Experimental work

Parenteral drug delivery systems offer assured levels of bioavailability. It provides a reliable route for drug administration in very ill and comatose patients who are not able to ingest anything orally.

Chemically granisetron hydrochloride is endo - N-(9-methyl-9-azabicyclo [3.3.1] non-3-yl)-1-methyl-1H-indazole-3-carboxamide hydrochloride. It is indicated for the prevention of nausea and vomiting associated with initial and repeat courses of emetogenic cancer therapy, including high dose cisplatin. It is also indicated for prevention and treatment of post operative nausea and vomiting.

From the literature survey it is observed that no published work that states about the multi-dose injection formulation, hence it is desired to develop the multi-dose injection formulation and chemical stability study should be carried out for the granisetron injection as anti emetic drug.

The aim of the present study was to formulate stable multi-dose injection formulation of Granisetron Hydrochloride and to determine its Chemical stability at intermediate, accelerated and stress conditions**.**

The following objectives were framed for the present investigation –

1. Design an optimum injection formulation of Granisetron hydrochloride which provides a better therapeutic effect.
2. Select the tubings which provide minimum sorption of Granisetron hydrochloride and preservative.

3. Select the filters used during filtration of Granisetron hydrochloride formulation which provide minimum adsorption of drug and preservative.
4. To develop a sterilization method for multi-dose packing.
5. The selected packaging material shall provide better stability to formulation at room, accelerated and stress conditions of temperature and humidity.

Experimental Work[78-102]

List of Chemicals used for the Study

Active Pharmaceutical Ingredient (API)		
1	Granisetron Hydrochloride	Symed Labs Limited, India
Excipients		
1	Sodium Chloride	Merck Ltd. Mumbai
2	Citric acid, Monohydrate	Merck Ltd. Mumbai
3	Methyl Paraben	Merck Ltd. Mumbai
4	Propyl paraben	Merck Ltd. Mumbai
5	m-Cresol	Merck Ltd. Mumbai
6	Benzyl alcohol	Merck Ltd. Mumbai

Requirement in the material, whima all the chemical, were purchesed from standed recognized firm and IR grade they and all IR grade.

List of Instruments used ins the Study

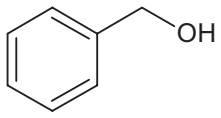
Sr. No.	Instruments	Make
1	Balance	AUW-220, Shimadzu, Japan
2	pH meter	Cyberscan®510^{PC} Eutech, Japan
3	Digital polarimeter	Rudolph Autopol®IV
4	HPLC	Shimadzu Prominence LC-20 model
5	Magnetic stirrer	Remi equipments, Bangalore
6	Laminar flow clean air station	Model no. 1500C-48-24-24 klenz Pvt. Ltd.
7	Autoclave	Thermopol Ltd.
8	Stability chambers	Newtronics company Pvt. Ltd. Mumbai
9	Osmometer	Model-3250, Advance instruments INC.
10	UV- spectrophotometer	1700,Pharmaspec, Shimadzu, Japan

List of Materials used for the Study

Sr. No.	Materials	Make
1	Silicon tubing	Saint Gobain Ltd. India
2	Nylon filter	Pall Corporation
3	Cellulose acetate filters	Millipore, Bangalore
4	PVDF filters	Millipore, Bangalore

Excipient Profile

Benzyl Alcohol

Empirical formula	C_7H_8O	
Chemical name	Benzenemethanol	
Molecular weight	108.14	
Structural formula		
CAS registry number	100-51-6	
Category	Antimicrobial preservative[81] [92], disinfectant; solvent	
Description	A clear, colorless, oily liquid with a faint aromatic odor and a sharp, burning taste.	
Solubility (at 20°C)	Chloroform	Miscible in all proportions
	Ethanol	Miscible in all proportions
	Ethanol (50%)	1 in 2.5
	Ether	Miscible in all proportions
	Fixed and volatile oils	Miscible in all proportions
	Water	1 in 25 at 25°C
Storage	Benzyl alcohol may be stored in metal or glass air-tight containers, protected from light, in a cool dry place.	

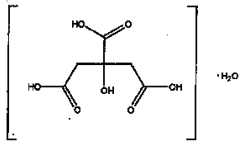
<table>
<tr><td>Antimicrobial activity</td><td colspan="2">The activity of benzyl alcohol may also be reduced by incompatibilities with some packaging materials, particularly polyethylene. Benzyl alcohol is bacteriostatic and is used as an antimicrobial preservative against Gram-positive bacteria, molds, fungi, and yeasts, although it possesses only modest bactericidal properties. Optimum activity occurs at pH below 5; little activity is shown above pH 8. Antimicrobial activity is reduced in the presence of nonionic surfactants, such as polysorbate 80.</td></tr>
<tr><td rowspan="6">Minimum inhibitory concentrations (MICs) of benzyl alcohol</td><td>Microorganism</td><td>MIC (µg/mL)</td></tr>
<tr><td>Aspergillus niger</td><td>5000</td></tr>
<tr><td>Candida albicans</td><td>2500</td></tr>
<tr><td>Escherichia coli</td><td>2000</td></tr>
<tr><td>Pseudomonas aeruginosa</td><td>2000</td></tr>
<tr><td>Staphylococcus aureus</td><td>25</td></tr>
<tr><td>Effect against bacteria</td><td colspan="2">Benzyl alcohol is moderately active against most Gram-positive organisms (typical MICs are 3–5 mg/mL)</td></tr>
<tr><td>Effect against fungi</td><td colspan="2">Benzyl alcohol is effective against moulds and yeasts; typical MICs (Miimum Inhibitory Concentration)are 3–5 mg/mL</td></tr>
<tr><td>Effect against spores</td><td colspan="2">Benzyl alcohol is inactive against spores, but activity may be enhanced by heating against Bacillus stearothermophilus at 100°C for 30 minutes.</td></tr>
</table>

Sodium chloride

Empirical formula	NaCl	
Chemical name	**Sodium chloride**	
Molecular weight	58.44	
CAS number	[7647-14-5	
Category	Tablet and capsule diluent; tonicity agent	
Description	Sodium chloride occurs as a white crystalline powder or colorless crystals; it has a saline taste. The crystal lattice is a face-centered cubic structure. Solid sodium chloride contains no water of crystallization although, below 0° C, salt may crystallize as a dihydrate.	
Solubility	Ethanol.	Slightly soluble
	Glycerin	1 in 10
	Water	1 in 2.8 1 in 2.6 at 100°C
pH	6.7-7.3 (saturated aqueous solution)	
Storage	The material should be stored in a well-closed container, in a cool, dry place	

Incompatibilities	Aqueous sodium chloride solutions are corrosive to iron. They also react to form precipitates with silver, lead, and mercury salts. Strong oxidizing agents liberate chlorine from acidified solutions of sodium chloride. The solubility of the antimicrobial preservative methylparaben is decreased in aqueous sodium chloride solutions and the viscosity of carbomer gels and solutions of hydroxyethyl cellulose or hydroxypropyl cellulose is reduced by the addition of sodium chloride.
Pharmaceutical applications	Sodium, chloride is widely used in a variety of parenteral and nonparenteral pharmaceutical formulations, where the primary use is to produce isotonic solutions. Sodium chloride has been used as a lubricant and diluent in capsules and direct-compression tablet formulations in the past, although this practice is no longer common. Sodium chloride has also been used as a channeling agent and as an osmotic agent in the cores of controlled-release tablets. It has been used as a porosity modifier in tablet coatings, and to control drug release from microcapsules. The addition of sodium chloride to aqueous spray-coating solutions containing hydroxypropyl cellulose or hypromellose suppresses the agglomeration of crystalline cellulose particles. Sodium chloride can also be used to modify drug release from gels and from multiple emulsions. It can be used to control micelle size, and to adjust the viscosity of polymer dispersions by altering the ionic character of a formulation.

Citric acid

Empirical formula	$C_6H_8O_7\,H_20$
Chemical Name	2-Hydroxy-1,2,3-propanetricarboxylic acid monohydrate
Molecular weight	210.14
Structural formula	
CAS registry number	5949-29-1
Category	Acidifying agent; antioxidant; buffering agent; chelating agent; flavor enhancer.
Description	Citric acid monohydrate occurs as colorless or translucent crystals, or as a white crystalline efflorescent powder. It is odorless and has a strong acidic taste. The crystal structure is orthorhombic.
Solubility	Soluble 1 in 1.5 parts of ethanol (95%) and 1 in less than 1 part of water; sparingly soluble in ether.
pH	2.2 (1% aqueous solution)
Storage	The bulk monohydrate or anhydrous material should be stored in airtight containers in a cool, dry place.

Incompatibilities	Citric acid is incompatible with potassium tartrate, alkali and alkaline earth carbonates and bicarbonates, acetates, and sulfides. Incompatibilities also include oxidizing agents, bases, reducing agents, and nitrates. It is potentially explosive in combination with metal nitrates. On storage, sucrose may crystallize from syrups in the presence of citric acid.
Pharmaceutical applications	Citric acid (as either the monohydrate or anhydrous material) is widely used in pharmaceutical formulations and food products, primarily 'to adjust the pH of solutions. Citric acid monohydrate is used in the preparation of effervescent granules, while anhydrous citric acid is widely used in the preparation of effervescent tablets. In food products, citric acid is used as a flavor enhancer for its tart, acidic taste. Citric acid monohydrate is used as a sequestering agent and antioxidant synergist. It is also a component of anticoagulant citrate solutions. Therapeutically, preparations containing citric acid have been used to dissolve renal calculi.

Sodium Hydroxide

Empirical formula	NaOH	
Chemical name	Sodium hydroxide	
Molecular weight	40	
CAS registry number	1310-73-2	
Category	Alkalizing agent; buffering agent	
Description	Sodium hydroxide occurs as a white or nearly white fused mass. It is available in small pellets, flakes, sticks, and other shapes or forms.	
Solubility	Ethanol	1 in 7.2
	Ether	Practically insoluble
	Glycerin	Soluble
	Methanol	1 in 4.2
	Water	1 in 0.9 and 1 in 0.3 at 100°C
pH	pH ≈ 12 (0.05% w/w aqueous solution)	
	pH ≈ 13 (0.5% w/w aqueous solution)	
	pH ≈ 14 (5% w/w aqueous solution)	
Storage	Sodium hydroxide should be stored in an airtight nonmetallic container in a cool, dry place.	
Incompatibilities	Sodium hydroxide is a strong base and is incompatible with any compound that readily undergoes hydrolysis or oxidation. It will react with acids, esters, and ethers, especially in aqueous solution.	
Pharmaceutical applications	Sodium hydroxide is widely used in pharmaceutical formulations to adjust the pH of solutions. It can also be used to react with weak acids to form salts.	

