Linearity, Specificity (Forced Degradation), Accuracy, Robustness, Precision, etc. Basic parameters of HPLC for this method were: Injection volume = 10 µL, wavelength 210 nm, Runtime = 25 minutes, Flow rate 0.8 mg/mL with gradient elution. Phenomenex's Gemini column (C18) was used for the method. The nominal standard concentration was 0.1 mg/mL. Linearity was performed (0.002 mg/mL to 0.1 mg/mL) to make sure R^2 value is greater than 0.999.

Table 2. Mobile Phase Gradient Programming in HPLC

Time (min)	Flow Rate (mL/min)	% Solution A (Buffer)	% Solution B (Acetonitrile)
0.00	0.80	60.0	40.0
13.00	0.80	60.0	40.0
20.00	0.80	5.0	95.0
20.01	0.80	60.0	40.0
25.00	0.80	60.0	40.0

Stock standards (0.5 mg/mL analyte concentration) of Progesterone, Estrone, Estradiol, Estriol, Testosterone and DHEA were prepared by weighing and diluting (by diluent) all standards in the volumetric flask. Stock solutions were diluted to make 0.1 mg/mL concentration of all analytes as a working standard. Fourteen *HRT HEAVY*™ formulations were prepared by weighing 0.25 g – 2.00 g of sample into 100 mL of volumetric flask. The aliquots were sonicated and further diluted to maintain the final analyte concentration of 0.1 mg/mL. All the working sample solutions were filtered through 0.45 µm PTFE syringe filter prior to the analysis. Expected retention times of Estriol is 3.2 minutes, Estradiol is 8.9 minutes, Testosterone is 10.3 minutes, Estrone is 11.8 minutes, DHEA is 12.9 minutes and Progesterone is 19.8 minutes. Example chromatogram of Estriol, Estradiol, Testosterone, Estrone, DHEA and Progesterone in *HRT HEAVY*™ base is shown in Figures 1.

Figure 1. Example Chromatogram of Estriol, Estradiol, Testosterone, Estrone, DHEA and Progesterone in HRT HEAVY™ Topical Cream Sample



Results and Discussions:

All samples met physical testing criteria at all testing time-points according to the Beyond-Use Date Stability testing protocol. Table 3 is the physical results of all hormone formulations.

Table 3. Physical Results of Hormone Formulations

Hormone Formulations		Physical Results			
High Conc.	**Low Conc.**	Description	Odor	pH	Weight Loss
High Conc. Estriol E-3 100 mg/g	Low Conc. Estriol E-3 0.5mg/g	Met Criteria	Met Criteria	7.6 – 7.7	<0.003%
High Conc. Estradiol E-2 100 mg/g	Low Conc. Estradiol E-2 0.5 mg/g	Met Criteria	Met Criteria	7.6 – 7.7	<0.005%
High Conc. Estrone E-1 10 mg/g	Low Conc. Estrone E-1 0.1 mg/g	Met Criteria	Met Criteria	7.6 – 7.7	<0.000%
High Conc. Progesterone 400 mg/g	Low Conc. Progesterone 10 mg/g	Met Criteria	Met Criteria	7.3 – 7.6	<0.089%
High Conc. Testosterone 200 mg/g	Low Conc. Testosterone 0.5 mg/g	Met Criteria	Met Criteria	7.6 – 7.8	<0.024%
High Conc. DHEA 50 mg/g	Low Conc. DHEA 1 mg/g	Met Criteria	Met Criteria	7.6 – 7.7	<0.007%
Hormones High Combo Formulation	Hormones Low Combo Formulation	Met Criteria	Met Criteria	7.2 – 7.6	<0.009%

Microbiological testing was conducted, including Total Combined Yeast and Mold (USP<61), Total Aerobic Microbial Count (USP<61>), *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* (USP <62>). Table 4 is the microbial results of all hormone formulations.

Table 4. Microbiological Results of Hormone Formulations

Hormone Formulations		Microbiological Results			
High Conc.	**Low Conc.**	TAMC	TYMC	Mannitol	Cetrimide
High Conc. Estriol E-3 100 mg/g	Low Conc. Estriol E-3 0.5mg/g	0 cfu/mL	0 cfu/mL	Neg.	Negative
High Conc. Estradiol E-2 100 mg/g	Low Conc. Estradiol E-2 0.5 mg/g	0 cfu/mL	0 cfu/mL	Negative	Negative
High Conc. Estrone E-1 10 mg/g	Low Conc. Estrone E-1 0.1 mg/g	0 cfu/mL	0 cfu/mL	Negative	Negative
High Conc. Progesterone 400 mg/g	Low Conc. Progesterone 10 mg/g	0 cfu/mL	0 cfu/mL	Negative	Negative
High Conc. Testosterone 200 mg/g	Low Conc. Testosterone 0.5 mg/g	0 cfu/mL	0 cfu/mL	Negative	Negative
High Conc. DHEA 50 mg/g	Low Conc. DHEA 1 mg/g	0 cfu/mL	0 cfu/mL	Negative	Negative
Hormones High Combo Formulation	Hormones Low Combo Formulation	0 cfu/mL	0 cfu/mL	Negative	Negative

The potency values (mg/g) as well as the % assay of initial concentration (% recovery) for each Hormone formulation at the Controlled Room Temperature (25°C/60%RH) storage condition are shown in Table 5 and Table 6.

