



Scientific Study

B4518, HRT Cream Base
(Hormone Stability-BUD Study)

Not appropriate for regulatory submission.
Please visit www.SpectrumRx.com or
contact Tech Services for the most up-to-
date information contained in this
information package.

Spectrum Pharmacy Products

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Estriol 1.0 % w/w

0.50g Estriol raw drug powder from Tecoland Chemical (bulk API, Lot # 20120524) was added to 49.50g Spectrum Chemical Mfg. Corp. HRT Botanical Cream and levigated with an electronic mortar and pestle, resulting in a final concentration of 1.0% w/w. The sample was then stored at room temperature in the same 50/70 mL Unguator container.

Analytical samples were prepared within 14 days of the scheduled pull by accurately weighing a 5.00 g of the Estriol 1% sample and transferring it to a 50.0 ml volumetric flask. The contents of the flask were diluted to volume with matrix matched mobile phase for HPLC determination.

The prepared samples were analyzed within 14 days of the scheduled pull. The specification for each pull point was no less than 90% and no more than 110% of the theoretical concentration of initial prepared sample. In this case no less than 0.90% w/w and no more than 1.10% w/w. Copies of chromatograms are attached showing no detectable co-elution, baseline interference, or degradation products.

Results were compared and samples were analyzed at various pull points as follows:

Estriol 1% w/w		Actual days	HRT Botanical Cream
Sample preparation	7/20/2012	0	N/A
Initial	8/1/2012	12	1.00%
120 day pull	11/21/2012	124	1.05%
150 day pull	12/14/2012	147	0.82%
180 day pull	1/16/2013	180	Not Tested

Summary:

Estriol 1.0% w/w in Spectrum Chemical Mfg. Corp. HRT Botanical Cream is within specifications at 120 days, but is out of specifications at 150 day pull. The 180 day pull was not tested.

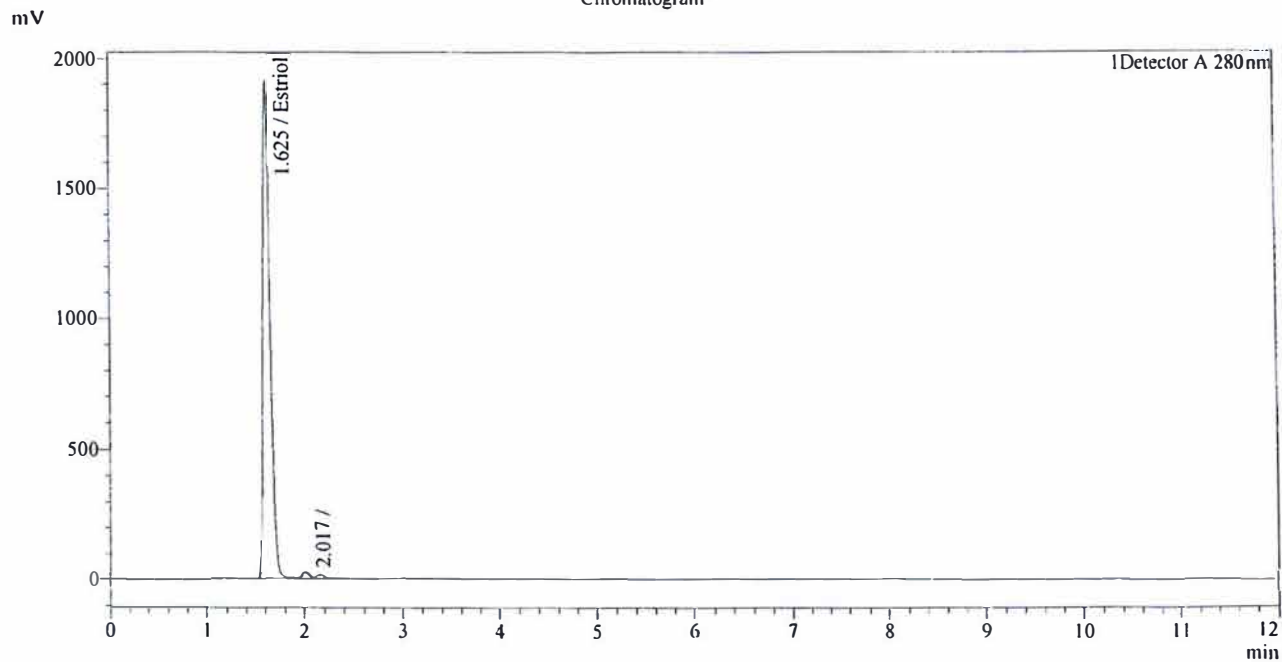
Attachments:

Copies of chromatographs of Estriol 1% in Spectrum Chemical Mfg. Corp. HRT Botanical Cream; Initial Standard, Initial Sample, 120-Day Standard, 120-Day Sample, 150-Day Standard, and 150-Day Sample.