Preformulation Studies

The Preformulation studies like characterization of drug sample including description, identification, pH, solubility, water content, melting range, residue on ignition, heavy metals, and assay by high performance liquid chromatography were performed.

1. Characterization of

Granisetron Hydrochloride was supplied by Symed Labs Limited, India was characterized for its identification and authenticity. The drug was characterized according to following specification.

Sr. No.	Test	Specifications
1	Description	White or almost white crystalline powder
2	Solubility	Freely soluble in water and slightly soluble in methanol
3	Identification A) Infrared absorption B) Chloride test	The infrared absorption spectrum of the preparation of the sample has exhibit maxima only at the same wavelengths as that of similar preparation of the Granisetron Hydrochloride working standard. It has responded to the test of chloride
4	Appearance of solution	Solution is be clear and colorless
5	pH	Between 4.0 and 6.5

6	Loss on drying	Not more than 0.5% w/w
7	Sulphated ash	Not more than 0.1% wlw
8	Assay by HPLC (On dried basis)	Not less than 98.0 % w/w and not more than 101.0 % w/w
9	Related compounds (%w/w) 1) Methylindazole 2) Single largest unknown impurity 3) Total Impurities	 Not more than 0.1% Not more than 0.1 % Not more than 0.8%
10	Residual solvents i) n-Hexane ii) isopropyl alcohol iii) Dichloromethane iv) Ethanol	 Not more than 100 ppm Not more than 1000 ppm Not more than 300 ppm Not more than 2000 ppm
11	Bacterial Endotoxins[86] [95]	Not more than 2 Endotoxin Unit per mg
12	Microbial limit test Total viable aerobic count (Bacterial and fungi) Enterobacteria Pseudomonas aeruginosa Escherichia coli Salmonella	Not more than 100 cfu/g

Description

Take about 5g sample in a Petri dish and observe for colour, odor and nature of material. It should be white or almost white crystalline powder.

Solubility

Accurately weigh about 1.0 g of sample and dissolve in 10 mL of water.

Accurately weigh about 0.01 g of sample and dissolve in 10 mL of methanol.

All above solutions should be clear.

Identification by Infrared absorption

Weigh about 2-3 mg of sample into small mortar pestle, add 200-300 mg of previously dried potassium bromide and crush to a very fine powder. Prepare a transparent thin pellet using compression mould. Place the KBr pellet in sample holder of the infrared spectrophotometer and scan through frequency 4000 cm^{-1} to 400 cm^{-1}, similarly scan the IR spectrum of Granisetron Hydrochloride working standard. Compare the infrared spectrum with reference spectrum of Granisetron Hydrochloride working standard.

The infrared absorption spectrum exhibits maxima only at the same wavelength as that of a similar preparation of Granisetron Hydrochloride working standard.

Identification by Chloride test

Weigh about 100 mg of sample and dissolve in 5 mL of water. Acidify with 2M nitric acid; add 0.4 ml of silver nitrate solution. Shake and allow to stand. A white; curdy precipitate is produced.

Appearance of solution

Accurately weigh about 0.2 g of sample and dissolve in water and dilute to 20 ml with the same solvent this solution should be clear and Colorless.

Determination of pH

Accurately weigh about 0.4 g of sample and dissolve in 40 ml water, Measure the pH value using a standardized pH meter and record.

Loss on drying

Place about 1.0 g of the sample in a previously dried and weighed glass stoppered weighing bottle, replace the cover and accurately weigh the bottle and the contents. Distribute the sample as evenly as practicable and place the loaded bottle in the drying chamber, removing the stopper and leaving it also in the chamber. Dry the sample at the temperature 100°C – 105°C for 4 hours, Open the drying chamber, close the bottle promptly and allow it to come to room temperature in a desiccator and weigh. Calculate the percentage of loss on drying as given below.

$$\% \text{ LOD (w/w)} = \frac{\text{Loss in weight (g)} \times 100}{\text{Wt of sample (g)}}$$

Sulphated ash

Weigh a platinum / silica crucible that has been previously ignited at 600°± 50°C and cooled in desiccator. Let the weight be W. Transfer accurately about 1.0 g of the substance into the empty crucible and note the weight of the crucible with the contents, Let the weight be 'W1'. Moisten -the sample with 1 ml. of sulfuric acid. Heat the substance, gently at first until the substance is thoroughly charred, cool, then moisten the residue with I mL of sulfuric acid. Heat gently until white fumes are no longer evolved, and ignite at 600 ± 50°C until the carbon is consumed, Cool in a desiccator, weigh and note the weight as 'W2'. Calculate the percentage of residue. If the amount of the residue so obtained exceeds the specified limit, again moistened residue with 1 mL sulfuric acid heat and ignite as before, and again calculate the percentage of residue.

Continue the ignition until two consecutive weightings do not differ by more than 0.50 mg per g of substance taken, the second weighing fallowing an additional 15 min. ignition period.

$$\% \text{ Residue on ignition (w/w)} = \frac{W2 - W}{W1 - W} \times 100$$

Assay by HPLC (On dried basis)

Chromatographic Conditions:

Instrument: Shimadzu Prominence LC-20 model High performance liquid chromatograph

Column: Hypersil BDS C-18 (250 mm x 4.6 mm) 5µ or equivalent.

Wavelength: UV at 305 nm

Injection volume; 10 µl

Temperature; 400°C

Run Time: 12 minutes

Flow Rate: 1.5 ml/minute

Mobile Phase: Transfer 1.6 ml of ortbophosphoric acid to 750 ml of Milli-Q Water. Add 250 ml of Acetonitrile mix. Add 1 ml of hexylamine adjust pH 7.50 (±0.05) with triethylamine and mix thoroughly. Filter through a membrane filter (0.45µ or finer porosity) and degas.

Diluents: Slime as mobile phase

Preparation of blank solution: Use diluent as blank

Preparation of standard solution:

Accurately weigh and transfer about 25 mg of Granisetron Hydrochloride (API) working standard to a 25 ml volumetric flask. Dissolve in and dilute with diluent up to the mark and mix. Dilute 5.0 ml of the above solution to 50 ml volumetric flask. Dilute with diluent makeup the volume with the same and mix.

Preparation of a sample solution:

Accurately weigh and transfer about 25 mg of sample to a 2.5ml volumetric flask. Dissolve in and dilute with diluent up to the mark and mix.

Dilute 5.0 ml of the above solution to 50 ml volumetric flask, Dilute with diluent and makeup the volume with the same and mix.

Procedure: Inject blank solution. Inject standard solution five times and record the peak areas. The relative standard deviation for five replicate injections determined from the Granisetron Hydrochloride peak should not be than 2.0%, and the asymmetry (tailing factor) of the Granisetron Hydrochloride peak should not be more than 2.0. Inject the sample solution in duplicates and record the chromatograms. Calculate the assay of Granisetron Hydrochloride.

The retention time of Granisetron Hydrochloride is in between 4-6 minute.

Calculation:

% Assay of Granisetron hydrochloride on dried basis:

$$= \frac{Au}{As} \times \frac{\text{Weight of Standard}}{25} \times \frac{5}{50} \times \frac{25}{\text{weight of sample}} \times \frac{50}{5} \times \frac{100}{(100 - \%\ LOD)} \times \text{Potency of standard}$$

Where, Au = Average area of Granisetron Hydrochloride peak in sample preparation

As = Average area of Granisetron Hydrochloride peak in standard preparation

Related compounds

Chromatographic Conditions:

Instrument: Shimadzu Prominence LC-20 model High performance liquid chromatograph

Column: Hypersil BDS C18 (250 mm x 4.6 mm) 5µ or equivalent.

Wavelength: UV at 305 nm

Temperature: 40°C

Flow Rate: 15 ml/minute

Run time: 40 min. [15 minute for reference solution (a)]

Injection volume: 20 µl

Mobile phase: Transfer 1.60 ml of orthophosphoric acid to 800 ml of Milli Q water add 200 ml of acetonitrile. Add 1 ml of hexylamine and mix thoroughly. Adjust pH 7.50 (± 0.05) with triethylamine. Filter through a membrane filter (0.45 µm or finer porosity) and degas.

Preparation of blank solution: Same as mobile phase.

Preparation of rising solvent: Prepare a mixture of acetonitrile and water as 10:90 v/v and mix and degas.

Reference solution (a): Accurately weigh and transfer about 12.50 mg each of Granisetron hydrochloride (API) working standard and Methylindazole working standard to a 50 ml volumetric flask. Dissolve in and dilute with mobile phase up to the mark and mix. Transfer 5.0 ml of the above solution to 50 ml volumetric flask. Dilute with mobile phase and makeup volume with the same and mix, Further transfer 1.0 m1 of the above solution to 25 ml volumetric flask. Dilute with mobile phase and makeup volume with the same and mix.

Sample preparation: Accurately weigh and transfer accurately about 25 mg of sample to a 25 ml volumetric flask. Dissolve in and dilute with mobile phase up to the mark and mix.

Reference solution (b): Accurately weigh and transfer about 25 mg of sample to a 50 ml volumetric flask, Dissolve in and dilute with mobile phase up to the mark and mix. Transfer 2.0 ml of the above solution to a colorless glass vial, stopper and expose the solution to sunlight for 34 hours (partial degradation of granisetron hydrochloride to impurity carboxamide).

Note: A degradation of at least about 0.3% of Granisetron hydrochloride to impurity carboxamide must be obtained as shown by appearance of corresponding peak in the chromatogram. If not, expose the solution once again to sunlight.

Procedure:

Evaluation of system suitability: Inject blank solution. Chromatograph the reference solution (b) and record the peak areas. The resolution between the peaks due to impurity carnoxamide and Granisetron hydrochloride should not be less than 3.5. (RRT about 0.72 minute carboxamide) inject the reference solution (a), the asymmetry (Tailing factor) due to Granisetron hydrochloride peak should not be more than 2. the relative standard deviation of area from six: replicate injections should not be more than 5%.

