HOLD TIME STUDY PROTOCOL FOR GELATIN MASS

SUPERSEDE PROTOCOL No.	**NIL**		
DATE OF STUDY			
HOLD TIME STUDY BATCH No.			

HOLD TIME STUDY PROTOCOL FOR GELATIN MASS

CONTENTS

PHARMADEVILS

QULITY ASSURANCE DEPARTMENT

HOLD TIME STUDY PROTOCOL FOR GELATIN MASS

1.0 PROTOCOL APPROVAL:

INITIATED BY:

DESIGNATION	NAME	SIGNATURE	DATE
OFFICER/EXECUTIVE (QUALITY ASSURANCE)			

REVIEWED BY:

DESIGNATION	NAME	SIGNATURE	DATE
HEAD (PRODUCTION)			
HEAD (QUALITY CONTROL)			
HEAD (ENGINEERING)			

APPROVED BY:

DESIGNATION	NAME	SIGNATURE	DATE
HEAD (QUALITY ASSURANCE)			

HOLD TIME STUDY PROTOCOL FOR GELATIN MASS

2.0 OBJECTIVE:

- To provide documented evidence with high degree of assurance that the **Gelatin mass (Absolut 3G Softgel Capsules)** when kept on hold for defined period under controlled environmental condition, shall consistently meet the predefined acceptance criteria.
- To confirm that the holding time and storage conditions for **Gelatin mass (Absolut 3G Softgel Capsules)** in the manufacturing area are suitable and appropriate for storage and pre determined usage period.

3.0 SCOPE:

- The scope of this protocol is limited to the Hold Time Study of **Gelatin mass,** manufactured in the Softgel capsules manufacturing area of Oral Solid Dosage Section.

HOLD TIME STUDY PROTOCOL FOR GELATIN MASS

4.0 RESPONSIBILITY:

DEPARTMENTS	RESPONSIBILITIES
Quality Assurance	• Preparation, review, approval and Authorization of the Hold Time Study Protocol. • Co-ordination with Microbiology, Quality Control, Production and Engineering to carryout Hold Time Study activity. • Monitoring of Hold Time Study activity. • Sampling of holded material as per sampling plan mentioned in this protocol.
Production	• Review of Protocol. • To plan the Production and proper storage of the Hold Time study material. • To co-ordinate and support Hold Time Study activity.
Engineering	• Review of Protocol. • To co-ordinate and support Hold Time Study activity.
Quality Control	• Review of Protocol. • Analysis of samples and give results to QA on time for compilation.

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HOLD TIME STUDY PROTOCOL FOR GELATIN MASS

5.0 REASON FOR STUDY:

- To establish the storage conditions and holding time, up to which the intermediate products can be stored and all the parameters related to the Intermediate product, remain within its predefined acceptance criteria.

6.0 SITE OF STUDY:

- Gelatin Mass Preparation area, Oral Solid Dosage Section.

7.0 TRAINING DETAILS:

- The Validation Team shall be authorized by Head-QA or his/her designee.
- All the personnel involved in the Hold Time Study activity, sampling and testing of Hold Time samples Should be appropriately trained both in their job related activities and on the Hold Time Study by Head-QA or his/her designee.

8.0 METHODOLOGY:

- Only after Protocol approval, the activities of Hold Time Study shall be carried out.
- Hold time study shall be carried out on one batch.
- During the course of Hold Time Study, the documentation systems, laboratory controls, in-process checks shall be evaluated.
- QA members shall take samples as per approved Hold Time Study Protocol and QC department shall analyze the samples as per specification and give results to QA on time for compilation.
- All results shall be recorded in approved Hold Time Study Report.

HOLD TIME STUDY PROTOCOL FOR GELATIN MASS

9.0 PRODUCT INFORMATION:

BRAND NAME : **…….**

GENERIC NAME : Omega – 3 Marine Triglycerides, Green Tea Extract, Ginkgo, Ginseng, Grape Seed Extract, Antioxidants, Vitamins, Minerals & Trace Elements Capsules