Table 5. Analytical Results of Single-API Hormone Formulations

Single-API Bud Testing	DAY 0	Day 30	Day 60	Day 90	Day 120	Day 170	Day 180
High Conc. Estriol E-3 100 mg/g	100.0%	102.7%	98.4%	98.5%	95.6%	100.9%	99.8%
High Conc. Estradiol E-2 100 mg/g	100.0%	100.9%	102.2%	100.3%	104.4%	101.4%	101.9%
High Conc. Estrone E-1 10 mg/g	100.0%	98.4%	96.0%	98.0%	103.0%	97.0%	98.2%
High Conc. Progesterone 400 mg/g	100.0%	96.9%	102.4%	100.4%	101.0%	99.7%	100.2%
High Conc. Testosterone 200 mg/g	100.0%	102.1%	105.5%	102.5%	108.0%	100.9%	103.2%
High Conc. DHEA 50 mg/g	100.0%	100.8%	90.6%	98.8%	98.7%	101.0%	97.9%
Low Conc. Estriol E-3 0.5 mg/g	100.0%	90.2%	94.6%	92.6%	93.0%	101.7%	108.9%
Low Conc. Estradiol E-2 0.5 mg/g	100.0%	103.5%	102.4%	105.6%	102.3%	98.0%	98.8%
Low Conc. Estrone E-1 0.1 mg/g	100.0%	93.2%	96.8%	101.3%	96.7%	91.0%	94.7%
Low Conc. Progesterone 10 mg/g	100.0%	100.4%	102.9%	98.0%	98.4%	98.2%	106.7%
Low Conc. Testosterone 0.5 mg/g	100.0%	102.0%	110.3%	95.7%	90.2%	92.5%	93.7%
Low Conc. DHEA 1 mg/g	100.0%	94.0%	89.6%	95.9%	97.7%	96.3%	92.0%

Table 6. Analytical Results of Combination-API Hormone Formulations

Combination-API Bud Testing	DAY 0	Day 30	Day 60	Day 90	Day 120	Day 170	Day 180
High Conc. Estriol E-3 5.25 mg/g	100.0%	105.3%	103.3%	106.2%	96.0%	99.4%	99.2%
High Conc. Estradiol E-2 1.50 mg/g	100.0%	100.0%	104.2%	106.8%	95.4%	102.4%	99.0%
High Conc. Estrone E-1 0.75 mg/g	100.0%	102.5%	108.5%	107.7%	90.5%	105.3%	93.1%
High Conc. Progesterone 200.00 mg/g	100.0%	100.6%	99.7%	99.3%	98.0%	98.6%	99.9%
High Conc. Testosterone 20.00 mg/g	100.0%	100.5%	106.1%	103.0%	106.4%	100.0%	103.8%
High Conc. DHEA 50.00 mg/g	100.0%	99.8%	90.0%	99.7%	100.2%	101.4%	102.4%
Low Conc. Estriol E-3 1.40 mg/g	100.0%	103.6%	103.5%	104.1%	96.6%	96.2%	99.5%
Low Conc. Estradiol E-2 0.40 mg/g	100.0%	101.7%	105.3%	105.4%	104.3%	99.6%	100.8%
Low Conc. Estrone E-1 0.20 mg/g	100.0%	93.2%	96.4%	99.8%	93.2%	95.3%	93.0%
Low Conc. Progesterone 10.00 mg/g	100.0%	98.4%	98.0%	96.3%	97.2%	99.8%	99.3%
Low Conc. Testosterone 0.50 mg/g	100.0%	97.6%	105.4%	99.9%	92.4%	93.0%	98.1%
Low Conc. DHEA 1.00 mg/g	100.0%	97.4%	93.3%	99.1%	99.0%	96.6%	99.4%

All of the Hormone Concentrate samples, high conc. samples, low conc. samples, high combo, and low combo stored at the Controlled Room Temperature met the acceptance criteria (% label of initial concentration: 90-110%) over the 180 day study duration.

Conclusion:

The above Hormone formulations (high/low/bracketed) are shown to be stable at Controlled Room Temperature for at least 180 days.