Inject sample preparation into the chromatograph and record the chromatograms. Disregard any peaks, if any obtained in the sample solution corresponding to the peaks obtained from blank solution. Calculate the amount of related compounds. No peaks should be observed at the RT's of related compounds from blank solution,

The retention time of Granisetron hydrochloride is about 8.5 minute,

Components	RRT	LOQ (%w/w)
Methylindazole	0.38	0.007
Granisetron hydrochloride (API)	1.00	0.018

Calculation:

1. Methylindazole (%w/w):

$$= \frac{Atm}{Asm} \times \frac{\text{Weight of Methylindazole}}{50} \times \frac{5}{50} \times \frac{1}{25} \times \frac{25}{\text{Weight sample}} \times \text{Purity of Methylindazole}$$

Where,

Atm is the Area of Methylindazole in sample chromatogram

Asm is the average Area of Methylindazole in reference solution (a) chromatogram

2. Single largest unknown impurity (%w/w):

$$= \frac{Au}{As} \times \frac{\text{Weight of Granisetron hydrochloride}}{50} \times \frac{5}{50} \times \frac{1}{25} \times \frac{25}{\text{Weight sample}} \times P$$

Where,

Au is the Area of single largest unknown impurity in sample chromatogram

As is the average Area of Granisetron hydrochloride in reference solution (a) chromatogram

P is the potency of Granisetron hydrochloride working standard

3. Total unknown impurities (%w/w):

$$= \frac{Ast}{As} \times \frac{\text{Weight of Granisetron hydrochloride}}{50} \times \frac{5}{50} \times \frac{1}{25} \times \frac{25}{\text{Weight sample}} \times P$$

Where,

Ast is the Sum of area of all unknown peaks in sample chromatogram

As is the average Area of Granisetron hydrochloride in reference solution (a) chromatogram

P is the potency of Granisetron hydrochloride working standard

4. Total impurities; 1 + 3

Residual solvents

Instrument	:	Perkin Elmer's Clarus 500 gas Chromatograph with flame ionization detector or equivalent.
Headspace	:	Perkin Elmer's. Turbo matrix headspace Sampler 40 or equivalent
Data handling system	:	Perkin Elmer's Turbochrom workstation. version 6.2.1.0.104:0104 or equivalent
Column	:	Fused silica capillary column- 30 m long 0,32 mm internal diameter with proprietary polysiloxane (6% cyano propyl phenyl 94 % dimethyl polysiloxane) stationary phase of 1.8 µ film thickness. (Use DB-624 or equivalent)
Instrument parameter		
Oven temperature	:	40°C
Time 1	:	15 min.
Rate 1	:	15°C /min

Oven temperature 2	:	180°C
Time 2	:	10 min.
Injector temperature	:	180°C
FID temperature	:	270°C
Carrier gas(N_2 flow)		0.5 ml/min. ±0.05 ml/min
Split flow (total)	:	50 ml/min. (Through split vent)
FlD Range	:	1
Attenuation	:	1
Total Run time	:	34.33 min.
Head Space Parameters		
Sample temperature	:	80°C
Needle temperature	:	95°C
Transfer line temperature	:	100°C
GC cycle time	:	50 min.
Thermostat Time	:	20 min.
Pressurize time	:	1.0 min.
Inject time	:	0.10 min.
Withdrawal time	:	0.50 min.
Headspace mode	:	Constant
Vial vent	:	OFF
Sampler shaker	:	ON
HS carrier pressure	:	10 psi

Reagents:

1. Ethanol AR Grade

2. Isopropyl alcohol-AR Grade

3. Dichloromethane-AR Grade

4. n-Hexane AR Grade

5. N,N-Dimethylformamide AR Grade

6. Water Milli-Q

Diluent: Prepare a mixture of N,N-Dimethylformamide and Milli-Q water in the ratio of 75:25 v/v.

Blank: Transfer 1.01ml of diluent in HS vial, seal with a septum and crimp cap.

Standard stock solution: Weigh accurately about 1.250 g of Ethanol, 0.6250 g of Isopropyl alcohol 0.l875 g of Dichloromethane and 0.0625 g of n-Hexane in 25 ml volumetric flask. Dissolve in N,NDimethylformamide and makeup volume with the same, Dilute 1.0 ml of the above solution to l0 ml with N,N Dimethylformamide.

Standard solution: Transfer 1.0 ml of standard stock solution to 25 ml volumetric flask. Dissolve in diluent and makeup the volume with diluent. Transfer 1.0 ml of the above solution into a vial seal the vial with a septum and crimp cap.

Test Solution: Transfer about 0.1g of the sample accurately weighed to a headspace vial. Add I ml of diluent, seal with a septum and crimp.

Procedure:

System Suitability: Inject Blank. inject the standard solution six times and record the peak areas. The resolution between isopropyl alcohol and dichloromethane should not be less than 1.5. The relative standard deviation of the peak area of each solvents from six replicate injections should not be more than 15%.

Separately inject test solution into the chromatograph. Record the chromatograms. Measure the peak areas and calculate: the amount of residual solvents.

The retention time of isopropyl alcohol is about 11 minute. RRT's (with respect to Isopropyl alcohol) and LOQ's of residual solvents are shown below.

Components	**RRT**	**LOQ (µg/g)**
Ethanol	0.81	84.7
Isopropyl alcohol	1.00	43.7
Dichloromethane	1.12	5.4
n-Hexane	1.39	0.7
N,N-Dimethylformamide	2.19	-

Calculation:

$$\text{Concentration of IPA in ppm} = \frac{\text{Peak area of A in Test solution}}{\text{Average Peak area of A in Std. solution}} \times \frac{\text{Weight of Standard IPA}}{25} \times \frac{1}{10} \times \frac{1}{25} \times \frac{1}{\text{Weight of sample taken}} \times P \times 10^4$$

Where, A = IPA

P = Purity of Solvent

$$\text{Concentration of Ethanol in ppm} = \frac{\text{Peak area of B in Test solution}}{\text{Average Peak area of B in Std. solution}} \times \frac{\text{Weight of Standard B}}{25} \times \frac{1}{10} \times \frac{1}{25} \times \frac{1}{\text{Weight of sample taken}} \times P \times 10^4$$

Where, B = Ethanol

P = Purity of Solvent

$$\text{Concentration of Dichloromethane in ppm} = \frac{\text{Peak area of C in Test solution}}{\text{Average Peak area of C in Std. solution}} \times \frac{\text{Weight of Standard C}}{25} \times \frac{1}{10} \times \frac{1}{25} \times \frac{1}{\text{Weight of sample taken}} \times P \times 10^4$$

Where, C = Dichloromethane

P = Purity of Solvent

$$\text{Concentration of n-Hexane in ppm} = \frac{\text{Peak area of D in Test solution}}{\text{Average Peak area of D in Std. solution}} \times \frac{\text{Weight of Standard D}}{25} \times \frac{1}{10} \times \frac{1}{25} \times \frac{1}{\text{Weight of sample taken}} \times P \times 10^4$$

Where, D = n-Hexane

P = Purity of Solvent

Bacterial Endotoxins

1. Apparatus and glassware: Depyrogenate all glassware and other heat stable materials in a hot-air oven.

2, Reagents: Control standard Endotoxin: Use Control Standard Endotoxin (CSE) which is standardized against Reference Standard of Endotoxin. This CSE is supplied by manufacturer with certificate of analysis.

   Reconstitute CSE as per manufacturer's instruction. Reconstituted CSE should be stored at 6-10°C and should be used within 4 weeks.

Note: One International Unit (l.U.) of endotoxin is equal to one endotoxin unit (EU)

a. LAL Reagent (Lysate): Use LAL reagent having a sensitivity of 0.125 EU/m1. Reconstituted Lysate should be used on the same day. Reconstituted lysate can be stored at 2-8°C for not more than 24 hours.

b. LAL Reagent Water (LRW); Use LRW for preparing sample solution and dilutions for test.

Determination of Bacterial Endotoxin in the sample:

1. After confirming the label sensitivity of LAL reagent, proceed for analysis of sample,
2. Prepare 250 g/ml solution of Granisetron with1 ml of LAL reagent water.
3. Calculate the Maximum Valid Dilution (MVD) of the product to be tested.

$$MVD = \frac{\text{Stock Concentration x Endotoxin limit}}{\text{Labeled sensitivity}}$$

For Granisetron,

Bacterial endotoxins limit = 2 EU/ mg of Granisetron,

For Lysate sensitivity = O. 125 EU/ml

$$\text{MVD for Granisetron} = \frac{250 \text{ mg/ml x } 2 \text{ EU /mg}}{0.125 \text{ EU/ml}}$$

MVD = 4000, so MVD/2 = 2000.

For Lysate sensitivity = 0.03 EU/ml.

$$\text{MVD for Granisetron} = \frac{250 \text{ mg/ml x } 2 \text{ EU /mg}}{0.03 \text{ EU/ml}}$$

MVD = 16000, so MVD/2 = 8000.

4. Subject the sample to MVD /2 dilution.

5. Arrange the depyrogenated tubes in rack and identify by number.

6. Perform addition of reagent as follows:

a. Test specimen: 50 µl LRW and 50 µl sample.

b. Negative Water Control (NWC): Add 100 µl of LRW

c. Positive Water Control (PWC): 50 µl LRW + 50 µl CSE (λ)

d. Positive Product Control (PPC): 50 µl test dilution + 50 µl CSE (λ)

7. Vortex all samples for not less than 3 minutes.

8. Immediately add 100µl lysate to all tubes and incubate in dry heating block maintained at 37°C ± 1°C for 60 ± 2 minutes.

9. Observe tubes for gel formation by inverting promptly and carefully at 180° and record the results.

10. Test is valid only if NWC is negative. PPC is positive and PWC is positive.

11. Test sample passes the test for endotoxins if both the tubes of sample under test are negative,

12. If during observation one tube of duplicate is found positive and one negative repeat the test.

Drug Excipients compatibility Studies

The drug excipients compatibility study was carried out to check the compatibility of the API with different selected excipients used in the formulation.

