

## **Scrutiny of Protocols:**

**rh-Insulin Finished Formulations  
from Manufacturer's of Insulin injection-  
vials/prefilled syringe**

***P: 1-9***

- includes Protocol for rh-Insulin Bulk

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**Summary Protocol of the finished product  
(Final lot) of rh-Insulin**

1	Pharmacopoeia compliance	
2	Trade name	
3	Batch numbers <ul style="list-style-type: none"> <li>• Finished product</li> <li>• Final Bulk</li> </ul>	
4	Type of containers ( vial / Pre Filled Syringe)	
5	Total number of containers in this batch	
6	Total number of containers used in QC tests	
7	Filling volume per container	
8	Composition –Insulin units/ mL	
9	Formulation of Insulin	
10	Date of Manufacturing	
11	Date of Expiry	
12	Storage temp	
13	Product license number –marketing authorization	
14	Name and address of manufacturer	
15	Name and address of product license holder if different	
16	QC tests performed by the manufacturer <i>As per the list attached (Pharmacopoeia- IP/ EP/ BP/ USP)</i>	

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CHECKLIST FOR PROTOCOL SCRUTINY AS PER USP

QC Tests on rh-Insulin Final lot

Mfg Name:..... B. No:..... Mfg. dt: ..... Expiry dt. : .....

*Formulation: Regular/ NPH/ Lente/ Human Insulin Isophane Suspension & Human Insulin Injection*

S.No	Tests Method	Manufacturer result	Specifications/ limits	NIB Results	Remarks
1	Identification <i>HPLC</i>		The retention time of the major peak in the chromatogram with the Assay preparation (test solution) corresponds to that of the major peak obtained with the standard preparation (1.5 mg/ ml)		
2	Assay (Potency) <i>HPLC</i>		NLT 95% & NMT the equivalent of 105% of the amount of Insulin stated on the label a) 38IU/ml-42IU/ml (40IU) b) 95IU/ml-105IU/ml (100 IU)	.....IU/ml % Label claim .....%	
3	Insulin in supernatant <i>HPLC</i>		• NPH/ Lente: NMT 1.0 IU/ ml Human Insulin Isophane Suspension & Human Insulin Injection: the % of soluble insulin human is in the range $L \pm 5$ , where L is the % of soluble insulin human stated on the product label. a) 30/70: 10-14IU (for 40IU) & 25-35IU (for 100IU) b) 50/50: 18-22IU (for 40IU) & 45-55IU (for 100IU)		
4	Related Proteins In house <i>HPLC</i>		NMT 2%	A21desamido: .....%  Other: .....%	
5	HMWP <i>HPLC</i>		• Regular: NMT 1.7% • NPH, Human Insulin Isophane Suspension & Human Insulin Injection : NMT 3% • Lente: NMT 1.5%	.....%	
6	Zinc <i>At. Absorption spectrometry</i>		• Regular: 10 µg - 40 µg/100 IU • NPH: 0.021mg- 0.04mg/100IU • Lente: 0.12mg-0.25mg/100 IU (total Zn) 20%-65% of total Zn (Zn in solution)		
7	Bacterial Endotoxin <i>Gel clot</i>		< 80USP EU/ 100USP Insulin units (< 32USP EU/ 40USP Insulin units- after conversion)		
8	Sterility <i>Membrane filtration</i>		Should comply membrane filtration method in USP		
9	Description (Colour/ Appearance/ Clarity) <i>Visual</i>		<b>Soluble:</b> 1) A colorless liquid, 2) clear as that of water & opalescence NMT suspension 1, 3) free from turbidity & foreign matter; 4) During storage, traces of a very fine sediment may be deposited. <b>Suspensions:</b> 1) A white suspension, 2) which on standing deposits a white sediment, 3) & leaves a colorless or almost colorless supernatant liquid, 4) The sediment is readily resuspended by gently shaking.		
10.1	Particulate matter ( <i>Light Obscuration</i> )		<i>Done for formulation in Regular: SVI</i> >10 µm size particles: NMT 6000/ vial >25 µm size particles: NMT 600/ vial		
10.2	Particulate matter ( <i>Microscopic method</i> )		<i>Done for formulation in suspension (&lt;100ml solution)</i> >10 µm size particles: NMT 3000/ vial >25 µm size particles: NMT 300/ vial		
11	pH <i>Potentiometric</i>		Regular, Lente: Between 7.0 & 7.8 NPH : Between 7.0 & 7.5		

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CHECKLIST FOR PROTOCOL SCRUTINY AS PER EP/BP/IP

QC Tests on rh-Insulin Final lot

Mfg Name:..... B. No:..... Mfg. dt: ..... Expiry dt. : .....