Sample Information

Acquired by : System Administrator
 Sample Name :
 Sample ID : Estriol Std
 Tray# : 1
 Vial# : 1
 Injection Volume : 5
 Data File : August 1-12 Estriol 101.lcd
 Method File : Estriol.lcm
 Batch File : 8-1-12 Estriol.lcb
 Report Format File : DEFAULT.lsr
 Date Acquired : 8/1/2012 3:58:02 PM
 Date Processed : 8/1/2012 6:08:42 PM
 Sample Amount : 1
 Dilution Factor : 1

Chromatogram



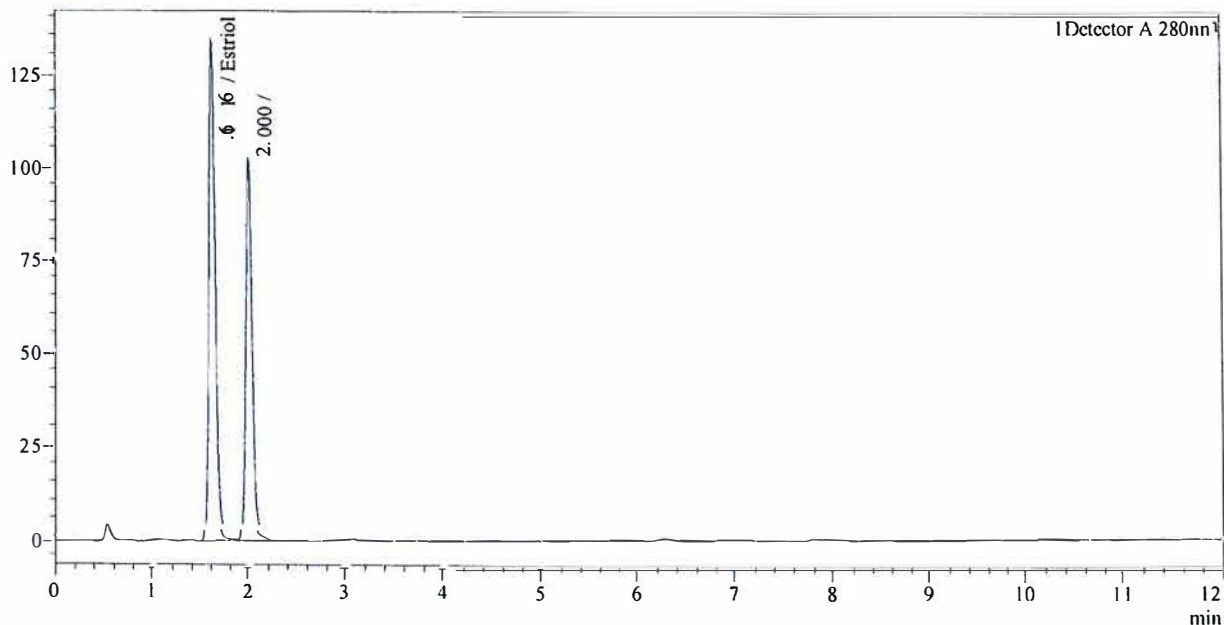
Detector A		QuantitativeResult					
ID#	Name	Ret. Time	Area	Tailing Factor	Conc.	Unit	
1	Estriol	1.625	9606967	1.239	8.172	mg/mL	
Total			9606967				

Sample Information

Acquired by System Administrator
 Sample Name 1% Estriol in HRT
 Sample ID Botanical Cream
 Tray# 1
 Vial# 3
 Injection Volume 5
 Data File August 1-12 Estriol 107.lcd
 Method File Estriol.lcm
 Batch File 8-1-12 Estriol.lcb
 Report Format File DEFAULT.lsr
 Date Acquired 8/1/2012 5:27:13 PM
 Date Processed 8/1/2012 6:08:52 PM 5.0855
 Sample Amount 10
 Dilution Factor