The compatibility study was carried out as per the combination mentioned below

Solid state compatibility:

Sr. No.	Combination	Quantity/Vial
1	Granisetron Hydrochloride	1 mg
2	Granisetron Hydrochloride + Sodium chloride	1 mg + 18 mg
3	Granisetron Hydrochloride + Citric acid	1 mg + 4 mg
4	Granisetron Hydrochloride + Methyl Paraben	1 mg + 0.36 mg
5	Granisetron Hydrochloride + Propyl Paraben	1 mg + 0.04mg
6	Granisetron Hydrochloride + m-Cresol	1 mg + 0.5mg
7	Granisetron Hydrochloride + Benzyl Alcohol	1 mg + 20mg
8	Granisetron Hydrochloride + Methyl Paraben + Propyl Paraben	1 mg + 0.36mg + 0.04mg
9	Granisetron Hydrochloride + Sodium chloride + Citric acid	1 mg + 18mg + 4mg
10	Granisetron Hydrochloride + Sodium chloride + Citric acid + Benzyl alcohol	1 mg + 18mg + 4mg + 20mg
11	Granisetron Hydrochloride + Sodium chloride + Citric acid + Methyl Paraben	1 mg + 18mg + 4mg + 0.36mg
12	Granisetron Hydrochloride + Sodium chloride + Citric acid + Propyl Paraben	1 mg + 18mg + 4mg + 0.04mg

13	Granisetron Hydrochloride + Sodium chloride + Citric acid + Methyl Paraben + Propyl Paraben	1 mg + 18mg + 4mg + 0.36mg + 0.04mg
14	Granisetron Hydrochloride + Sodium chloride + Citric acid + m-Cresol	1 mg + 18mg + 4mg + 0.5mg
15	Granisetron Hydrochloride + Sodium chloride + Benzyl alcohol	1 mg + 18 mg + 20mg
16	Granisetron Hydrochloride + Sodium chloride + Methyl Paraben	1 mg + 18 mg + 0.36mg
17	Granisetron Hydrochloride + Sodium chloride + Propyl Paraben	1 mg + 18 mg + 0.04mg
18	Granisetron Hydrochloride + Sodium chloride + Methyl Paraben + Propyl Paraben	1 mg + 18 mg + 0.36mg + 0.04mg
19	Granisetron Hydrochloride + Sodium chloride + m-Cresol	1 mg + 18 mg + 0.5 mg
20	Granisetron Hydrochloride + Citric acid + Benzyl alcohol	1 mg + 4mg + 20mg
21	Granisetron Hydrochloride + Citric acid + Methyl Paraben	1 mg + 4mg + 0.36mg
22	Granisetron Hydrochloride + Citric acid + Propyl Paraben	1 mg + 4mg + 0.04mg
23	Granisetron Hydrochloride + Citric acid + Methyl Paraben + Propyl Paraben	1 mg + 4mg + 0.36mg + 0.04mg
24	Granisetron Hydrochloride + Citric acid + m-Cresol	1 mg + 4mg + 0.5mg

Solution state compatibility:

Sr. No.	Combination	Quantity/Vial
1	Granisetron Hydrochloride + Water for injection	1 mg
2	Granisetron Hydrochloride + Sodium chloride + Water for injection	1 mg + 18 mg
3	Granisetron Hydrochloride + Citric acid + Water for injection	1 mg + 4 mg
4	Granisetron Hydrochloride + Methyl Paraben + Water for injection	1 mg + 0.36 mg
5	Granisetron Hydrochloride + Propyl Paraben + Water for injection	1 mg + 0.04mg
6	Granisetron Hydrochloride + m-Cresol + Water for injection	1 mg + 0.5mg
7	Granisetron Hydrochloride + Benzyl Alcohol + Water for injection	1 mg + 20mg
8	Granisetron Hydrochloride + Methyl Paraben + Propyl Paraben + Water for injection	1 mg + 0.36mg + 0.04mg
9	Granisetron Hydrochloride + Sodium chloride + Citric acid + Water for injection	1 mg + 18mg + 4mg
10	Granisetron Hydrochloride + Sodium chloride + Citric acid + Benzyl alcohol + Water for injection	1 mg + 18mg + 4mg + 20mg
11	Granisetron Hydrochloride + Sodium chloride + Citric acid + Methyl Paraben + Water for injection	1 mg + 18mg + 4mg + 0.36mg
12	Granisetron Hydrochloride + Sodium chloride + Citric acid + Propyl Paraben + Water for injection	1 mg + 18mg + 4mg + 0.04mg
13	Granisetron Hydrochloride + Sodium chloride + Citric acid + Methyl Paraben + Propyl Paraben	1 mg + 18mg + 4mg + 0.36mg + 0.04mg

14	Granisetron Hydrochloride + Sodium chloride + Citric acid + m-Cresol + Water for injection	1 mg + 18mg + 4mg + 0.5mg
15	Granisetron Hydrochloride + Sodium chloride + Benzyl alcohol + Water for injection	1 mg + 18 mg + 20mg
16	Granisetron Hydrochloride + Sodium chloride + Methyl Paraben + Water for injection	1 mg + 18 mg + 0.36mg
17	Granisetron Hydrochloride + Sodium chloride + Propyl Paraben + Water for injection	1 mg + 18 mg + 0.04mg
18	Granisetron Hydrochloride + Sodium chloride + Methyl Paraben + Propyl Paraben + Water for injection	1 mg + 18 mg + 0.36mg + 0.04mg
19	Granisetron Hydrochloride + Sodium chloride + m-Cresol + Water for injection	1 mg + 18 mg + 0.5 mg
20	Granisetron Hydrochloride + Citric acid + Benzyl alcohol + Water for injection	1 mg + 4mg + 20mg
21	Granisetron Hydrochloride + Citric acid + Methyl Paraben + Water for injection	1 mg + 4mg + 0.36mg
22	Granisetron Hydrochloride + Citric acid + Propyl Paraben + Water for injection	1 mg + 4mg + 0.04mg
23	Granisetron Hydrochloride + Citric acid + Methyl Paraben + Propyl Paraben + Water for injection	1 mg + 4mg + 0.36mg + 0.04mg
24	Granisetron Hydrochloride + Citric acid + m-Cresol + Water for injection	1 mg + 4mg + 0.5mg

Procedure:

All the compositions mentioned in the above table were mixed individually and filled in to a 5mL Glass vials and placed in stability along with placebo at 60°C and 40°C/75%RH for 1week, 2week, 4 week and 8 week.

After specified time interval samples were withdrawn and analysed for assay and related substances.

Preservative efficacy study

All useful antimicrobial agents are toxic substances. For maximum protection of patients, the concentration of preservative shown to be effective in the final packaged product should be below a level that may be toxic to human being.

The concentration of an added antimicrobial preservative can be kept at minimum if active ingredients of the formulation possess an intrinsic antimicrobial activity.

The preservative efficacy study was performed to optimize the concentration of preservatives in the formulation. The formulation was prepared as per the following concentrations of preservatives.

Sr. no.	Ingredients / Concentration (mg per mL)						
	Granisetron hydrochloride	Sodium chloride	Citric acid	Methyl paraben	Propyl paraben	m-Cresol	Benzyl alcohol
1	1.0	9.0	2.0	-	-	-	-
2	1.0	9.0	2.0	0.21	0.024	-	-
3	1.0	9.0	2.0	0.180	0.020	-	-
4	1.0	9.0	2.0	0.144	0.016	-	-
5	1.0	9.0	2.0	0.108	0.012	-	-
6	1.0	9.0	2.0	0.072	0.008	-	-
7	1.0	9.0	2.0	0.036	0.004	-	-
8	1.0	9.0	2.0	-	-	0.30	-
9	1.0	9.0	2.0	-	-	0.25	-
10	1.0	9.0	2.0	-	-	0.20	-
11	1.0	9.0	2.0	-	-	0.15	-
12	1.0	9.0	2.0	-	-	0.10	-
13	1.0	9.0	2.0	-	-	0.05	-
14	1.0	9.0	2.0	-	-	-	12.0
15	1.0	9.0	2.0	-	-	-	10.0
16	1.0	9.0	2.0	-	-	-	8.0
17	1.0	9.0	2.0	-	-	-	6.0
18	1.0	9.0	2.0	-	-	-	4.0
19	1.0	9.0	2.0	-	-	-	2.0

Procedure:

The most stringent criteria of British pharmacopoeia were followed for experiment. The preservative efficacy study was performed with following procedure.

1. Requirements:

Soyabean casein digest agar and Soyabean casein digest broth

Saboraud dextrose agar medium Sterilized normal saline tubes and Sterilized peptone water tubes

Sterilized Petri plates

Sterilized pipettes and Sterilized pipette tips

Polysorbate 80

Polysorbate 20 and Soya lecithin I Fluid. casein digest-soy lecithin-polysorbate 20 medium (Neutralizer).

2. Procedure for culture suspension preparation of *E-coli, P.aeruginosa, S. aureus, C.afbicans* and environmental isolate

Subculture the following cultures in Soyabean casein digest broth.

S. No.	Name of the organism	ATCC number
1	*E. coli*	8739
2	*Ps. Aeruginosa*	9027
3	*S. aureus*	6538
4	*C. albicans*	10231
5	Environmental isolate	Not Applicable

2.1. Incubate the inoculated broth tubes at 30 - 35°C for 48 to 72 hours.

2.2. After incubation, transfer 1 ml of *E.coli* culture to 9ml of 0.1 %w/v peptone water and mark the tube as 10^{-1}.

2.3. Vortex the 10^{-1} dilution tube and transfer 1ml each to 2 petri plates and 1ml to another tube containing 9ml of 0.1% peptone water and mark the tube as 10^{-2}.

2.4. Vortex the 10" dilution tube and transfer 1ml each to 2 petri plates and 1ml to another tube containing 9ml of 0.1% w/v peptone water and mark the tube as 10^{-3}.

2.5. Vortex the 10^{-3} dilution tube and transfer 1 ml each to 2 petri plates and 1ml to another tube containing 9ml of 0.1 % w/v peptone water and mark the tube as 10^{-4}.

2.6. Vortex the 10^{-4} dilution tube and transfer 1 ml each to 2 petri plates and 1 ml to another tube containing 9mI of 0.1% w/v peptone water and mark the tube as 10^{-5}.

2.7. Vortex the 10^{-5} dilution tube and transfer 1 ml each to 2 petri plates and 1 ml to another tube containing 9ml of 0.1% w/v peptone water and mark the tube as 10^{-6}.

2.8. Vortex the 10^{-6} dilution tube and transfer 1 ml each to 2 petri plates and 1 ml to another tube containing 9 ml of 0.1 % w/v peptone water and mark the tube as 10^{-7}.

2.9. Vortex the 10^{-7} dilution tube and transfer 1 ml each to 2 petri plates and 1 ml to another tube containing 9ml of 0.1 % w/v peptone water and mark the tube as 10^{-8}.

2.10. Vortex the 10^{-8} dilution tube and transfer 1 ml each to 2 petri plates and 1 ml to another tube containing 9ml of 0.1 % w/v peptone water and mark the tube as 10^{-9}.

2.11. Vortex the 10^{-9} dilution tube and transfer 1 ml each to 2 petri plates and 1 ml to another tube containing 9ml of 0.1 % w/v peptone water and mark the tube as 10^{-10}.