CATEGORY : **Multivitamin**

LABEL CLAIM : **Each soft gelatin capsule contains:**

Omega – 3 Marine Triglycerides BP	
Providing Eicosapentaenoic Acid	90 mg
Docosahexaenoic Acid	60 mg
Green Tea Extract eq. to Polyphenols	10 mg
Powdered American Ginseng Extract USP	42.5 mg
Ginkgo USP	10 mg
Grape Seed Extract	15 mg
Lactic Acid Bacillus	500 Lacs
Citrus Bioflavonoid (8 mg of Bioflavonoid)	20 mg
Natural Mixed Carotenoids (10%)	11.33 mg
Vitamin D_3 IP	200 IU
Refined Wheat Germ Oil BP	25 mg
Menadione Sodium Bisulphite (Vitamin K)	10 mcg
Benfotiamine	1.5 mg
Vitamin B_6 IP	1 mg
Vitamin B_{12} IP	1 mcg
Niacinamide IP	20 mg
Calcium Ascorbate USP	45 mg
Folic Acid IP	150 mcg
Biotin USP	100 mcg
Choline Bitartrate USP	25 mg
Lutein USP	250 mcg
Dibasic Calcium Phosphate IP	
eq. to Elemental Calcium	20 mg
& Elemental Phosphorus	15.45 mg
Ferrous Fumarate IP	30 mg
Zinc Oxide IP eq. to Elemental Zinc	15 mg
Potassium Iodide IP	
eq to Elemental Iodine	150 mcg
Magnesium Oxide IP	
eq. to Elemental Magnesium	30 mg
Manganese Sulphate USP	
eq. to Elemental Manganese	1.5 mg

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Copper Sulfate Pentahydrate BP eq. to Elemental Copper	0.5 mg
Chromium Picolinate USP eq. to Elemental Chromium	65 mcg
Sodium Molybdate Dihydrate BP eq to Elemental Molybdenum	25 mcg
Sodium Selenite Pentahydrate BP eq. to Elemental Selenium	20 mcg
Potassium Chloride IP eq. to Elemental Potassium	4 mg
& Elemental Chloride	3.6 mg
Sodium Borate BP eq. to Elemental Boron	150 mcg
Colloidal Silicon Dioxide IP eq. to Elemental Silicon	2 mg
Nickel Sulfate eq. to Elemental Nickel	5 mcg
Stannous Chloride Dihydrate BP eq. to Elemental Tin	10 mcg
Sodium Metavanadate eq. to Elemental Vanadium	10 mcg
Piperine	5 mg

(Methylparaben IP & Propylparaben IP used as preservatives)
Colour: Approved colour used in gelatin shell

COLOUR	**:**	Approved colours used in gelatin shell
DOSAGE FORM	**:**	Oral Solid Dosage Form
MANUFACTURING LICENSE NO.	**:**	**……**
BATCH SIZE	**:**	1, 00,000 Capsules
SHELF LIFE	**:**	21 Months
MARKET	**:**	Domestic
DESCRIPTION	**:**	Dark Green coloured Soft Gelatin Capsules containing Brownish colour viscous mass.
STORAGE CONDITION	**:**	Store at a controlled room temperature.

HOLD TIME STUDY PROTOCOL FOR GELATIN MASS

10.0 Manufacturing Formula:

S. No.	Ingredients	Reference Grade	Function	Theoretical Quantity (For Batch Size: 1.0 Lac)	Unit
GELATIN MASS:					
1.	Gelatin (Soft Shell 4 Mesh) 150-180 Bloom	IP	Soft Shell	36.000	Kg
2.	Glycerin	IP	Solvent	12.000	Kg
3.	Sorbitol Solution 70% non crystallizing	IP	Solvent	8.000	Kg
4.	Methyl Paraben	IP	Preservative	0.240	Kg
5.	Propyl Paraben	IP	Preservative	0.120	Kg
6.	Ethyl Vanillin	USP	Flavorant	0.080	Kg
7.	Col. Brilliant Blue FCF	_	Colorant	43.000	g
8.	Col. Tartrazine	_	Colorant	215.000	g
9.	Titanium Dioxide	IP	Opacifier	0.157	Kg
10.	*Purified Water	_	Solvent	Q.S.	Ltr.

