Hormone Stability Studies
USP <795> New Guideline Updates

Updated as of March 2024

Fagron is on a mission to develop the most extensively studied creams for transdermal and vaginal hormones on the market. The revisions to USP <795> (official November 1st, 2023) indicate a changing regulatory climate and a trend towards increasingly high standards for the extension of beyond use dates (BUDs). Given the increased scrutiny and the additional requirements for establishing extended BUDs, additional testing is required to help pharmacies provide the best formulations with scientifically robust data to support an extended BUD for patient convenience. In this study we validate the suitability of two vehicles, Phytobase™ and HRT Heavy™ for compounded hormone replacement therapy by demonstrating stability and antimicrobial effectiveness per USP <51> of the vehicles with various common combinations of hormones. For a summary of results, see below!

New Guideline Compliant HRT BUD Studies in Phytobase

Currently, the below preparations in Phytobase™ already receive extended dating per guidelines official in November 2023. You can view the entire publication in IJPC March/April 2021.[3]

Active Ingredient Bracketed Range	Studied BUD	Container Closure Composition*
Estriol 0.5-20mg/g	180 days	Polypropylene (Topi-Click)
Estradiol 0.5-20mg/g	180 days	Polypropylene (Topi-Click)
Estrone 1-10mg/g	150 days	Polypropylene (Topi-Click)
Progesterone 1-200mg/g	180 days	Polypropylene (Topi-Click)
Testosterone 1-200mg/g	180 days	Polypropylene (Topi-Click)
Low: Estriol 0.5mg, Estradiol 0.5mg, Progesterone 5mg/g High: Estriol 10mg, Estradiol 20mg, Progesterone 200mg/g	180 days	Polypropylene (Topi-Click)

*The same BUD may be applied to preparations in another polypropylene based container such as UnoDose

Updates to Previous HRT Heavy™ Studies and New Combination Studies in Phytobase™ and HRT Heavy™

The previous HRT Heavy™ studies did not meet requirements for BUD extension per the November guideline update as they did not have associated antimicrobial effectiveness testing (AET) per USP <51>. In this study we add AET to validate those previous HRT Heavy studies as well as add on a variety of new combinations bracketed at high and low concentrations for both HRT Heavy™ and Phytobase™. Stability indicating testing for combination products in HRT Heavy™ and Phytobase™ added on in this new study was completed by an independent, third-party, FDA registered, and cGMP compliant laboratory and AET was completed by a combination of the same laboratory and internal testing by Fagron Solutions.

Combinations with a checkmark at the given timepoint have completed both HPLC stability indicating testing to that timepoint as well as AET per USP <51> in the noted container closure. While AET was performed at the 90- and 180-day timepoints, only HPLC stability indicating testing was performed at the 120-day timepoint. Completed stability testing at the 120-day timepoint is

noted with a green dot. Our 90-day timepoint results are published in the January/February 2024 edition of the International Journal of Pharmaceutical Compounding. The 180-day results are currently pending peer reviewed publication. The below **Table 1** and **Table 2** are a summary of the results with information regarding container closure, **Table 3** lists values obtained for each time point as an absolute percentage from previous HRT Heavy studies, and **Table 4** and **Table 5** list values reported as an absolute percentage for the current beyond use date study in HRT Heavy™ and Phytobase™ respectively. **Table 6** summarizes the AET per USP <51> for category 2 products (non-sterile aqueous topical products) passed by all individual API and combination products tested at both the 90- day and 180-day timepoints.