2.12. Vortex the 10^{-10} dilution tube and transfer 1 ml each to 2 petri plates.

13. Pour 15 - 20ml of pre-sterilized molten media (SCDA for bacteria and SDA for Candida albicans) maintained at temperature of about 45^{0}C to each of petri plate.

2.14. Gently rotate the plates in clockwise and anticlockwise direction on the LAF bench for uniform mixing of culture and media.

2.15. Allow the plates to solidify.

2.16. After solidification of plates, incubate all the plates at 30 - 35'C for 3 days for bacterial cultures and at 20 - 25C for 5 days for Candida albicans.

(NOTE: Harvested culture should not be more than 5 passages from the original culture.)

3.PROCEDURE FOR CULTURE SUSPENSION PREPARATION OF *Aspergillus niger:*

3.1 Take the sub cultured slant of Aspergillus niger ATCC 16404.

3.2 Harvest the culture in 10 ml of sterile saline (0.9%w/v Sodium chloride) containing 0.05% of Polysorbate 80, to obtain a microbial count of 1 x 10^{-7} to 1 x 10^{-8} CFU per ml (Stock).

NOTE: 1. Harvested culture should not be more than 5 passages from the original culture.

3.3 From the harvested stock solution, perform the serial dilution up to 10^{-10} by using sterile saline (0.9%wlv Sodium chloride).

3.4 From each dilution tube, transfer 1 ml each to a set of sterile petri plates.

3. 5 Pour 15-20ml of pre-sterilized molten SDA at about 45°C.

3.6 Gently rotate the plates in clockwise and anticlockwise direction on LAF bench for uniform mixing of culture and media.

3.7 Allow the plates to solidify.

3.8 After solidification of plates, incubate all the plates at 20 - 25°C for 5 days.

4. PRESERVATION OF THE DILUTED CULTURE SUSPENSION:

4.1. Store all the diluted culture suspension tubes of step 2 and 3 in the cooling cabinet (2-8°C) till the Final observations are made.

4.2. Record the incubation details, media and dilutions of each organism.

5. SELECTION OF DILUTION FOR TEST:

5.1. At the end of incubation period, check the plates visually and count the individual plates with the help of colony counter. Record the observations, calculate the average.

5.2. Based on the results, calculate the number of cells in the stock solution by using the following formula:

Number of cells in stock = Average Number of CFU per plate x Dilution factor.

For example, If the average number of CFU per plate at 10^{-9} dilution is 40.

Then the Number of cells in stock = 40 x 10^{-9}

5.3. Select the dilution tube having 1 x 10^{-7} to 1 x 10^{-8} CFU per ml as per the steps given below.

5.3.1 Select the plates having 10 CFU to 100 CFU for counting and record for each of the organism and calculate the number of cells in the stock. Like for example, If the average count for *Escherichia coli* is 40 and the corresponding dilution 10^{-8} is the numbers of cells in stock are 40 x 10^{-8}.

5.3.2 Corresponding to number of cells in stock as in step 3.7.3.1, find out the required dilution (1 x 10^{-7} to 1 x 10^{-8} per ml) as per the illustration below:

If the concentration of cells in tube (10^{-1}) is 40 x 10^{-7}, then the concentration of cells in 10^{-2} dilution tube is about 4 x10^{-7} Thus, 10^{-2} dilution tube should be considered as suitable dilution for test.

5.3.3 Record the details of dilution and estimated concentration in CFU/ml.

6. PROCEDURE FOR PRESERVATIVE EFFICACY TEST:

6.1 Take required number of product containers to collect 20ml of product and transfer, the product into the six sterile bacteriological containers.

NOTE: If the sample is bulk sample, collect 200ml of sample and transfer 20ml each into six sterile bacteriological containers.

6.2 Label all the containers with name of the organism to be added to container.

6.3 Vortex the selected tube of the respective culture and add 0.2ml culture suspension of organisms of the tubes containing 20ml of sample each.

6.4 Vortex the contents.

Note: The volume of culture suspension used should be in between 0.5% and 1% of the volume of the product in the container, For example, add 0.1 to 0.2ml of culture suspension for 20ml of product.

6.5 Record the calculation details of quantity of culture suspension required for each organism.

6.6 At the end of 6 hours (From the culture addition to the product), carryout the sample serial dilution as per illustration below.

6.6.1 Select the container which is inoculated with Escherichia coli, mix the solution by using vortex mixer and transfer 1 ml to 9ml of sterile % neutralizer. Label the tube with organism name and dilution (10^{-1}).

6.6.2 Vortex the tube (10^{-1}') and transfer 1 ml each of dilution to a set of sterile petridish labeled as 10^{-1} and to a tube containing 9ml of sterile neutralizer, Label the tube with organism name and 10^{-2}.

6.6.3 Follow step number 6.6.2 for serial dilution up to tube labeled as 10^{-6}.

6.6.4 Pour 15 - 20ml of pre-sterilized molten media (SCDA for bacteria and SDA for yeast and mold) maintained at temperature of approximately 45^0C to each of petri plate. 6.6.5 Gently rotate the plates in clockwise and anticlockwise direction on the LAF bench for uniform mixing of culture and media.

6.6.6 Allow the plates to solidify.

6.6.7 After solidification of plates, incubate all the plates at 30 - 35^0C for 3 days for bacterial cultures and at 20-25^0C for 5 days for fungal cultures.

6.6.8 Repeat the step 6.6.1 to 6.6.7 for the remaining microbial cultures listed in the above table.

6.7 Simultaneously, in place of product, perform a control with Peptone water (0.1 % w/v) for all cultures except *A. niger* (For *A-niger,* saline solution to be used for serial dilution) by following above steps 6.6.1 to 6.6.7 for all the listed organisms.

Note: This counting shall be considered as initial count and shall be documented.

6.8 Incubate the bacterial culture plates at 30 - 35°C for 3 days and fungal cultures at 20 - 25°C for 5 days.

6.9 The inoculated sample containers has to be stored at 20 -25°C up to 28 days for further testing as per schedule time points.

6.10 Repeat the procedure 3.8.6.1 to 3.8.6.7 for 24 hours, 7th day, 14th day, 21st day and 28th days counts and record the results.

6.11 Calculate the log reduction after each time point of test as per formula.

7. ACCEPTANCE CRITERIA:

For Parenteral preparations:

Microorganism		**Log Reduction**					
		6 hours	**24 hours**	**7 Day**	**14 Day**	**21 Day**	**28 Day**
Bacteria	A	2	3	-	-	-	NR*
	B	-	1	3	-	-	NI**
Fungi	A	-	-	2	-	-	NI**
	B	-	-	-	-	-	NI**

NR*: No recover

NI: No increase**

The A criteria express the recommended efficacy to be achieved. In justified cases where the A criteria cannot be attained, for example for reasons of an increased risk of adverse reactions, the B criteria must be satisfied.

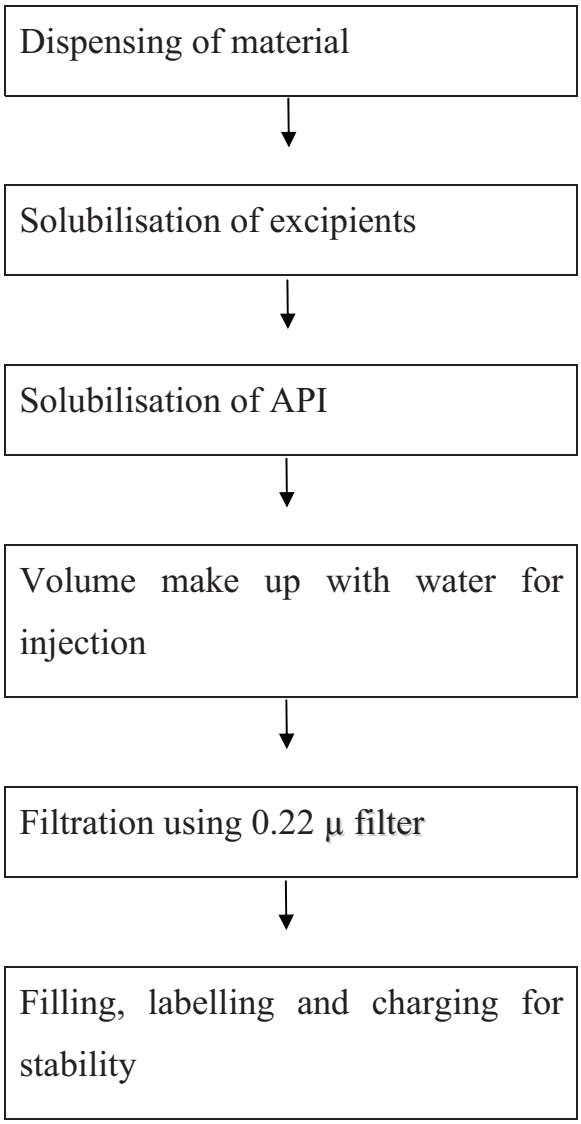
Prototype Formulation Development :

The following excipients were scientifically identified based on their functionality and Preformulation study. The rationale for selecting the excipients is given in the table below.

Formulation Components

Sr. No.	Name of the Excipients	Category	Uses
1	Sodium chloride	Isotonic agent	Use to match the tonicity of the formulation with blood.
2	Citric acid Monohydrate	Buffering agent	Used as buffering agent to prevent the change in ph of the solution during stability.
3	Benzyl alcohol[91] [100]	Preservative	Used prevent bacterial and fungal contamination of the product during shelf life.
4	Methyl paraben	Preservative	Used prevent bacterial and fungal contamination of the product during shelf life.
5	Propyl paraben	Preservative	Used prevent bacterial and fungal contamination of the product during shelf life.
6	m-Cresol	Preservative	Used prevent bacterial and fungal contamination of the product during shelf life.

Process Development Flow Chart



Batch Formulations

Sr. No.	Composition	Batch 1 (mg/ml)	Batch 2 (mg/ml)	Batch 3 (mg/ml)	Batch 4 (mg/ml)	Batch 5 (mg/ml)
1.	Granisetron Hydrochloride Equivalent to Granisetron	1.0	1.0	1.0	1.0	1.0
2.	Sodium Chloride	9.0	9.0	9.0	9.0	9.0
3.	Citric Acid (Monohydrate)	-	-	2.0	2.0	2.0
4.	Methyl Paraben	0.18	-	0.18	-	-
5.	Propyl Paraben	0.02	-	0.02	-	-
6.	m-Cresol	-	0.25	-	0.25	-
7.	Benzyl Alcohol	-	-	-	-	10
8.	Water for injection	q.s to 1mL	q.s to 1mL	q.s to 1mL	q.s to 1mL	q.s to 1mL

Development of Manufacturing Process

The preservative was dissolved in "Water for Injection" (WFI) and this solution was mixed with a solution of sodium chloride, and citric acid in WFI. To the resultant mixture, granisetron hydrochloride was added. The pH and Osmolality of this solution was measured and if required adjusted with sedum hydroxide, and volume was made using WFI. The solution was allowed to pass through 0.22 - micron membrane filter and the sterile solution so obtained was aseptically filled in the desired depyrogenated multi-dose containers. The containers are plugged aseptically with sterile stoppers and sealed.

