

**LEUPROLIDE (LEUPRORELIN) US
DRUG SUBSTANCE SPECIFICATION**



Molecular weight: 1209.4 (free peptide)

Page 1 of 2

Sequence: p-Glu-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NH₂

Last update: 09 JUN 2018

Available registration documents (CTD format): DMF

TEST	ACCEPTANCE CRITERION	ANALYTICAL PROCEDURE																
Appearance	White to off-white powder	Visual inspection																
Solubility	Soluble in water and 1% (v/v) aqueous acetic acid at a concentration of ≥ 10 mg/mL to give a clear, colorless solution	Visual inspection																
Identity, MS	Monoisotopic mass 1208.7 ± 1 u	USP																
Identity, Amino acid analysis	<table border="0"> <tr><td>Ser</td><td>Present</td></tr> <tr><td>Glx</td><td>0.85 to 1.1</td></tr> <tr><td>Pro</td><td>0.85 to 1.1</td></tr> <tr><td>Leu</td><td>1.8 to 2.2</td></tr> <tr><td>Tyr</td><td>0.85 to 1.1</td></tr> <tr><td>His</td><td>0.85 to 1.1</td></tr> <tr><td>Arg</td><td>0.85 to 1.1</td></tr> <tr><td>Trp</td><td>Present</td></tr> </table>	Ser	Present	Glx	0.85 to 1.1	Pro	0.85 to 1.1	Leu	1.8 to 2.2	Tyr	0.85 to 1.1	His	0.85 to 1.1	Arg	0.85 to 1.1	Trp	Present	USP
Ser	Present																	
Glx	0.85 to 1.1																	
Pro	0.85 to 1.1																	
Leu	1.8 to 2.2																	
Tyr	0.85 to 1.1																	
His	0.85 to 1.1																	
Arg	0.85 to 1.1																	
Trp	Present																	
Identity, HPLC	Co-elutes with reference standard	USP																
Peptide purity, HPLC	$\geq 97.5\%$	USP/Ph. Eur.																
Total impurities, HPLC	$\leq 2.5\%$	USP/Ph. Eur.																
Related substances, HPLC	<table border="0"> <tr><td>Acetyl-Leuprolide</td><td>$\leq 1.0\%$</td></tr> <tr><td>D-His-Leuprolide</td><td>$\leq 0.5\%$</td></tr> <tr><td>L-Leu⁶-Leuprolide</td><td>$\leq 0.5\%$</td></tr> <tr><td>D-Ser-Leuprolide</td><td>$\leq 0.5\%$</td></tr> <tr><td>Any other impurity</td><td>$\leq 0.5\%$</td></tr> </table>	Acetyl-Leuprolide	$\leq 1.0\%$	D-His-Leuprolide	$\leq 0.5\%$	L-Leu ⁶ -Leuprolide	$\leq 0.5\%$	D-Ser-Leuprolide	$\leq 0.5\%$	Any other impurity	$\leq 0.5\%$	USP/Ph. Eur.						
Acetyl-Leuprolide	$\leq 1.0\%$																	
D-His-Leuprolide	$\leq 0.5\%$																	
L-Leu ⁶ -Leuprolide	$\leq 0.5\%$																	
D-Ser-Leuprolide	$\leq 0.5\%$																	
Any other impurity	$\leq 0.5\%$																	
Acetic acid, HPLC	4.7% to 9.0%	In-house																

**LEUPROLIDE (LEUPRORELIN) US
DRUG SUBSTANCE SPECIFICATION**



Molecular weight: 1209.4 (free peptide)

Page 2 of 2

Sequence: p-Glu-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NHEt

Last update: 09 JUN 2018

Available registration documents (CTD format): DMF

TEST	ACCEPTANCE CRITERION	ANALYTICAL PROCEDURE
Water, Karl Fischer	≤ 8.0%	USP
Leuprolide content, HPLC	≥ 82%	USP/Ph. Eur.
Leuprolide content (With reference to the anhydrous, acetic acid free substance)	97.0% to 103.0%	Calculation
Specific optical rotation $[\alpha]_D^{20}$ (c = 1, 1% acetic acid) With reference to the anhydrous, acetic acid free substance	-38.0 to -42.0	USP
Residual solvents, GC		In-house
	Acetonitrile ≤ 410 ppm (w/w)	
	Ethanol ≤ 5000 ppm (w/w)	
	Methanol ≤ 1000 ppm (w/w)	
	Triethylamine ≤ 320 ppm (w/w)	
Trifluoroacetic acid, HPLC	≤ 0.25%	In-house
Phosphate, IC	≤ 0.1%	In-house
Residue on ignition	≤ 0.3%	USP
Bacterial endotoxins	≤ 166.7 IU/mg	USP