11.0 MASTER DOCUMENT VERIFICATION:

Verify the following Master Documents and record the details in Hold Time Study Report.

a. Manufacturing License Number

b. Master Formula Record

c. Batch Production and Control Record

d. In-process Specification and STPs

12.0 SPECIFICATIONS & STANDARD TEST PROCEDURES:

12.1 For Gelatin Mass:

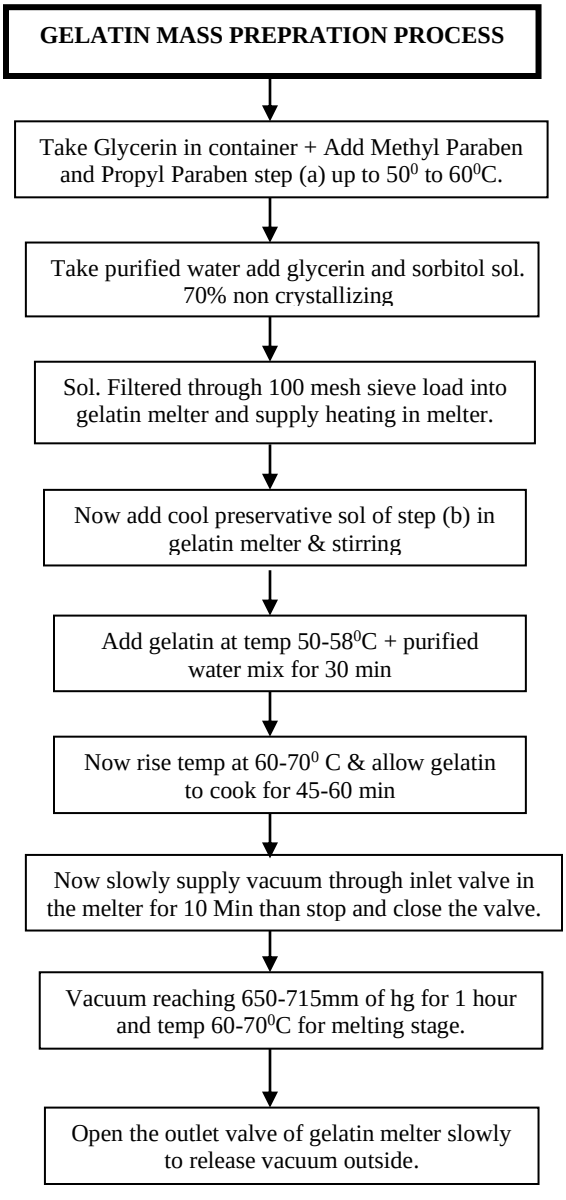
S.No.	Name of Product	Specification No.	STP No.
1.	Gelatin Mass		

13.0 GELATIN MASS: Gelatin is defined as a product obtained by the partial hydrolysis of collagen derived From the skin, white connective tissue and bones of animals. Gelatin derived from an acid-treated precursor is known as type (a) and gelatin derived from an alkali-treated process is known as type (b).

HOLD TIME STUDY PROTOCOL FOR GELATIN MASS

13.1 GELATIN PRODUCTION: As described in the introduction, gelatin is derived from collagen which is the principal Constituent of connective tissues and bones of vertebrate animals. Collagen is distinctive in that it contains an unusually high level of the cyclic amino acids proline and hydroxyproline. Collagen consists of three helical polypeptide chains wound around each other and connected by intermolecular crosslinks. Gelatin is recovered from collagen by hydrolysis. There are several varieties of gelatin, the composition of which depends on the source of collagen and the hydrolytic treatment used. Strict attention is paid to Good Manufacturing Practices and HACCP programs to ensure the purity of the gelatin. The product is tested at various intervals during production and as a finished product to ensure compliance with customer

13.2 MANUFACTURING PROCESS:



HOLD TIME STUDY PROTOCOL FOR GELATIN MASS

Take sample of mass and observe the absence of air bubble from melted mass completely.

↓

ADDITION OF ETHYL VANILLIN

↓

Add ethyl vanillin in glycerin and mix with step 3.2.5

↓

Now Unload the cooked gelatin mass in holding tank at 50-54^{0}C

↓

COLOUR ADDITION

↓

Take glycerin in S.S container

↓

Disperse col. Brilliant blue FCF col. Tartrazine & titanium dioxide mix in uniform dispersion

↓

In Filtered mixed sol. Add color in one part of unloaded gelatin mass till uniform gel mass prepared at 50-54^{0}C

↓

Now keep it aside for 2 hours

HOLD TIME STUDY PROTOCOL FOR GELATIN MASS

14.0 SAMPLING EQUIPMENT:

S. No.	Equipment name	Equipment I.D	Equipment qualification Done date	Equipment qualification due date
1.	Gelatin mass reactor			