Discussion

All 12 new combination formulations both high and low (24 total preparations) tested within range at the 90-day mark. All 17 bracketed high and low formulations (34 total preparations) demonstrated antimicrobial effectiveness per USP <51> for the entire duration of the study and passed testing at both the 90-day and 180-day timepoints. In HRT Heavy, the estriol/estradiol (biest) combination is limited to 120 days due to estriol testing just below acceptable range at the 180-day mark and the estriol/estradiol/progesterone/testosterone/DHEA combination is limited to 90 days due to the high concentration testing high out of range at the 120-day mark. The reason for this has not been fully elucidated, however, we posit that it may be related to the relative total API load as compared to the cream base. The high concentration product contains 31% weight by weight as active pharmaceutical ingredient. Though our container closure of choice (Topi-Click) does offer a tight seal, it may be that the high overall API to cream base ratio exacerbated minimal water loss over the course of the study contributing to higher-than-expected API concentrations. We posited that this water loss may be exacerbated by how the product was stored and used throughout the test. During the test, the product was kept sealed in the Topi-Click devices, however, material was only removed for testing and not used on a daily basis as we would commonly see for regular patient use of HRT products. This means that cream at the top of the container remained there for long periods of time without being used until the product was opened at the 90-, 120-, and 180-day timepoints, potentially contributing to that slight water loss we saw with the highest API load product. Future studies may consider a lower overall concentration or a procedure that mimics regular usage in which some material is removed from the top on a regular basis. It should also be noted at the 120 -day timepoint our lab did note that some of the Topi-Click containers had not been capped correctly after testing at the 90 -day timepoint, this may also have contributed to some water loss. In Phytobase, the biest/progesterone/testosterone low combination had estriol test low at the 180-day timepoint, and estradiol/testosterone high combination had both active ingredients test slightly above range at the 180-day timepoint, perhaps due to similar factors that influenced the high combination product HRT Heavy, though the reason for this was not entirely clear as similar combinations remained acceptably within range for the entire duration of the study. Overall, all formulations were found to be stable for 90 days and most preparations tested within range for the full 180-day duration of the study.

Conclusion

This study found a wide range of compatibility of hormones in both HRT Heavy™ and Phytobase™, demonstrating the suitability of these vehicles both from a stability and an antimicrobial effectiveness perspective for a wide range of compounded hormone combinations and preparations.

Summary of Results

Table 1. HRT Heavy Bracketed Stability Study Summary

Active Ingredient Bracketed Range[1]	90 days	120 Days	180 days	Container Closure Composition[3]
Estriol 0.5-100mg/g	✓	N/A[2]	✓	Polypropylene/Polyethylene (Unguator)
Estradiol 0.5-100mg/g	✓	N/A[2]	✓	Polypropylene/Polyethylene (Unguator)
Progesterone 10-400mg/g	✓	N/A[2]	✓	Polypropylene/Polyethylene (Unguator)
Testosterone 0.5-200mg/g	✓	N/A[2]	✓	Polypropylene/Polyethylene (Unguator)
Dehydroepiandrosterone (DHEA) 1-50mg/g	✓	N/A[2]	✓	Polypropylene/Polyethylene (Unguator)
Low: Estriol 0.1mg, Estradiol 0.1mg/g High: Estriol 20mg, Estradiol 20mg/g	✓	●	Limited to 120-day BUD	Polypropylene (Topi-Click)
Low: Estriol 0.1mg, Estradiol 0.1mg, Testosterone 0.1mg, Progesterone 1mg/g High: Estriol 20mg, Estradiol 20mg, Testosterone 20mg, Progesterone 200mg	✓	●	✓	Polypropylene (Topi-Click)
Low: Estriol 0.1mg, Estradiol 0.1mg, Testosterone 0.1mg High: Estriol 20mg, Estradiol 20mg, Testosterone 20mg/g	✓	●	✓	Polypropylene (Topi-Click)
Low: Estriol 0.1mg, Estradiol 0.1mg, Testosterone 0.1mg, Progesterone 1mg, DHEA 1mg/g High: Estriol 20mg, Estradiol 20mg, Testosterone 20mg, Progesterone 200mg, DHEA 50mg/g	✓	Limited to 90-day BUD	Limited to 90-day BUD	Polypropylene (Topi-Click)
Low: Estradiol 0.1mg, Testosterone 0.1mg/g High: Estradiol 20mg, Testosterone 20mg/g	✓	●	✓	Polypropylene (Topi-Click)
Low: Estradiol 0.1mg, Testosterone 0.1mg, Progesterone 1mg/g High: Estradiol 20mg, Testosterone 20mg, Progesterone 200mg/g	✓	●	✓	Polypropylene (Topi-Click)

[1] Studies in HRT Heavy did not use any additional solvent

[2] Stability indicating testing has been completed to 180 days in previous studies, only AET is needed to make these studies compliant with new guidelines

[3] The same BUD may be applied to preparations in another polypropylene based container such as UnoDose

✓ At these timepoints, both stability indicating testing and antimicrobial effectiveness testing per USP <51> was performed.