Autoclave suitability Test

All the formulations of batch 1-5 were subjected to autoclaving suitability study to test the effect of terminal sterilization with steam. Batches are filled into USP type –I glass vials, sealed with bromobutyl rubber plugs and subjected for autoclaving at 121°C for 15, 30, 45 and 60 minutes. The autoclaved samples were tested for appearance, pH, assay and RS.

Optimization of formula and process

Based on the Preformulation studies the following Formula and process was taken for further studies.

Sr. No.	**Composition**	**Quantity (mg/ml)**
1.	Granisetron Hydrochloride Equivalent to Granisetron	1.0
2.	Sodium Chloride	9.0
3.	Citric Acid (Monohydrate)	2.0
4.	Methyl Paraben	0.18
5.	Propyl Paraben	0.02
4.	Sodium Hydroxide	q.s
6.	Water for injection	q.s to 1mL

The brief manufacturing process is as follows:

1) Collect water for injection in a SS vessel. Bubble the nitrogen for 30 minutes

2) Add and dissolve Methyl paraben and propyl paraben in water for injection (75-80°C). Cool to room temperature.

3) Add and dissolve calculated quantity of Sodium Chloride and Citric Acid Monohydrate in water for injection under continuous stirring, to get a clear solution. Mix the solution of step 2 and 3 stir to get clear solution.

4) Add and dissolve calculated quantity of Granisetron hydrochloride in the bulk solution of step 3 under continuous stirring, to get a clear solution.

5) Make up the volume of the solution to 97% and check the pH. If necessary adjust the pH 4.0 to 6.0 with 1N Sodium Hydroxide Solution.

6) Make up the volume of the injection with water for injection and check the pH. Sparge the nitrogen in the bulk solution.

7) Filter the resultant bulk solution through 0.22µ filter.

8) Fill the Granisetron Hydrochloride Injection in multi-dose vial.

Compatibility Study with Tubing

The prepared solution was then subjected to the compatibility study with different types of silicon tubing.

1. Platinum cured tube

2. Peroxide cured tube

3. Tygon tube

4. Braided tygon tube

5. Pharmed Tube

Procedure:

Silicon tubing with inner diameter of 20 mm and a wall thickness of 30 mm was used. Prior to extraction studies the tubing was rinsed for 3 min. with Water for Injection and steam sterilized at 121^0C for 30 minutes.

Static study:

In this study the product was filled in to the silicon tube and samples were with drawn at different time intervals of 5, 10, 20, 30, 60 and 120 minutes and samples were analyzed for assay, RS and preservative content.

Dynamic study:

In this study the product was made to flow through the silicon tube at the rate of 2mL/min and the samples were with drawn at different time intervals of 5, 10, 20, 30, 60 and 120 minutes and samples were analyzed for assay, RS and preservative content.

Compatibility Study with Filters

Filter 47 mm, PVDF, nylon and cellulose acetate was dipped in 100 mL granisetron injection formulation in a 500 mL glass beaker. Glass beaker was appropriately bound with aluminium foil. Sample was stored at 25°C ± 2°C. Sampling was done at intervals of 24 hours and 48 hours solution was analyzed for pH, Assay, RS and Preservative content.

Compatibility with Different type of container system

The optimized Batch of granisetron injection was filled in to Glass vials, Three Piece LDPE container and LDPE BFS containers. The material of construction used for containers are as follows

Three piece containers	:	PE 1810E, PE 1840 H, PE 3020D
BFS containers	:	PE 3020 D
Glass Vials	:	Type I: Borosilicate glass

Above mentioned PE 1810 E, PE 1840 H and PE 3020 D are the grades of low density polyethylene. Polyethylene is a long chain polymer of repeating groups, each connected to two hydrogen atoms. The individual molecules are very long with a carbon "backbone" formed by the carbon atoms connecting to each other. The polymer contains millions of these long molecular chains, each hopelessly entangled with all of its neighbours. The strength of the moulded part lies in the complexity of that entanglement. When cross linking occurs, the molecular weight increases with resulting improvement of the physical properties of the polyethylene.

Cross linking polyethylene compounds contain chemical agents designed to create a molecular change during the moulding process, which results in the polymer molecules becoming interlocked (Cross linked) with each other. Other polyethylene resins bond with each other during the moulding process by surface attachment only, while Crosslink creates a chemical interlocking bond between the molecules that is "in a sense" one giant molecule.

Procedure:

The Batches of granisetron injection was filled in to above mentioned different type of containers and charged for stability at 60°C, 40°C/75%RH, 40°C/25%RH, 30°C/65%RH, 25°C/60%RH and 25°C/40%RH for 3 months.

After specified time interval samples were withdrawn and analysed for chemical and physical parameters.

Compatibility with Different type of closure system

The optimized Batch of granisetron injection was tested for compatibility with following types of rubber closures.

1. Bromobutyl rubber stoppers
2. Chlorobutyl rubber stoppers
3. Teflon coated rubber stoppers
4. Latex rubber stoppers

Procedure:

The 30 g of Rubber stoppers were soaked in 30mL of injection, in a USP Type –I glass vials individually and charged for stability along with placebo at 60°C and 40°C/75%RH for 1week, 2week, 4 week and 8 week.

After specified time interval samples were withdrawn and analysed for pH, assay and related substances.

Stability of Granisetron Hydrochloride Injection[78]

The final formulation of Granisetron Hydrochloride was filled into USP type I clear glass vials and subjected to stability testing

following International Conference on Harmonization (ICH) guidelines.

The design of the formal stability studies for the drug product should be based on knowledge of the behaviour and properties of drug substance and from stability studies on the drug substances. The likely changes on storage and the rationale attributes to be tested in formal stability studies should be stated.

Specifications

Stability studies should include testing of those attributes of the drug product that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy. The testing should cover, as appropriate, the physical, chemical and biological attributes, preservative content and functionality tests. Analytical procedures should be fully validated and stability indicating.

Storage Conditions

In general drug product should be evaluated under storage conditions (with appropriate tolerances) that it's thermal stability and, if applicable, its sensitivity to moisture. The storage conditions and the length of studies chosen should be sufficient to cover storage, shipment, and subsequent use.

Storage Conditions :

Study	Storage condition	Minimum time period covered
Accelerated	40^0C± 2^0C/75 % RH ±5% RH	6 Month
Intermediate	30^0C± 2^0C/65 % RH ±5% RH	18 Month
Long term	25^0C± 2^0C/60 % RH ±5% RH	24 Months
Stress condition	60^0C± 2^0C	1 Month

Finished product analysis

Finished product was analyzed before the stability charging. These tests were performed initially and at every time point of the stability. All the analytical procedures were validated. Analytical parameters to be tested are as follows.

Appearance

Sample under test was inspected visually for colour and clarity

pH :

The pH is a number which represents conventionally the hydrogen ion concentration of an aqueous solution. For practical purposes, its definition is an experimental one. The pH of a solution to be examined is related to that of a reference solution (pH_S) by the following equation:

$$PH = pH8\frac{I-Eo}{R}$$

in which E is the potential, expressed in volts, of the cell containing the solution to be examined and Es is the potential, expressed in volts, of the cell containing the solution of known pH (pHs), k is the change in potential per unit change in pH expressed in volts, and calculated from the Nernst equation.

Operating procedure:

Note: This procedure is applicable to pH meter Make: Eutech, Model: pH Cyberscan l100

1. Ensure that the pH meter is clean, kept in proper position and calibration status is valid.
2. Rinse the electrode with purified water to remove any impurities, adhered on the electrode body from prior use.
3. Switch ON the instrument by pressing the ON/OFF key and ensure that meter is in (MEAS) mode to measure the sample pH.
4. Dip the electrode and the temperature probe in to the sample making sure that the bulb of the electrode is completely dipped in the sample solution.
4. Allow the reading to stabilize until the pH value stops blinking.
5. After checking the sample pH, wash the electrode with purified water and dip in 3% potassium chloride solution during each pH check of the sample, first check 1 pH buffer before and after the estimated pH of the sample solution.

Principle

Osmolality of the solution was determined by the freezing point depression method. Freezing point is the temperature at which the solid form of the pure solvent coexists in equilibrium with a solution at affixed external pressure. A system for measuring temperature consisting of a resistor sensitive to temperature (thermistor), with an appropriate current or potential-difference measurement device that may be graduated in temperature depression or directly in osmolality.

Instrument

Osmometer Model-3250, Advance Instruments Inc.

Specifications

Sample size	250 µl
Temperature	25°C

Procedure

1) Instrument was switched on and waited till initialization completed.
2) START' key was pressed and waited till Running Diagnostic completed.
3) The probe and stir/freeze wire was cleaned and wiped.
4) 250 µL of the sample was pipette out in sample tube with help of micro pipette and micro-tip and sample tube was placed in position.
5) 'START' key was pressed.
6) Reading was noted for osmolality in mOsm on digital display.

Transmittance

Principle

If the incident light with a wavelength λ and intensity Io impinges on a solution with concentration c, and path length 1cm, the radiant energy of the light decreases in the exponential manner. Mathematically the radiant concentration and radiant path length relation can be expressed as -

$dI/dC = -K_1I$ and $dI/dl = -K_2l$

Instrument : **-** UV Visible Spectrophotometer

Specifications

Path Length	:	10.0 nm
Mode of Measurement	:	Transmission
Detection	:	At 650 nm
Blank	:	Air as blank

Procedure

Concomitantly measure the absorbance of the test preparation at 650 nm using blank preparation.

Colour Index

Principle

If the incident light with a wavelength λ and intensity Io impinges on a solution with concentration c, and path length 1cm, the

radiant energy of the light decreases in the exponential manner. Mathematically the radiant concentration and radiant path length relation can be expressed as

$dI/dC = -K_1I$ and $dI/dl = -K_2l$

Instrument: - UV Visible spectrophotometer

Specifications

Path Length	**:**	10.0 nm
Mode of Measurement	**:**	Absorption
Detection	**:**	At 430 nm
Blank	**:**	Air as blank

Procedure

Concomitantly measure the absorbance of the test preparation at 430 nm using blank preparation.