15.0 SAMPLING PLAN:

15.1 For Gelatin Mass:

The Samples of Gelatin Mass shall be collected from S.S containers as per following sampling plan:

S.No.	Hold Time	Quantity of Sample	Test Parameters
1.	0 hr	10 gm + 10 gm	1. Description 2. Microbial Limit Test 3. Pathogen test
2.	6 hrs	10 gm + 10 gm	
3.	12 hrs	10 gm + 10 gm	
4.	18 hrs	10 gm + 10 gm	
5.	24 hrs	10 gm + 10 gm	
6.	36 hrs	10 gm + 10 gm	
7.	48 hrs	10 gm + 10 gm	
8.	60 hrs	10 gm + 10 gm	
9.	72 hrs	10 gm + 10 gm	
10.	84 hrs	10 gm + 10 gm	
11.	96 hrs	10 gm + 10 gm	

***Note:** Two samples is to be provided to Q.C one for microbial analysis**.**

HOLD TIME STUDY PROTOCOL FOR GELATIN MASS

16.0 ACCEPTANCE CRITERIA:

16.1 For Gelatin Mass:

S.No.	Test Parameters	Acceptance Criteria
1.	Description	Dark Green coloured Soft Gelatin Capsules containing brownish colour viscous mass
2.	**Microbial Limit Test:**	
	1) Total Aerobic Microbial Count (Bacteria & Fungi).	NMT 1000 cfu/gm for Bacteria NMT 100 cfu/gm for fungi
	2) Pathogens:	
	i) *Escherichia coli*	Should be Absent/gm
	ii) *Salmonella*	Should be Absent/10 gm
	iii) *Pseudomonas aeruginosa*	Should be Absent/gm
	iv) *Staphylococcus aureus*	Should be Absent/gm
	v) Candida albicans	Should be Absent/gm
	vi) Clostridium tetani	Should be Absent/gm
	vii) Enterobacteria	Should be Absent/gm

17.0 METHODOLOGY/HOLD TIME STUDY PROCEDURE:

17.1 SOP/SPECIFICATION TO BE FOLLOWED:

- Procedure for Preparation of Media as per **Current version of SOP.**
- Procedure for Cleaning of Utensils used in Microbiology Lab as per **Current version of SOP.**
- Procedure for Entry and Exit into Microbiology Lab Area as per **Current version of SOP.**
- Operation & Cleaning of Double Door Steam Sterilizer **Current version of SOP.**

17.2 MATERIAL REQUIRED:

- Pre Sterilized Swabs.
- 100 ml 0.9 % w/v Sterile Normal Saline.
- Pre-Incubated Plates of Soya Bean Casein Digest Agar (SCA).

17.3 ENVIRONMENTAL MONITORING:

During Validation study environmental conditions shall be monitored by following methods:

- Temperature and RH as per current SOP.

HOLD TIME STUDY PROTOCOL FOR GELATIN MASS

17.4 HOLDING OF EQUIPMENT:

- After Cleaning keep the Gelatin Mass under controlled environmental condition in **Gelatin mass preparation area** at Softgel.

17.5 PREPARATION OF STERILE MEDIA PLATES:

- Prepare Soya Bean Casein Digest Agar (SCA) Plates as per SOP for "Preparation of Media" **Current version of SOP.**

17.6 PRETREATMENT OF SAMPLE:

- **Water Soluble Products:**
  Dissolve 10 gm or dilute 10 ml of sample in buffered sodium chloride peptone solution pH 7.0 and adjust the volume to 100 ml **(Solution A)**.

17.7 FOR TAMC AND TYMC:

17.7.1 MEMBRANE FILTRATION METHOD:

- For TAMC. From above pre-treated sample transfer 10 ml to membrane filtration assembly and filter it. Repeat the process for TYMC. Wash both time membrane filters with 3 x 100 ml of suitable solution such as sterilized Sodium Chloride Peptone Solution pH 7.0.
- Transfer one Membrane Filter to Pre incubated Soyabean Casein Digest Agar (SCA) medium and other membrane filter to Sabouraud Dextrose Agar (SBD) plate.

