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 • Finished product • Final Bulk	
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 *As per the list attached (*Pharmacopoeia- *IP/ EP/ BP/ USP)*	

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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 *HPLC*		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) *HPLC*		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 *HPLC*		• NPH/ Lente: NMT 1.0 IU/ ml Human Insulin Isophane Suspension & Human Insulin Injection: the % of soluble insulin human is in the range L±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 *HPLC*		NMT 2%	A21desamido: …….....% Other: …….....%	
5	HMWP *HPLC*		• Regular: NMT 1.7% • NPH, Human Insulin Isophane Suspension & Human Insulin Injection : NMT 3% • Lente: NMT 1.5%	……%	
6	Zinc *At. Absorption spectrometery*		• 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 *Gel clot*		< 80USP EU/ 100USP Insulin units *(< 32USP EU/ 40USP Insulin units- after conversion)*		
8	Sterility *Membrane filtration*		Should comply membrane filtration method in USP		
9	Description (Colour/ Appearance/ Clarity) *Visual*		**Soluble:** 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. **Suspensions**: 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 *(Light Obscuration)*		*Done for formulation in Regular: SVI* >10 µm size particles: NMT 6000/ vial >25 µm size particles: NMT 600/ vial		
10.2	Particulate matter *(Microscopic method*)		*Done for formulation in suspension (<100ml solution)* >10 µm size particles: NMT 3000/ vial >25 µm size particles: NMT 300/ vial		
11	pH *Potentiometric*		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 *HPLC*		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) *HPLC*		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 *HPLC*		•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 *HPLC*		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 *HPLC*		• Non-Protamine (Regular, Lente): NMT 2% • Protamine (NPH, biphasic isophane): NMT 3%	……%	
6	Zinc *At. Absorption spectrometery*		• 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 *Gel clot*		Less than 80IU per 100IU of Insulin *(< 32IU per 40 IU of Insulin- after conversion)*		
8	Sterility *Membrane filtration*		Should comply membrane filtration method		
9	Description (Colour/ Appearance/ Clarity) *Visual & Chemical*		**Soluble:** 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. **Suspensions**: 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 *(Light obscuration)*		*Done for formulation in Regular: SVI* *≥10 µm size particles: NMT 6000/ vial* *≥25 µm size particles: NMT 600/ vial*		
10.2	Particulate matter *(Microscopic)*		*Done for formulation in suspension (<100ml solution)* ≥10 µm size particles: NMT 3000/ vial ≥25 µm size particles: NMT 300/ vial		
	Crystal: Shape & size		**NPH, Biphasic isophane**: rod-shaped , maximum > 1 µm, rarely > 60µm, free from large aggregates **Lente:** rhombohedral, maximum >10µm, rarely > 40µm; no uniform shape & rarely >2µm		
11	pH *Potentiometric*		Between 6.9 & 7.8		

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RECOMBINANT PRODUCT LAB
Summary Protocol of Bulk of rh-Insulin

Test			
	Physical Quality		
1	APPEARANCE/ SOLUBILITY		
	Date		
	Qty. used in this test		
	Method of analysis		
	Specification		
	Result		
2	LOSS ON DRYING		
	Date		
	Qty. used in this test		
	Method of analysis		
	Specification		
	Result		
3	SULPHATED ASH		
	Date		
	Qty. used in this test		
	Method of analysis		
	Specification		
	Result		
Test	**Identification**		
4	PEPTIDE MAP		
	Date		
	Qty. used in this test		
	Method of analysis		
	a) HPLC Column Cat. No., Column dimensions	Source	Column temp.
	b) Mobile Phase	Name	Ratio used
	c) *Staphylococcus aureus* strain V8 protease/ *Staphylococcus aureus* V8 protease	Source	Activity
	d) Reference Standard Cat. No./ B. No.	Source	Assigned potency
	e) Reference Solutions prepared		
	f) Sample Qty used		
	g) Peptide map chromatogram	Ref. Std	Sample
	Specification		
	Result		

Test **Identification ….contd.**

5 AMINO ACID COMPOSITION

Date

Qty. used in this test

Method of analysis

Specification

Result

Test **Potency**

6 RABBIT BIOIDENTITY

Date

Qty. used in this test

Method of analysis

Species, Strain, Sex, Wt.

Schedule of injections

Ref. Std. used B. No. Assigned potency

No. of animals used per batch

Date of glucose estimation

Potency of sample vs. Reference

Specification

Result

7 ASSAY

Date

Qty. used in this test

Method of analysis

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

Specification

Result

Test **Purity**

8 RELATED PROTEINS

Date .....

Qty. used in this test .....

Method of analysis .....

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 .....

Specification .....

Result .....

9 HR MOLECULAR WEIGHT PROTEINS (HMWP)

Date .....

Qty. used in this test .....

Method of analysis .....

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 .....

Specification .....

Result .....

Test **Purity……contd.**

10 ZINC CONTENT
Date
Qty. used in this test
Method of analysis
Reference Standard Source Conc. of Zn/ml
Cat. No.

Reference Solutions prepared

Specification

Result

11 HOST CELL DERIVED PROTEINS
Date
Qty. used in this test
Method of analysis
Kit used (with details)

Specification

Result

12 SINGLE CHAIN PRECURSOR
Date
Qty. used in this test
Method of analysis
Specification

Result

13 PROINSULIN LIKE IMMUNOREACTIVITY
Date
Qty. used in this test
Method of analysis
Kit used (with details)

Specification

Result

Test **Contamination**

14.1 MICROBIAL LIMITS
Date
Qty. used in this test
Method of analysis
Specification

Result

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

Test **Tertiary structure**

18 *Date*

Qty. used in this test

Method of analysis

Specification

Result