Hormone Stability Studies
USP <795> New Guideline Updates

Updated as of May 2024

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 HRT Heavy™ for compounded hormone replacement therapy by demonstrating stability and antimicrobial effectiveness per USP <51> of the vehicle with various common combinations of hormones. For a summary of results, see below!

Updates to Previous HRT Heavy™ Studies and New Combination Studies in 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 in HRT Heavy™. Stability indicating testing for combination products in HRT Heavy™ 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** is a summary of the results with information regarding container closure, **Table 2** lists values obtained for each time point as an absolute percentage from previous HRT Heavy studies, and **Table 3** lists values reported as an absolute percentage for the current beyond use date study in HRT Heavy™. **Table 4** 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.

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.

Data

Table 2. 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 3. 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 4. 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. Taylor S, Haselhorst R. Compatibility of Various Hormones in Phytobase and HRT Heavy Cream Bases. IJPC. 2024; 28(1): 75-81.