● At these timepoints, only stability indicating testing was performed.

Table 2. Phytobase Bracketed Stability Study Summary

Active Ingredient Bracketed Range[1]	90 days	120 Days	180 days	Container Closure Composition[2]
Estriol 0.5-20mg/g[3]	●	●	✓	Polypropylene (Topi-Click)
Estradiol 0.5-20mg/g[3]	●	●	✓	Polypropylene (Topi-Click)
Estrone 1-10mg/g[3]	●	●	✓	Polypropylene (Topi-Click)
Progesterone 1-200mg/g[3]	●	●	✓	Polypropylene (Topi-Click)
Testosterone 1-200mg/g[3]	●	●	✓	Polypropylene (Topi-Click)
Low: Estriol 0.1mg, Estradiol 0.1mg/g High: Estriol 20mg, Estradiol 20mg/g	✓	●	✓	Polypropylene (Topi-Click)
Low: Estriol 0.1mg, Estradiol 0.1mg, Testosterone 0.1mg, Progesterone 1mg/g High: Estriol 20mg, Estradiol 20mg, Testosterone 20mg, Progesterone 200mg	✓	●	Limited to 120-day BUD	Polypropylene (Topi-Click)
Low: Estriol 0.1mg, Estradiol 0.1mg, Testosterone 0.1mg High: Estriol 20mg, Estradiol 20mg, Testosterone 20mg/g	✓	●	✓	Polypropylene (Topi-Click)
Low: Estradiol 0.1mg, Testosterone 0.1mg/g High: Estradiol 20mg, Testosterone 20mg/g	✓	●	Limited to 120-day BUD	Polypropylene (Topi-Click)
Low: Progesterone 1mg, Testosterone 0.1mg/g High: Progesterone 200mg, Testosterone 20mg/g	✓	●	✓	Polypropylene (Topi-Click)
Low: Estradiol 0.1mg, Testosterone 0.1mg, Progesterone 1mg/g High: Estradiol 20mg, Testosterone 20mg, Progesterone 200mg/g	✓	●	✓	Polypropylene (Topi-Click)
Low: Estriol 0.5mg, Estradiol 0.5mg, Progesterone 5mg/g[3] High: Estriol 10mg, Estradiol 20mg, Progesterone 200mg/g[3]	●	●	✓	Polypropylene (Topi-Click)

[1] Studies in Phytobase used 5 to 10% weight by weight of ethoxy diglycol as solvent

[2] The same BUD may be applied to preparations in another polypropylene based container such as UnoDose

[3] Completed as part of the previous Phytobase study published in 2021, see reference {3} for more details

✓ At these timepoints, both stability indicating testing and antimicrobial effectiveness testing per USP <51> was performed.

● At these timepoints, only stability indicating testing was performed.

Data

Table 3. HRT Heavy Single API High and Low Bracketed Results

API	Elapsed Time (days)	Low Concentration (%)	High Concentration (%)
Estriol (0.5-100mg/g)	0	100.0	100.0
	30	90.2	102.7
	90	92.6	98.5
	120	93.0	95.6
	170	101.7	100.9
	180	108.9	99.8
Estradiol (0.5-100mg/g)	0	100.0	100.0
	30	103.5	100.9
	90	105.6	100.3
	120	102.3	104.4
	170	98.0	101.4
	180	98.8	101.9
Progesterone (10-400mg/g)	0	100.0	100.0
	30	100.4	96.9
	90	98.0	100.4
	120	98.4	101.0
	170	98.2	99.7
	180	106.7	100.2
Testosterone (0.5-200mg/g)	0	100.0	100.0
	30	102.0	102.1
	90	95.7	102.5
	120	90.2	108
	170	92.5	100.9
	180	93.7	103.2
Dehydroepiandrosterone (DHEA) (1-50mg/g)	0	100.0	100.0
	30	94.0	100.8
	90	95.9	98.8
	120	97.7	98.7
	170	96.3	101.0
	180	92.0	97.9