17.7.2 Negative Control: Add 10 ml of the chosen diluent to Membrane filtration assembly and filter it. Repeat the process for TYMC. Wash both time membrane filters with 3 x 100 ml of suitable solution Such as sterilized Sodium Chloride Peptone Solution pH 7.0. Transfer one Membrane Filter to Pre-incubated Soyabean Casein Digest Agar (SCA) medium and Membrane Filter to Sabouraud Dextrose Agar (SBD) Plate.

17.7.3 Positive Control: Performed Positive control as per **"SOP".**

- Incubate Soyabean Casein Digest Agar (SCA) Plates at 30°C to 35°C for 5 days and Sabouraud Dextrose Agar (SBD) plates at 20°C to 25°C for 7 days.
- After incubation calculate the CFU per gm or per ml of sample being examined.

17.8 POUR PLATE METHOD:

- Stir the pretreated sample on vortex mixer.

- Use two pre-sterilized Petri plates (diameter 90-100 mm) each for TAMC and TYMC.
- For TAMC, pour 1 ml of each pre-treated sample into two pre-sterilized Petri plates and then pour 20-25 ml sterilized Soyabean Casein Digest Agar (Cool up to 45°C) and rotate the plate gently in clockwise and anticlockwise direction for proper mixing of sample.
- **Negative Control:** Add 1 ml of the chosen diluent into sterile Petri-plates and add about 15 ml of liquefied sterile Soyabean Casein Digest Agar (Cool upto 45°C) the Petri-plates and add about 15 ml of liquefied sterile Soyabean Casein Digest Agar (Cool upto 45°C) the Petri dishes and allow the medium to solidify.
- **Positive Control:** Performed positive control as per **"SOP".**
- Allow to solidify and then incubate at 30-35°C for 5 days in inverted position.
- For TYMC, pour 1 ml each of pretreated samples into pre-sterilized Petri plates and then pour 20-25 ml sterilized Sabouraud Dextrose Agar or Sabouraud Chloramphenicol Agar (Cool upto 45°C) and rotate the plate gently in clockwise and anticlockwise direction for proper mixing of sample.
- Allow to solidify and incubate all the plates at 20°C to 25°C for 5-7 days in inverted position.
- After incubation calculate the CFU per gm or per ml of sample being examined.
- **Negative Control:** Add 1 ml of the chosen diluent to sterile petri-plates and add about 15 ml of liquefied sterile Sabouraud Dextrose Agar (Cool upto 45°C) in the Petri dishes and allow the medium to solidify.
- **Positive Control:** Performed positive control as per **SOP.**

17.9 TESTS FOR SPECIFIED MICRO ORGANISMS (PATHOGENS):

Sample Preparation: Transfer 10 ml of **Solution A** to 90 ml of Soya bean Casein Digest Broth Medium and incubate at 30°C to 35°C for 18 to 24 hrs. Solution B.

17.9.1 Test for Escherichia coli:

- After incubation of Solution B, Shake the broth and transfer 1 ml to 100 ml of Pre-incubated Mac Conkey Broth. Incubate at 42°C to 44°C for 24 to 48 hrs.
- Subculture on a plate of Pre-incubated MacConkey Agar plate and incubate at 30°C to 35°C for 18 to 72 hrs. The growth of Pink, a non-mucoid colony indicates the possible presence of .coli. This should Confirmed by identification test.
- If the above media shows Pink non-mucoid colonies indicates the presence of E.coli which is Confirmed by Indole production test.
- If the above media shows Pink non-mucoid colonies indicates the presence of E.coli which is

Confirmed by Indole production test.

- **Confirmation Test:** Add 0.1 ml of contents of Mac Conkey broth to 5 ml of sterile 1% peptone water. Add 0.5 ml of Kovac's reagent to the test tube, shake well and allow to stand for one minute. If a cherry red color ring is observed at upper layer of the reagent, it indicates presence of E.coli.
- **Negative Control:** Use 1 ml chosen diluent to inoculate in 100 ml of sterile MacConkey broth and incubate at 42-44°C for 24-48 hrs. After incubation subculture on plates of Mac Conkey agar and incubate at 30-35°C for 18-72 hrs.
- **Positive Control:** Performed positive control as per **SOP.**