Chromatogram

mV



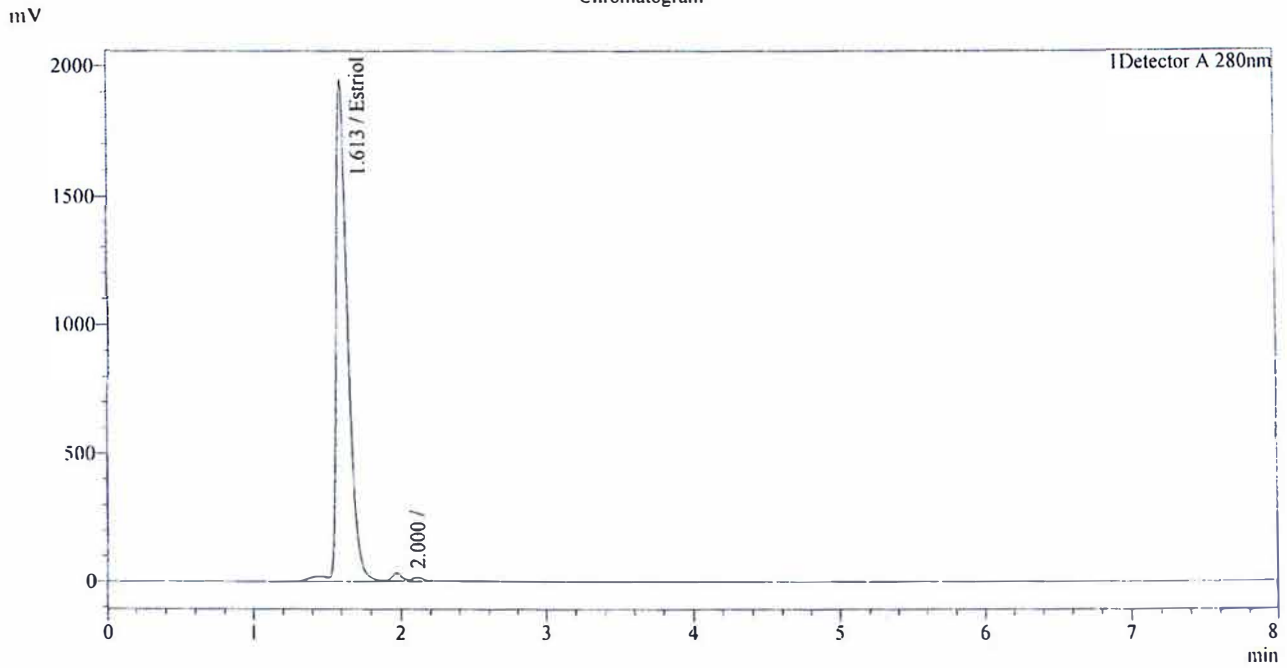
QuantitativeResult

Detector A ID#	Name	Ret. Time	Area	Tailing Factor	Conc.	Unit
1	Estriol	1.616	597707	1.188	0.999	mg/mL
Total			597707			

Sample Information

Acquired by : System Administrator
 Sample Name : Estriol Std
 Sample ID : 1
 Tray# : 1
 Vial# : 1
 Injection Volume : 5
 Data File : November 21-12 Estriol 101.lcd
 Method File : Estriol.lcm
 Batch File : 11-21-12 Estriol.lcb
 Report Format File : DEFAULT.lsr
 Date Acquired : 11/21/2012 9:31:22 AM
 Date Processed : 11/21/2012 10:58:17 AM
 Sample Amount : 1
 Dilution Factor : 1

Chromatogram



Detector A		QuantitativeResult				
ID#	Name	Ret. Time	Area	Tailing Factor	Conc.	Unit
1	Estriol	1.613	10541671	1.348	8.172	mg/mL
Total			10541671			