Table 4. HRT Heavy Combination Product High and Low Bracketed Results

Combination (High and Low)	Estriol (%)	Estradiol (%)	Progesterone (%)	Testosterone (%)	DHEA (%)
Estriol 0.1mg Estradiol 0.1mg/g					
T =0	102	102			
T=90	98	100			
T= 120	94	95			
T = 180	85	89			
Estriol 20mg, Estradiol 20mg/g					
T =0	101	101			
T=90	100.5	103			
T=120	102.5	104			
T =180	107	108			
Estriol 0.1mg, Estradiol 0.1mg, Progesterone 1mg, Testosterone 0.1mg/g					
T=0	96	101	99.3	104	
T =90	98	92	102.6	99	
T= 120	101	97	106.7	101	

T= 180	97	99	103.6	97	
Estriol 20mg, Estradiol 20mg, Progesterone 200mg, Testosterone 20mg/g					
T =0	101.5	102	97.6	102	
T = 90	102.5	104.5	103.1	103.5	
T=120	104.5	105.5	105.7	104	
T =180	107.5	108.5	106.6	106.5	
Estriol 0.1mg, Estradiol 0.1mg, Testosterone 0.1mg/g					
T =0	102	98		109	
T=90	96	102		101	
T=120	95	97		101	
T = 180	99	100		98	
Estriol 20mg, Estradiol 20mg, Testosterone 20mg/g					
T=0	100.5	101		100.5	
T=90	101	102		100.5	
T=120	103	103		102	
T = 180	106.5	108.5		107.5	
Estriol 0.1mg, Estradiol 0.1mg, Testosterone 0.1mg, Progesterone 1mg, DHEA 1mg					
T =0	101	97	95.5	105	96.7
T=90	98	103	105.5	103	103.7
T = 120	97	99	101.4	107	96.9
T = 180	88	96	101.2	95	97.3
Estriol 20mg, Estradiol 20mg, Testosterone 20mg, Progesterone 200mg, DHEA 50mg/g					
T =0	100.5	101	101.2	100.5	98.4
T=90	106	108	106.2	107	106.4
T =120	113	115	114.3	113	114.2
Estradiol 0.1mg, Testosterone 0.1mg/g					
T =0		96		103	
T =90		98		95	
T= 120		99		100	
T = 180		99		94	
Estradiol 20mg, Testosterone 20mg/g					
T =0		101		100.5	
T=90		101.5		101.5	
T = 120		107.5		106	
T = 180		107.5		105.5	
Estradiol 0.1mg, Testosterone 0.1mg, Progesterone 1mg/g					
T =0		96	96.3	106	
T =90		99	103.8	99	
T = 120		97	101	104	
T= 180		101	107.1	98	
Estradiol 20mg, Testosterone 20mg, Progesterone 200mg/g					
T =0		102	108.6	103	
T =90		102	102.9	101.5	
T=120		102.5	105.2	101.5	
T =180		109.5	109	108	