17.9.2 Test for Salmonella (If Limit is Absent/10 g):

- **Preparation of Sample:** Dissolve 10 g of test sample 100 ml of sterile Soyabean Casein Digest Medium. Homogenize and incubate at 30-35°°C for 18-24 hours (Solution C).
- Transfer 0.1 ml of the enrichment culture to 10 ml of Pre-incubated Rappaport Vassiliadis Salmonella Enrichment Broth and incubate at 30-35°C for 18-24 hrs. After incubation shake the test tube & subculture on a sterile petriplate of Pre-incubated Xylose Lysine Deoxycholate Agar and incubate at 30-35°C for 18-48 hours. Growth of well developed red colonies with or without black centers indicates the possibility of Salmonella.
- **Conformation Test:** Prepare the slant of Tripple Sugar Iron Agar and after sterilization allow to solidify. Swab the suspected colony by means of inoculating needle in to the butt and streak the surface of slant. Possible presence of Salmonella is indicated by black butt and Yellow slant.
- Add 0.1 ml of culture detected on Triple Sugar Iron Agar in 5 ml sterile urea broth and incubate at 35-37°C for 18-24 hrs. Acid & gas formation (color of the broth change from yellow to red) confirmed the presence of Salmonella. Product passes the test for absence of Salmonella if no acid & Gas formations occur in urea broth.
- **(If Limit is Absent/g)** Transfer 0.1 ml of the enrichment culture from Solution B to 10 ml of Pre-incubated Rappaport Vassiliadis Salmonella Enrichment Broth and incubate at 30-35°C for 18-24 hrs. After incubation shake the test tube & sub culture on a sterile petri plate of Pre-incubated Xylose Lysine Deoxycholate Agar and incubate at 30-35°C for 18-48 hours. Growth of well developed red colonies with or without black centers indicates the possibility of Salmonella.
- **Conformation Test:** Prepare the slant of Triple Sugar Iron Agar and after sterilization allow to solidify. Stab the suspected colony by means of inoculating needle in to the butt and streak the surface of slant. Possible presence of Salmonella is indicated by black butt and yellow slant.

- Add 0.1 ml of culture detected on Triple Sugar Iron Agar in 5 ml a sterile urea broth and incubate at 35-37°C for 18-24 hours. Acid & gas formation (color of the broth change from yellow to red) confirmed the presence of Salmonella. Product passes the test for absence of Salmonella if no acid & gas formation occur in urea broth.
- **Negative Control:** Use 1 ml chosen diluents to inoculate in 10 ml of sterile RVS Broth and incubate at 30-35°C for 24-48 hrs. After incubation subculture on plates of Pre-incubated XLD (Xylose Lyseine Deoxycholate Agar) and incubate at 30-35°C for 18-48 hrs.
- **Positive Control:** Performed positive control as per **SOP.**

17.9.3 **Test for Staphylococcus aureus:**

- After incubation of Solution B, shake the broth and subculture a loop full growth on the surface of pre-incubated Mannitol Salt Agar medium at 30°C to35°C for 18 to 72 hrs.
- If the above media shows Yellow or White colonies surrounded by a Yellow zone indicates the presence of Staphylococcus aureus which is confirmed by Coagulase test.
- **Confirmation Test:** Add 2 to 3 drops of Staphylococcus aureus culture + 0.5 ml of mammalian rabbit plasma. Incubate in water bath at 35-37°C, examine the tube at 3 hrs & subsequently at suitable interval upto 24 hrs. If white precipitate formation started after 3 hrs shows the presence of Staphylococcus aureus.
- **Negative Control:** Use blank pre-incubated Mannitol Salt Agar medium plate and incubate at 30 to 35°C for 18 to 72 hrs.
- **Positive Control:** Performed positive control as per “SOP”.

17.9.4 Test for Pseudomonas aeruginosa:

- After incubation of Solution B, shake the broth and subculture on pre-incubated Cetrimide Agar medium plate and incubate at 30 to 35°C for 18 to 72 hrs.
- Presence of growth on agar media indicates the possibility of presence of Pseudomonas aeruginosa. If there are no such types of growth, or identification test are negative, it indicates absence of Pseudomonas aeruginosa.
- If the above media shows Green colonies indicates the presence of Pseudomonas aeruginosa which is confirmed by confirmation test.
- **Confirmation Test:** Place a suspected colony by means of needle on to Oxidase Disc. If the disc turns to blue in colour, it confirms the presence of Pseudomonas aeruginosa.

- **Negative Control:** Use blank pre-incubated Cetrimide Agar medium plate and incubate at 30°C to 35°C for 18 to 72 hrs.
- **Positive Control:** Performed positive control as per **"SOP"**.