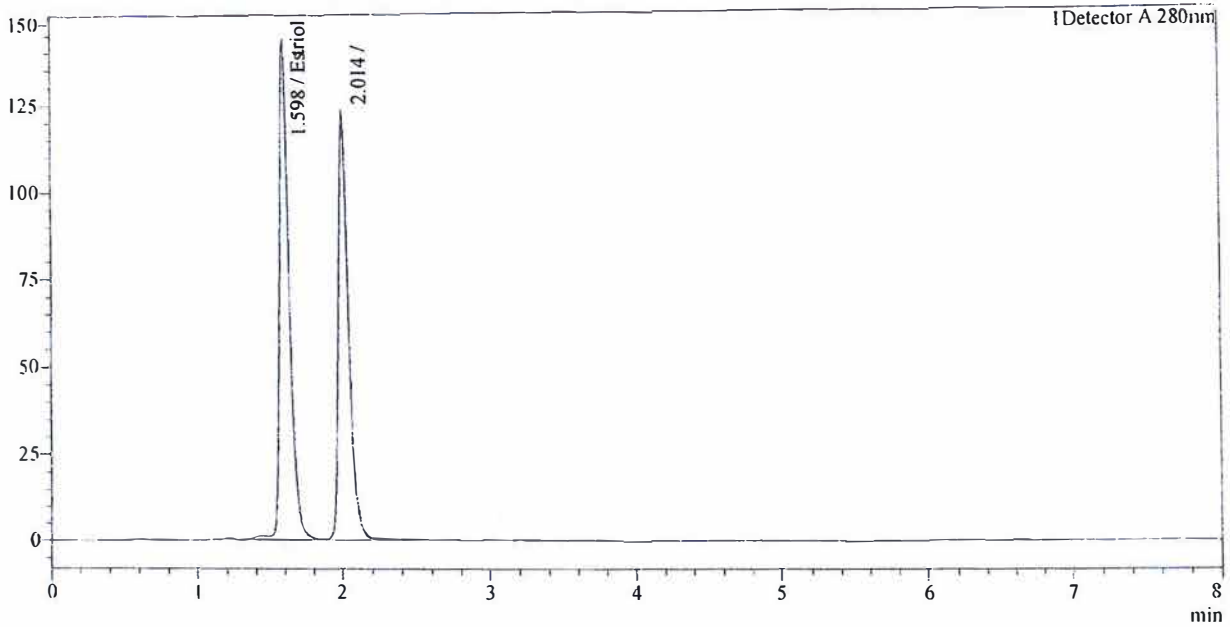
System Administrator

Sample Information

Acquired by : 1% Estriol in HRT
Sample Name : Botanical Cream
Sample ID :
Tray# : 1
Vial# : 3
Injection Volume : 5
Data File : November 21-12 Estriol 107.lcd
Method File : Estriol.lcm
Batch File : 11-21-12 Estriol.lcb
Report Format File : DEFAULT.lsr
Date Acquired : 11/21/2012 10:29:35 AM
Date Processed : 11/21/2012 10:58:26 AM
Sample Amount : 5.1207
Dilution Factor : 10