BACTERIAL ENDOTOXIN TEST: [81]

PURPOSE:

To provide a detailed procedure for performing bacterial endotoxin test by Gel clot method.

PROCEDURE:

1. Introduction:

The gel- clot technique detects or quantifies endotoxins based on clotting of the LAL reagent in the presence of endotoxin. The concentration of endotoxin required to cause the rate to clot under standard conditions is the labeled sensitivity of the LAL reagent.

2. LAL (Limulus Amebocyte Lysate):

Limulus Amebocyte Lysate (LAL) obtained from the aqueous extracts of circulating amebocytes of horse shoe crab (Limulus polyphemus or tachypleus tridentatus) which has been prepared and characterized for use as an LAL Reagent.

3. CSE (Control Standard Endotoxin)

It is a used for preparation of positive controls and endotoxin standard solutions.

4. LRW (LAL Reagent Water)

It is a non LAL reactive and endotoxin free water.

5. Preparation of Control Standard Endotoxin (CSE)

5.1 Reconstitution:

5.1.1 A CSE of ***E.coIi,*** 055:65, is available from supplier of endotoxin kit, which is suitable for confirmation of LAL labeled sensitivity and for preparation of positive controls. CSE from the supplier has predetermined amount of endotoxin, as described in the Certificate of analysis (CoA) which was standardized with US reference endotoxin.

5.1.2 Reconstitute the lyophilized control standard endotoxin *(E.coIi,* 055:B5) with volume of LAL reagent water indicated on the certificate of analysis.

5.1.3 Vortex vigorously for minimum 5 minutes before further dilution.

5.1.4 Each CSE lot is specific to lysate lot. Refer the CoA before using the CSE and lysate.

6. Preparation of standard CSE dilutions:

6.1 Mix vigorously the reconstituted CSE vial, using a vortex mixer for not less than 1 minute before making appropriate serial dilutions.

6.2 Prepare dilutions of standard CSE stock solution LAL reagent water as indicated below.

(If the concentration of the stock solution is 20 EU/mL)

Tube No.	**Control Standard Endotoxin**	**LAL reagent water (mL)**	**Final concentration (EU/mL)**
01	0.1 ml Of Endotoxin Standard Stock Solution	1.9	1.0 (8 λ)
02	1.0 mL from tube No- 1	1.0	0.5 (4 λ)
03	1.0 mL from tube No- 2	1.0	0.25 (2 λ)
04	1.0 mL from tube No- 3	1.0	0.125 (λ)
05	1.0 mL from tube No- 4	1.0	0.06 (1/2 λ)
06	1.0 mL from tube No- 5	1.0	0.03 (1/4 λ)

6.3 Accurately transfer 0.1 mL from tube No-3 into a depyrogenated glass tube in duplicate and label it as 2 λ**.**

6.4 Transfer 0.1 mL from tube No-4 into a depyrogenated glass tube induplicate and label it as λ.

6.5 Transfer 0.1 mL from tube No-5 into a depyrogenated glass tube induplicate and label it as λ/2**.**

6.6 Transfer 0.1 mL from tube No4 into a depyrogenated glass tube induplicate and label it as λ /4.

7. Preparation of Endosafe Lal Reagent

7.1 Collect LAL powder into the bottom of the vial by gently tapping the vial on a hard surface.

7.2 Open the seal of the vial and slowly open the rubber stopper.

7.3 Rehydrate with the indicated amount of LAL reagent water just before use by LAL Reagent Water.

7.4 Swirl gently to dissolve, avoiding liquid contact with stopper.

8. Storage Condition Of CSE And Lal Reagent

8.1 Lyophilized LAL should be stored at 28 **C;** avoid exposures to temperatures above 25°C

8.2 Rehydrated LAL reagent ideally should be stored in refrigerator at 2-8 ^{0}C during intermittent use, up to 24 hours. Otherwise store a rehydrated LAL reagent at -20°C or below up to 28 days. Reagent may be frozen and thawed only once.

8.3 The reconstituted CSE vial can be stored in a refrigerator at 2-8°C for 28 days.

9. Endotoxin limit

The general endotoxin limit for parenteral drugs is 5 EU/kg body weight dose, except for a 0.2 EU/kg body weight limit for intrathecal drugs. Medical device eluates must not exceed 0.5 EU/mL; a 0.06 EU/mL limit applies to device that contact cerebrospinal fluid. The endotoxin limits for different drugs vary and are calculated based on dosage considering the above values as constants.

10. Calculation of Maximum Valid Dilution (MVD)

10.1 MVD (Maximum valid dilution) is the dilution up to which the product can be diluted and still detects the endotoxin.

10.2 For drug products that have a published limit, the MVD may be calculated by following formula:

$$\text{MVD} = \frac{\text{Endotoxin Limit (EU/mg)} \times \text{Potency}}{\lambda\,(\text{Labeled sensitivity of Lysate})}$$

10.3 For drug products not having the published limit, the MVD shall be calculated by th following formula,

$$\text{MUD} = \frac{K}{M}$$

Where,

K- is the threshold human pyrogenic dose of endotoxin per kg of body weight

M - is equal to the maximum recommended human dose of product per kg of body weight in a single hour period.

11. Calculation of Minimum Valid Concentration (MVC)

11.1 When the product is subjected for the test of interference, calculate the minimum valid

concentration by using following formula:

$$\text{MVC} = \frac{\text{Sensitivity of Lysate}\,(\lambda)}{\text{Endotoxin Limit}}$$

11.2 With the help of this formula the non inhibitory dilution of the product shall be taken for the method validation of the product for bacterial endotoxin test using Gel clot method.

12. Requirements for LAL Test

12.1 Depyrogenated glassware, Endotoxin dilution and reaction test tubes, glass pipettes for dilutions.

12.2 LAL reagent

12.3 LAL reagent water

12.4 Control standard endotoxin

12.5 Vortex mixer

12.6 Heating block

12.7 Micropipette and tips

12.8 Rubber bulb

13. Sample Preparation

For finish product of parenteral preparation collect 5 vials / ampoules from each batch to be tested and pool the sample in depyrogenated container.

14. Sample Dilution

14.1 Prepare dilutions of pooled sample using LAL reagent water up to the MVD or as per the validation.

14.2 Accurately transfer 0.1 mL from final dilution tube to assay tube in duplicate.

15. Positive Product Control

Accurately transfer 50 µL from the final dilution tube in the assay tube in duplicate and add 50 µL of 4λ CSE in both the tubes, and add 0.1, mL of lysate in that mixture.

16. Negative Control

Add 0.1 mL of LRW and 0.1 mL of lysate in a test tube.

17. Incubation Of Sample Tubes

17.1 Add 0.1 mL of lysate In all the finally diluted standard and sample tubes and shake gently before incubation.

17.2 The tubes are incubated at 37^0C ± 1^0C for 60±1 minutes on heating block.

18. Observation

A positive reaction is characterized by the formation of firm gel that remains intact when inverted through 180 Angle. Record such a result as positive (+). A negative result is characterized by the absence of such a Gel. Record such a result as Negative (-).

19. Interpretation of the Test

The article meets the requirements of the test if the concentration of endotoxin is not more than that specified in the specifications. Results are valid only when CSE are positive a 2 fold of labeled sensitivity of Lysate.

STERILITY TESTING

PROCEDURE:

A) REQUIREMENTS:

1. Pre-incubated 100 ml Soya bean Casein Digest medium tubes. (SCDM)
2. Pre-incubated 100 ml Fluid Thioglycollate Medium tubes (FTGM)
3. Pre incubated Rinsing fluid -0.1% Peptone water.
4. Sterilized filtration assembly (Manifold, Filtration cones, Filtration flask with silicon tubing).
5. Sterilized Membrane Filters (0.45micron, 47 mm Diameter)
6. Sterilized Scissors, Forceps, Syringes, Needles, Seal opener and ampoule cutter.
7. Vacuum Pump and burner.
8. Steritest equipment and steritest canisters.
9. Sterile filtered 70% v/v IPA solution.
10. Sterile filtered Disinfectant (Sporicidal) solution.
11. Standard Organisms.

B) TRANSFER OF SAMPLE:

1. Immerse the sample vials (Glass) / ampoules in 70% v/v IPA for a contact period of 30 minutes.
2. After the contact period takeout the sample units and place it in a sanitized beakers (For each batch *I* sample use separate beakers).
3. Enter into the Sterility test area as per the standard operating procedures.
4. Immerse the sample units in Bacillocid 2.5% or any other sporicidal agent for a contact period of 10 minutes.
5. Immerse the sample units once again in the 70% v/v IPA for a contact period of 10 minutes.

C) MEMBRANE FILTRATION PROCEDURE -A (MANIFOLD METHOD):

1. Sanitize the Laminar air flow bench, burner and SS trolley by mopping with the help of a sterile lint free mop soaked in 70% vlv IPA and allow it air dry.
2. Transfer the sample units from pass box to Laminar air flow bench.
3. Unload the items required for sterility test from the autoclave and transfer the materials from cool zone to laminar air f1ow by using trolley.
4. Transfer the required media and samples from the pass box to laminar air flow by using the same SS trolley. If there is no space in the laminar air flow keep the trolley with media bottles *I* tubes near the laminar air flow.

5. Arrange the filtration cup bases on the manifold.
6. Using sterile forceps aseptically transfer the 0.45micron membrane on to the filtration cup base and fix the filtration cup top upon the membrane.
7. Lighten the burner with foot operating switch whenever required.
8. Pre-wet the membrane with 100ml of 0.1 % pre sterilized peptone water and filter the solution through the membrane filtration cones.

For Vials:

a. By using the sterile syringe and needle aseptically transfer the solution from the vial to filtration cup.

b. Repeat the above step for the other vials.

9. Rinse the membrane filter three times with 100ml 0.1 % pre-sterilized peptone water.
10. Remove the 'filtration cup from the base and cut the membrane into' two halves with help of sterile forceps and sterile scissors.
11. Aseptically 'transfer one half of the membrane into the SCDM tube and other half into the FTGM tube.

Negative Control:

a. Aseptically filter 4 x 100 ml of Sterilized 0.1% peptone water through a separate membrane filter unit.

b. Cut the membrane into two halves with help of sterile forceps and sterile scissors.

c. Aseptically transfer one half of the membrane into the SCOM tube and other half into the FTGM tube and incubate at 20-25°C & 30-35°C respectively for 14 days.