17.9.5 Test for Bile Tolerant Gram Negative Bacteria (Enterobacteria):

- Prepared the sample take Solution B, mix well and keep at 20 to 25°C for 2 to 5 hrs.
- Transfer 10 ml of Solution A to 90 ml pre-incubated Enterobacteria Enrichment Broth Mossel medium and incubate the medium at 30 to 35°C for 24 to 48 hrs.
- After incubation, subculture on the plate of pre-incubated Violet Red Bile Glucose Agar and incubates the plate at 30 to 35°C for 18 to 24 hrs.
- After incubation observe the plates and carryout the gram staining. If there are no such types of growth, or does not show gram negative bacteria, it indicates absence of Enterobacteria.
- **Negative Control:** Use 1 ml chosen diluents to inoculate in to 100 ml of pre-incubated Enterobacteria enrichment Broth Mossel broth and incubate at 30-35°C for 24-48 hrs. After incubation shake the test tube and subculture on a plate of pre-incubated Violet Red Bile Glucose Agra and incubate at 30-35°C for 18-24 hrs.
- **Positive Control:** Performed positive control as per "SOP".

17.9.6 Test for Clostridia:

- Take two equal portions of 10 ml from solution A and heat one portion at 80°C for 10 minutes and cool rapidly. Do not heat the other portion.
- Transfer each of homogenized portion in two tubes containing 100 ml pre-incubated Reinforced medium from Clostridia. Incubate the tubes under anaerobic condition at 30 to 35 °C for 48 hrs.
- After incubation, make sub-subculture from each container on pre-incubated Columbia Agar plates. Incubated under anaerobic conditions at 30 to 35°C for 48 hrs.
- The presence of anaerobic growth of Gram positive bacilli with or without endospore, giving a negative catalase test indicates the possibilities of presence of Clostridia. If there are no such types of anaerobic growth on Columbia agar or identification test are negative, it indicates absences of Clostridia.
- **Negative Control:** Use 1 ml chosen diluents to inoculate in to 100 ml of pre-incubated Reinforced medium for Clostridia incubate the tubes under anaerobic conditions at 30 to 35°C for 48 hrs. After incubation. Make sub-subculture from each container on pre-incubated Columbia Agar plates. Incubate under anaerobic conditions at 30 to 35°C for 48 hrs.
- **Positive Control:** Performed positive control as per **"SOP"**.

HOLD TIME STUDY PROTOCOL FOR GELATIN MASS

18.0 RESTUDY CRITERIA:

- If required, re study shall be considered and carried out when any of the following conditions occur or prevail:
- Change in storage conditions
- Change in manufacturing process which may affect the quality of the products.

Note: *In case of the requirements for revalidation, because of above mentioned reasons, the validation of the critical steps shall be undertaken through addendum protocol to this protocol or a separate protocol.*

19.0 FREQUENCY OF STUDY:

- Once

20.0 NON COMPLIANCE:

- In case of any deviation observed during Hold Time Study, inform to Department Head and Head Quality for necessary action.
- Document the deviation detail in observed deviation section.
- The Head Quality and the Department Head will study the impact of deviation. If deviation is acceptable and it does not have an impact on quality of product as well as on overall performance of the study, prepare final conclusion.

21.0 DOCUMENTS TO BE ATTACHED:

- Raw data generated during analysis of hold time study samples and execution of the protocol.

22.0 REFERENCES:

- Schedule–M–"Good Manufacturing Practices and Requirements of Premises, Plant and Equipment for Pharmaceutical Products."
- WHO Essential Drugs and Medicines Policy, QA of Pharmaceuticals, Vol 2 – Good Manufacturing Practices and Inspection.
- In-process Specifications.
- In-process Standard Testing Procedure.

23.0 ABBREVIATIONS:

QC	:	Quality Control
Ltd.	:	Limited
QA	:	Quality Assurance

HOLD TIME STUDY PROTOCOL FOR GELATIN MASS

IP	:	Indian Pharmacopoeia
USP	:	United State Pharmacopoeia
mg	:	Milligram
Kg	:	Kilogram
No.	:	Number
STP	:	Standard Testing Procedure
RH	:	Relative Humidity
NMT	:	Not More Than
ID.	:	Identification
Caps	:	Capsules
WHO	:	World Health Organization
Vol	:	Volume