Chromatogram

mV



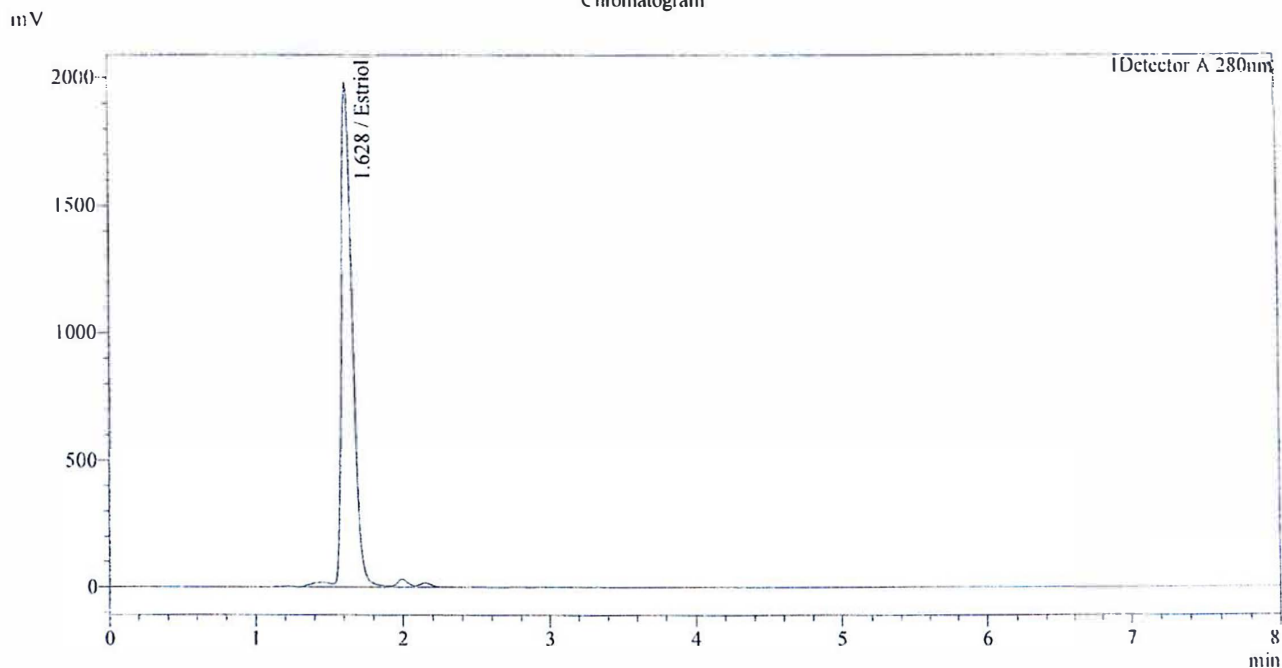
QuantitativeResult

Detector A ID#	Name	Ret. Time	Area	Tailing Factor	Conc.	Unit
1	Estriol	1.598	693948	1.398	1.047	mg/mL
Total			693948			

Sample Information

Acquired by : System Administrator
Sample Name : Estriol Std
Sample ID :
Tray# : 1
Vial# : 1
Injection Volume : 5
Data File : December 14-12 Estriol 101.lcd
Method File : Estriol.lcm
Batch File : 12-14-12 Estriol.lcb
Report Format File : DEFAULT.lsr
Date Acquired : 12/14/2012 8:46:34 AM
Date Processed : 12/14/2012 10:52:21 AM
Sample Amount : 1
Dilution Factor : 1

Chromatogram



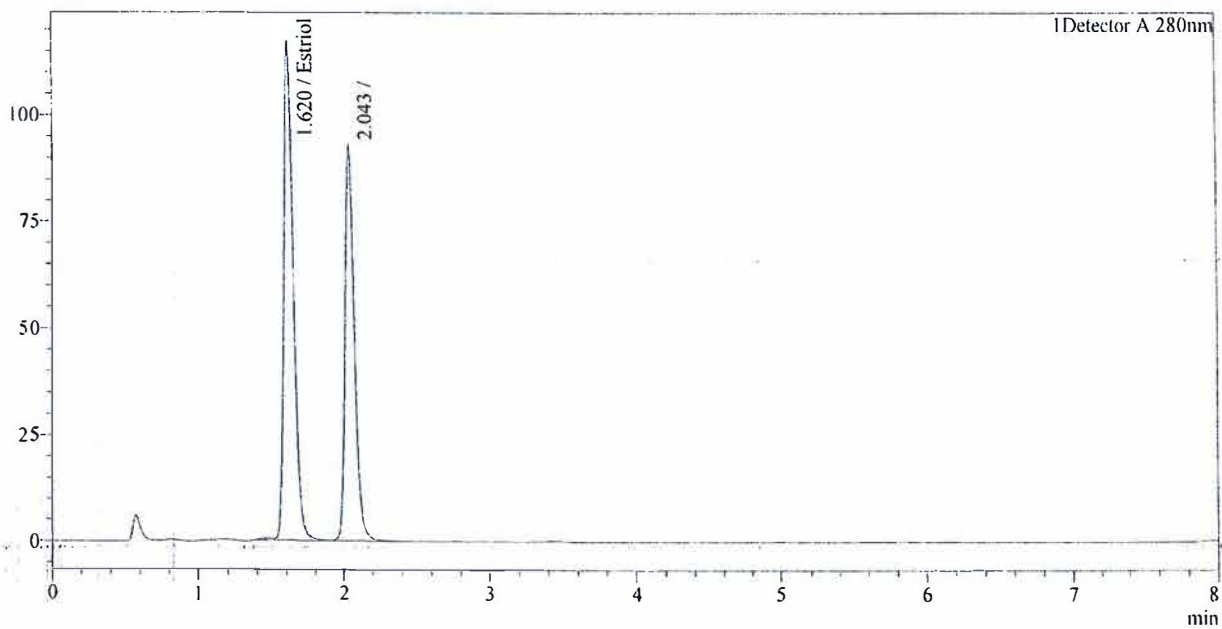
Detector A		QuantitativeResult				
ID#	Name	Ret. Time	Area	Tailing factor	Conc.	Unit
1	Estriol	1.628	10450333	1.274	8.172	mg/mL
Total			10450333			