Positive Control:

Inoculate 10 to 100 CFU/mL of *Bacillus subtilis* ATCC 6633 or *Candida albicans* ATCC 10231 or *Aspergillus niger* ATCC 16404 for SCOM and *Pseudomonas aeruginosa* ATCC 9027 or *Clostridium sporogens* ATCC11437 or *S.aureus* ATCC 6538 for FTGM (Rotate the organism every month for each medium).

Note: 1. An altemate to *S.aureus* ATCC 6538 is *Bacillus subtilis* ATCC 6633.

2**.** This activity must be done in Microbial limit test Area under laminar air flow.

Incubation:

Transfer the sterility test tube through the pass box (MQ-072) and incubate the SCOM tubes at 20° -25°C and FTGM tubes at 30°-35°C for 14 days.

D) MEMBRANE FILTRATION PROCEDURE – B (STERITEST METHOD):

1. This method is an alternate method to manifold method.
2. Sanitize the Laminar air flow bench ,burner and SS trolley by mopping with the help of a sterile lint free mop soaked in 70% v/v IPA and allow it air dry.
3. Transfer the sample units to Laminar air flow bench.

4. Unload the items required for sterility test from the autoclave and transfer the materials from cool zone to laminar air flow by using SS trolley.
5. Transfer the required media, canisters and samples from the pass box to laminar air flow by using the same SS trolley. If there is no space in the laminar air flow keep the trolley with media bottles and samples near the laminar air flow.
6. Lighten the burner with foot operating switch whenever required.
7. Operate the steritest instrument as per the standard operating procedures.
8. Pre-wet the membrane with 100 ml of 0.1 % pre sterilized peptone water and filter the solution.

For Vials:

a. In case of glass vial remove flip off and insert the steritest canister needle through the rubber stopper' and aseptically aspirate the contents of the vial and filter through the 0.45 micron membrane of the canister.

b. Repeat the above step for other vials.

9. Rinse the membrane three times with 100 ml 0.1% pre-sterilized peptone water.
10. Filter the solution and close the bottom of the canister with the lid provided.
11. With help of clip (Provided with canister), close the one line and through the other line add FTGM into one canister.

12. Close the FTGM line with help clip and open the other line and add SCOM into the other canister.

13. Cut the tubing and insert the tube end into the vent filter provided at the top of the canister.

Incubation:

Transfer the sterility test canister through the pass box (MQ-072) and incubate the SCOM canister at 20 -25°C and FTGM canister at 30 - 35°C for 14 days.

Negative Control:

a. Aseptically filter 4 x1 OOml of Sterilized 0.1 % peptone water through the separate

set of canister.

b. Add the SCOM and FTGM into canister and incubate at at 20-25°C and 30-35°C

respectively for 14 days.

Positive Control:

Inoculate 10 to 100 CFU/mL of *Bacillus subtilis* ATCC 6633 or *Candida albicans* ATCC 10231 or *Aspergillus niger* ATCC 16404 for SCOM and *Pseudomonas aeruginosa* ATCC 9027 or *Clostridium sporogens* ATCC11437 or *S.aureus* ATCC 6538 for FTGM (Rotate the organism for every month for each medium)

Note: 1. An alternate to *S.aureus* ATCC 6538 is *Bacillus subtilis* ATCC6633. '

2. This activity must be done in Microbial limit test Area under laminar air flow.

E) DIRECT INOCULATION METHOD:

i. Transfer the quantity of preparation to be examined directly in to the culture medium so that the volume of the product is not more than 10 percent of the volume of the medium. If the product to be examined has antimicrobial[83] activity, carry out the test after neutralizing this with a suitable neutralizing substance or by dilution in a sufficient quantity of culture medium. When it is necessary to use a large volume of the product it may be preferable to use a concentrated culture medium prepared in such a way that it takes account of the subsequent dilution. Where appropriate the concentrated medium may be added directly to the product in its container.

ii. **For aqueous solutions**:

Remove the liquid from the test containers with a sterile pipette or with a sterile syringe or a needle. Aseptically transfer the specified volume of the material from each container to a vessel of the culture medium. Mix the liquid with the medium but do not aerate excessively. Incubate the inoculated media for not less than 14 days, at 30°C to 35°C in the case of fluid thioglycolate medium and at 20°C to 25°C in the case of soyabean-casein digest medium 5.5.3.

iii. When the material being examined renders the medium· turbid so that the presence or absence of microbial growth cannot be determined readily by visual examination, transfer suitable portions of the medium to fresh vessels of the same medium between the third and seventh days after the test is started. Continue incubation of the transfer vessels for

not less than 7 additional days after the transfer and for a total of not less than 14 days.

Negative Control:

Keep un inoculated SCOM tube and FTGM tube as a negative control.

Positive Control:

Inoculate 10 to 100 CFU/mL of *Bacillus subtilis* ATCC 6633 or *Candida albicans* ATCC 10231 or *Aspergillus niger* ATCC 16404 for SCOM and *Pseudomonas aeruginosa* ATCC 9027 or *Clostridium sporogens* A TCC 11437 or *S.aureus* A TCC 6538 for FTGM (Rotate the organism for every month for each medium).

Note: 1. An alternate to *S.aureus* ATCC 6538 is *Bacillus subtilis* ATCC 6633.

2. This activity must be done in Microbial limit test Area under laminar air flow.

PHOTO STABILITY STUDY[77] [79]

1. LIST OF EQUIPMENT:

Photo stability chamber

2. SAMPLE PRESENTATION:

i. Drug product in the immediate pack.

ii. Drug product in the marketed pack.

iii. Drug product in the immediate pack wrapped in aluminium foil (As dark control)

Sr. No.	Sample description	Packing information	Orientation
1	Immediate pack	Clear glass vial	Transverse
2	Marketed Pack	Clear glass vial in carton	Transverse
3	Immediate pack wrapped in aluminium foil (As dark control)	Clear glass vial wrapped in aluminium foil	Transverse

3. STABILITY STUDY PROCEDURE:

Calibrate the photo stability chamber with both cool white fluorescent and near ultraviolet lamp.

LIGHT EXPOSURE:

To provide an overall illumination of not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 300 watt hours *I* square meter.

SAMPLING:

After exposure for the no. of days *I* hours to achieve an overall illumination of not less than1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt hours *I* square meter, sampling of both the groups is done.

4. TESTING PARAMETERS:

i. Description, Identification and pH

ii. Particulate contamination

iii. Assay and Related substances

iv. Light transmission and color index

v. Osmolality

FREEZE THAW AND THERMAL CYCLING STUDY

Sample presentation: Drug product in immediate pack

Sr. No.	Experiment	Stability study design
1	STUDY –I	Refrigerator Temperature: 2°C to 8°C Accelerated Temperature: 40°C ± 2°C *I* 75% RH ± 5%RH
2	STUDY –II	Freezing Temperature: -20°C to -10°C Accelerated Temperature: 40°C ± 2°C *I* 75% RH ± 5%RH

PROCEDURE: STUDY –I

CYCLE 1

1. Place the samples in upright orientation in the refrigerator maintained at temperature between 2°C to 8°C for 48 hours.

2 After 48 hours remove these samples from the refrigerator and place them in the 40°C ±2°C *I* NMT 25% RH stability chamber for next 48 hours.

3 Record the observations and storage period of the vials removed from refrigerator.

CYCLE 2

1. After 48 hours remove all the vials from the 40°C ± 2°C I NMT 25% RH stability chamber and store them in refrigerator at 2°C to 8°C for next 48 hours.
2. Record the observations and storage period of the vials removed from 40°C ± 2°C I NMT 25% RH chamber.
3. After 48 hours remove all vials from the refrigerator and place them in the 40°C ± 2°C I NMT 25% RH chamber for next 48 hours.
4. Record the observations and storage period of the vials removed from the refrigerator.

CYCLE 3

1. Repeat Cycle 2
2. Upon completion of Cycle 3, remove all samples from the 40°C ± 2°C I NMT 25%RH chamber. and send the samples for analysis.

STUDY –II

CYCLE 1

1. Place the samples in upright orientation in the deep freezer maintained at temperature between -20°C to 10°C for 48 hours.
2. After 48 hours remove these samples from the deep freezer and place them in the 40°C ± 2°C I NMT 25%RH stability chamber for next 48 hours.
3. Record the observations and storage period of the vials removed from deep freezer.

CYCLE 2

1 After 48 hours remove all the vials from the 40°C ± 2°C I NMT 25%RH stability chamber and store them in deep freezer of -20°C to -10°C for next 48 hours.

2 Record the observations and storage period of the vials removed from 40°C ± 2°C I NMT 25%RH chamber.

3 After 48 hours remove all vials from the deep freezer and place them in the 40°C ± 2°C I NMT 25%RH chamber for next 48 hours.

4 Record the observations and storage period of the vials removed from the deep freezer.

CYCLE 3

1 Repeat Cycle 2

2 Upon completion of Cycle 3, remove all samples from the 40°C ± 2°C I NMT 25%RH stability chamber, and send for analysis.

TESTING PARAMETERS:

i. Description, Identification and pH

ii. Particulate contamination

iii. Assay and Related substances

iv Light transmission and color index

v Osmolality

RHEOLOGICAL EVALUATION OF GRANISETRON INJECTION

The viscosity of Granisetron Injection, With Preservative (1 mg/mL) and Granisetron Injection, Preservative Free (0.1 mg/mL) was determined by using Brookfield Viscometer. Brookfield viscometer provides the facility of small sample adapter which is generally

employed for the rheological evaluation where sample volume is limited.

The details of viscosity[97] [] measurement are summarized as follows:

Brookfield Viscometer Model : LVDV-II + Pro

Spindle : SC4-18

Sample Chamber : SC4-13R (P) (with RTD temperature probe)

Sample Volume : 6.7 mL

Shear Rate (sec^{-1}) : 1.32 N

Method for Viscosity Determination:

The sample adapter was mounted and the spindle was tied as per the instructions given in the manual of Brookfield viscometer. Exact 6.7 mL of Granisetron Injection, With Preservative (1.0 mg/mL) was added in to the sample chamber, supplied with RTD temperature probe. The temperature was kept constant at 27.8^0C (room temperature). The viscosity was then determined by using different rotational speeds of spindle i.e. 100 rpm, 150 rpm, 180 rpm and 200 rpm.

The same procedure was employed for the viscosity determination of Granisetron Injection, Preservative Free (0.1 mg/mL)