*Formulation: Regular/ NPH/ Biphasic isophane/ Lente*

S.No	Tests Method	Manufacturer result	Specifications/ limits	NIB Results	Remarks
1	Identification <i>HPLC</i>		The position of the peak due to Insulin in the chromatogram obtained with test solution corresponds to that of principal peak obtained with appropriate Ref. solution.		
2	Assay (Potency) <i>HPLC</i>		NLT 90% & NMT the equivalent of 110% of the amount of Insulin stated on the label a) 36-44 IU/ml (40IU) b) 90-110IU/ml (100IU)	.....IU/ml % Label claim .....%	
3	Insulin in supernatant <i>HPLC</i>		•NPH, Lente: NMT 2.5% of total insulin content • Biphasic isophane: the defined ratios shall be demonstrated by a test method which is approved by the competent authority to comply with the label claim.	.....%	
4	Related Proteins <i>HPLC</i>		A21desamido HI: NMT 5% of total areas of peaks Others apart from HI & A21 Desamido HI: NMT 6% of total areas of peaks	A21desamido: .....% Other: .....%	
5	HMWP <i>HPLC</i>		• Non-Protamine (Regular, Lente): NMT 2% • Protamine (NPH, biphasic isophane): NMT 3%	.....%	
6	Zinc <i>At. Absorption spectrometry</i>		• Regular, NPH, biphasic isophane: NMT40µg/100IU • Lente: 0.12mg-0.25mg/100 IU (total Zn) 20%-65% of total Zn (Zn in solution)		
7	Bacterial Endotoxin <i>Gel clot</i>		Less than 80IU per 100IU of Insulin ( < 32IU per 40 IU of Insulin- after conversion)		
8	Sterility <i>Membrane filtration</i>		Should comply membrane filtration method		
9	Description (Colour/ Appearance/ Clarity) <i>Visual &amp; Chemical</i>		<b>Soluble:</b> 1) A colorless liquid, 2) clear as that of water & opalescence NMT suspension 1, 3) free from turbidity & foreign matter; 4) During storage, traces of a very fine sediment may be deposited. <b>Suspensions:</b> 1) A white suspension, 2) which on standing deposits a white sediment, 3) & leaves a colorless or almost colorless supernatant liquid, 4) The sediment is readily resuspended by gently shaking.		
10.1	Particulate matter ( <i>Light obscuration</i> )		<i>Done for formulation in Regular: SVI</i> ≥10 µm size particles: NMT 6000/ vial ≥25 µm size particles: NMT 600/ vial		
10.2	Particulate matter ( <i>Microscopic</i> )		<i>Done for formulation in suspension (&lt;100ml solution)</i> ≥10 µm size particles: NMT 3000/ vial ≥25 µm size particles: NMT 300/ vial		
	Crystal: Shape & size		<b>NPH, Biphasic isophane:</b> rod-shaped , maximum > 1 µm, rarely > 60µm, free from large aggregates <b>Lente:</b> rhombohedral, maximum >10µm, rarely > 40µm; no uniform shape & rarely >2µm		
11	pH <i>Potentiometric</i>		Between 6.9 & 7.8		

**Test 1 Physical Quality**  
**APPEARANCE/ SOLUBILITY**  
*Date*  
*Qty. used in this test*  
*Method of analysis*  
*Specification*  
  
*Result*

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**2 LOSS ON DRYING**  
*Date*  
*Qty. used in this test*  
*Method of analysis*  
*Specification*  
  
*Result*

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**3 SULPHATED ASH**  
*Date*  
*Qty. used in this test*  
*Method of analysis*  
*Specification*  
  
*Result*

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**Test 4 Identification**  
**PEPTIDE MAP**  
*Date*  
*Qty. used in this test*  
*Method of analysis*  
 a) HPLC Column  
 Cat. No., Column dimensions  
  
 b) Mobile Phase  
  
 c) *Staphylococcus aureus* strain V8  
 protease/ *Staphylococcus aureus* V8  
 protease  
 d) Reference Standard  
 Cat. No./ B. No.  
  
 e) Reference Solutions prepared  
  
 f) Sample Qty used  
  
 g) Peptide map chromatogram  
  
*Specification*  
  
*Result*

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Source	Column temp.
Name	Ratio used
Source	Activity
Source	Assigned potency
Ref. Std	Sample

