Scrutiny of Protocols :

rh-Insulin- Bulk From Bulk Manufacturer's

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Summary Protocol of the rh-Insulin Bulk

1 1.1 1.2	Expression system used for insulin production • E.coli (strain) • Yeast species and (strain) • Source			
2	Insulin prepared by • Two chain method • Proinsulin method –intracellular / • Proinsulin method –secreted			
2.1	Plasmid stability • Method of analysis • % retention			
2.2	Manufacturing Date of Bulk			
2.3	Expiry assigned to bulk –API • Date • Method of analysis			
3	Initial Production Run			
3.1	Fermentor capacity			
3.2	Medium used			
3.3	Manufacturing cycle			
4	Harvest			
4.1	Quantum generated			
4.2	Name of QC tests performed • Identification • Purity • Safety • Physicochemical • Any other	Name of test	Result	Specification
5	Purification & Concentration			
5.1	Method used			
6	Purified bulk			
6.1	Quantum generated			
6.2	Name of QC tests performed • Identification • Purity • Safety • Physicochemical • Any other	Name of test	Result	Specification

7	Purity of Crystallized end product			
7.1	Recovery yield (%)			
7.2	Recovery (Kg) / batch			
7.3	Method used			
7.4	Specification			
7.5	Results			
8	Finished bulk	Name of test (list attached)	Result	Specification
8.1	Volume			
8.2	Constituents/ composition			
9	Containerization			
9.1	No. of vials filled			
9.2	Date of Containerization			
10	Reference material representative of Bulk			
10.1	Batch No			
10.2	Brief description : • Wt of Human Insulin crystals, analysis of %insulin, %desamido, insulin related impurities			
10.3	Total number of containers prepared • mg material packed in each container			
10.4	Assigned unitage			
	• As such / Dried • Method of analysis /Pharmacopoeia compliance • Specifications • Results			
10.5	Peptide map of Reference material • Traceable ref standard • Chromatogram of both			
10.6	Stability of Ref material • Predicted loss of activity per year			

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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