Table 5. Phytobase Combination Product High and Low Bracketed Results

Combination (High and Low)	Estriol (%)	Estradiol (%)	Progesterone (%)	Testosterone (%)
Estriol 0.1mg Estradiol 0.1mg/g				
T =0	98	93		
T=90	94	90		
T= 120	93	91		
T = 180	100	100		
Estriol 20mg, Estradiol 20mg/g				
T =0	101.5	102.5		
T=90	98.5	99.5		
T=120	102.5	102.5		
T = 180	103.5	105		
Estriol 0.1mg, Estradiol 0.1mg, Progesterone 1mg, Testosterone 0.1mg/g				
T=0	97	95	91.9	96
T =90	101	97	96.5	99
T=120	103	100	97.3	99
T = 180	89	93	93.3	94
Estriol 20mg, Estradiol 20mg, Progesterone 200mg, Testosterone 20mg/g				
T =0	98.5	99.5	101.7	100
T = 90	106.5	108	110	109.5
T =120	103	100	97.3	99
T = 180	105	106.5	108.8	106
Estriol 0.1mg, Estradiol 0.1mg, Testosterone 0.1mg/g				
T =0	101	99		100
T=90	98	104		100
T= 120	91	104		110
T = 180	99	103		101
Estriol 20mg, Estradiol 20mg, Testosterone 20mg/g				
T=0	95	100		99
T=90	105.5	101.5		101.5
T=120	107	102		106.5
T = 180	99.5	107.5		103
Estradiol 0.1mg, Testosterone 0.1mg/g				
T =0		101		102
T =90		98		102
T = 120		102		105
T = 180		103		107
Estradiol 20mg, Testosterone 20mg/g				
T =0		101		101
T=90		105		105.5
T=120		107.5		107
T =180		112.5		113
Estradiol 0.1mg, Testosterone 0.1mg, Progesterone 1mg/g				
T =0		97	98.1	100
T =90		101	109.4	104
T = 120		90	98.7	97
T = 180		90	98.9	96
Estradiol 20mg, Testosterone 20mg, Progesterone 200mg/g				
T =0		101.5	99.6	101.5
T =90		105.5	100.5	105.5
T= 120		107.5	107.4	107
T =180		105	104.6	104.5

Progesterone 0.1mg, Testosterone 0.1mg/g				
T =0			99.6	95
T= 90			105.4	96
T = 120			101.6	102
T = 180			106.4	107
Progesterone 200mg, Testosterone 20mg/g				
T =0			99.5	101.5
T=90			100.8	105.5
T= 120			104.8	103.5
T =180			104.1	103.5

Table 6. Antimicrobial Effectiveness Testing per USP <51> Performed on All Formulations

Organism	Time	Results	Acceptable Limit
Candida albicans	14 days	Pass	No increase in the number of CFU initially inoculated
Candida albicans	28 days	Pass	No increase in the number of CFU initially inoculated
Aspergillus brasiliensis	14 days	Pass	No increase in the number of CFU initially inoculated
Aspergillus brasiliensis	28 days	Pass	No increase in the number of CFU initially inoculated
Escherichia coli	14 days	Pass	NLT 2 log reduction in the number of CFU initially inoculated
Escherichia coli	28 days	Pass	No increase from the 14 days count
Pseudomonas aeruginosa	14 days	Pass	NLT 2 log reduction in the number of CFU initially inoculated
Pseudomonas aeruginosa	28 days	Pass	No increase from the 14 days count
Staphylococcus aureus	14 days	Pass	NLT 2 log reduction in the number of CFU initially inoculated
Staphylococcus aureus	28 days	Pass	No increase from the 14 days count

Antimicrobial effectiveness testing per USP <51> is intended to test the viability of the preservative system of a preparation. The process involves inoculation with the above listed organisms and then monitoring for 28 days after inoculation to see if the preservative system can adequately prevent microbial growth and replication. In our study, we did the first round of AET at day 90 (inoculation occurred at the 90 day timepoint, and then the preparation was monitored for 28 days in accordance with USP <51>), then we did a second round of AET at day 180 (inoculation occurred at the 180 day timepoint, and then the preparation was monitored for 28 days in accordance with USP <51>).

References:

1. *United States Pharmacopeia and National Formulary* (USP 43-NF 38). Pharmacopeial Forum Vol No 47(6). Accessed September 25, 2023. https://online.uspnf.com/uspnf/document/1_GUID-98DCB48D-DC23-4A63-AD2E-01CA8979FB7E_5_en-US?source=Search%20Results&highlight=795
2. *United States Pharmacopeia and National Formulary* (USP 43-NF 38). Pharmacopeial Forum Vol No 47(6). Accessed September 25, 2023. https://online.uspnf.com/uspnf/document/1_GUID-98DCB48D-DC23-4A63-AD2E-01CA8979FB7E_6_en-US?source=Search%20Results&highlight=795
3. Wynn T, Taylor S, Zander C. Stability of Fagron's Phytobase Cream Compounded with Various Hormones. IJPC. 2021;25(2): 156-162.
4. Taylor S, Haselhorst R. Compatibility of Various Hormones in Phytobase and HRT Heavy Cream Bases. IJPC. 2024; 28(1): 75-81.