Acquired by : System Administrator
Sample Name :
Sample ID : 1% Estriol in HRT Botanical
Tray# : Cream
Vial# : 1
Injection Volume : 3
Data File : 5
Method File : December 14-12 Estriol 107.lcd
Batch File : Estriol.lcm
Report Format File : 12-14-12 Estriol.lcb
Date Acquired : DEFAULT.Isr
Date Processed : 12/14/2012 10:25:43 AM
Sample Amount : 12/14/2012 10:52:31 AM 5.1999
Dilution Factor : 10

Sample Information

Chromatogram

mV



Detector A		QuantitativeResult				
ID#	Name	Ret. Time	Area	Tailing Factor	Conc.	Unit
1	Estriol	1.620	530198	1.208	0.820	mg/mL
Total			530198			

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 Spectrum Chemical Mfg. Corp. HRT Cream. The BUD/stability study is being conducted on High Concentrated Hormones, 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

High concentrations, 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 Spectrum Chemical Mfg. Corp. HRT Cream.”

Analytical Testing Method:

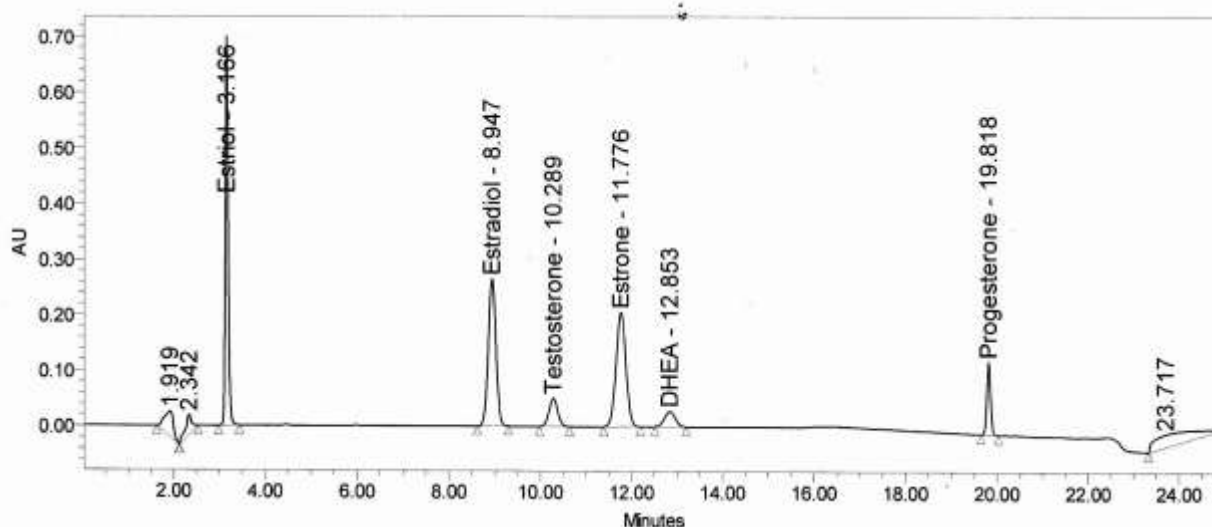
Above hormone formulations were tested using a Stability Indicating Validated method “Simultaneous Determination of Male and Female Hormones P4, E1, E2, E3, DHEA, and T in Hormone Formulations by RP-HPLC –DAD Instrument”. Method was developed and validated in-house prior to use for the stability study testing. Method was validated based on the ICH guidelines Q2 (R1) Validation of Analytical Procedures. The method validation 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 R² 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 *Spectrum Chemical Mfg. Corp. HRT Cream* 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 *Spectrum Chemical Mfg. Corp. HRT Cream* is shown in Figures 1.

Figure 1. Example Chromatogram of Estriol, Estradiol, Testosterone, Estrone, DHEA and Progesterone in *Spectrum Chemical Mfg. Corp. HRT 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 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.