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<b>Test</b>	<b>Purity</b>		
8	RELATED PROTEINS		
	<i>Date</i>	-----	
	<i>Qty. used in this test</i>	-----	
	<i>Method of analysis</i>	-----	
	a) HPLC Column	Source	Column temp.
	Cat. No., Column dimensions	-----	
	b) Mobile Phase	Name	Ratio used
		-----	
	c) Reference Standards	Source	Assigned potency
	Cat. No./ B. No.	-----	
	d) Reference Solutions prepared	-----	
		-----	
	e) Chromatograms of validity criteria	-----	
		-----	
	<i>Specification</i>	-----	
		-----	
	<i>Result</i>	-----	
		-----	
9	HR MOLECULAR WEIGHT PROTEINS (HMWP)		
	<i>Date</i>	-----	
	<i>Qty. used in this test</i>	-----	
	<i>Method of analysis</i>	-----	
	a) HPLC Column	Source	Column temp.
	Cat. No., Column dimensions	-----	
	b) Mobile Phase	Name	Ratio used
		-----	
	c) Reference Standards	Source	Assigned potency
	Cat. No./ B. No.	-----	
	d) Reference Solutions prepared	-----	
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	e) Chromatograms of validity criteria	-----	
		-----	
	<i>Specification</i>	-----	
		-----	
	<i>Result</i>	-----	
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<b>Test</b>	<b>Purity.....contd.</b>		
10	ZINC CONTENT		
	<i>Date</i>	-----	
	<i>Qty. used in this test</i>	-----	
	<i>Method of analysis</i>	-----	
	Reference Standard	Source	Conc. of Zn/ml
	Cat. No.		
	Reference Solutions prepared	-----	
	<i>Specification</i>	-----	
	<i>Result</i>	-----	
11	HOST CELL DERIVED PROTEINS		
	<i>Date</i>	-----	
	<i>Qty. used in this test</i>	-----	
	<i>Method of analysis</i>	-----	
	Kit used (with details)	-----	
	<i>Specification</i>	-----	
	<i>Result</i>	-----	
12	SINGLE CHAIN PRECURSOR		
	<i>Date</i>	-----	
	<i>Qty. used in this test</i>	-----	
	<i>Method of analysis</i>	-----	
	<i>Specification</i>	-----	
	<i>Result</i>	-----	
13	PROINSULIN LIKE IMMUNOREACTIVITY		
	<i>Date</i>	-----	
	<i>Qty. used in this test</i>	-----	
	<i>Method of analysis</i>	-----	
	Kit used (with details)	-----	
	<i>Specification</i>	-----	
	<i>Result</i>	-----	
<b>Test</b>	<b>Contamination</b>		
14.1	MICROBIAL LIMITS		
	<i>Date</i>	-----	
	<i>Qty. used in this test</i>	-----	
	<i>Method of analysis</i>	-----	
	<i>Specification</i>	-----	
	<i>Result</i>	-----	

**Test**      **Contamination ....contd.**  
14.2      BACTERIAL ENDOTOXIN TEST*Date**Qty. used in this test**Method of analysis*

Bacterial Endotoxin Kit used

CSE

Reconstitution Date

Lot No

Expiry Date

Lysate

Sensitivity (IU/ml)

Lot No

Expiry Date

Date of reconstitution of lysate

Incubation at 37°C

Dry block/ waterbath

*Specification**Result*

## 14.3      PROTEIN CONTAMINATION

*Date**Qty. used in this test**Method of analysis**Specification**Result*

## 14.4      MICROBIAL CONTAMINATION

*Date**Qty. used in this test**Method of analysis**Specification**Result*

## 14.5      CHEMICAL CONTAMINATION: Residual solvents, Column leachable material

*Date**Qty. used in this test**Method of analysis**Specification**Result***Test**      **Intact Mol.Wt**

15

*Date**Qty. used in this test**Method of analysis**Specification**Result***Test**      **Sulphaydryl Group & Disulfide  
Bridge Arrangement**

16

*Date**Qty. used in this test**Method of analysis**Specification**Result*

**Test    Carbohydrate content**

17    *Date*  
      *Qty. used in this test*  
      *Method of analysis*  
      *Specification*

*Result*

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**Test    Tertiary structure**

18    *Date*  
      *Qty. used in this test*  
      *Method of analysis*  
      *Specification*

*Result*

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