PROTOCOL

FOR

DECONTAMINATION OF STEROIDS RESIDUE

FROM THE OINTMENT FACILITY

SUPERSEDE PROTOCOL No.	NIL
DATE OF STUDY	

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1.0 PROTOCOL APPROVAL:

INITIATED BY:

DESIGNATION	NAME	SIGNATURE	DATE
EXECUTIVE (QUALITY ASSURANCE)			

REVIEWED BY:

DESIGNATION	NAME	SIGNATURE	DATE
HEAD (WAREHOUSE)			
HEAD (ENGINEERING)			
HEAD (PRODUCTION)			
HEAD (QUALITY CONTROL)			
MANAGER (QUALITY ASSURANCE)			

APPROVED BY:

DESIGNATION	NAME	SIGNATURE	DATE
HEAD (QUALITY ASSURANCE)			

2.0 OBJECTIVE:

The objective of this Decontamination protocol is to describe the Background, Regulatory Requirements, Strategy and Theoretical Aspects of the Steroidal decontamination activity to be performed for Facility (Warehouse area, Manufacturing area, Service Floor & Utility area) of Block.

This Protocol shall provide the methodology for selection of area, selection of API's, selection of equipments, sampling Procedure, locations, acceptance criteria and Analytical Method to identification, selection & efficacy of decontaminating agent for decontamination of facility. Also ensure the traces residue of steroids after decontaminating the facility (warehouse area, Manufacturing area, Service floor and utility area).

3.0 SCOPE:

This protocol is applicable for Warehouse, Manufacturing facility, Service floor and Utility area of Ointment Section.

4.0 RESPONSIBILITY:

DEPARTMENTS	RESPONSIBILITIES
Quality Assurance	• Executive/Asst. Manager shall prepare the protocol. • Manager QA shall review the protocol. • Head QA shall approve the protocol. • To ensure the compliance of the QMS. • To review analytical records and results. • To conclude the decontamination activity.
Production	• Executive/Asst. Manager shall review the protocol. • Head Production shall review and approve the protocol. • Executive/Asst. Manager shall execute the protocol.
Engineering	• Executive/Asst. Manager shall review the protocol. • Engineering personnel shall execute the protocol. • Head Engineering shall review and approve the protocol. • To provide complete support for the decontamination activity for the facility. • To procure all the necessary consumable parts and replace the same as per protocol. • To re qualify the facility as required by the protocol
Warehouse	• To provide information about RM & FG Stock. • To provide complete support for the decontamination activity for the facility
Quality Control	• Analytical Method Development and Validation for detection of residual of steroids. • Sampling as per Sampling Plan, analyze the samples Compile the analysis data.

5.0 BACKGROUND:

- Decontamination of the Ointment facility has multifaceted and difficult challenges, which demands for detailed Technical Asessment, Technically sound methodology, Proper Planning, Systmatic implementation and Documentation. It also becomes imperative to study the background of the decontamination requirementTo avoid a high risk of cross contamination, decontamination study has been planned. Since Steriods are Super potent/Potent category product, therefore, an exhausitive decontamination study has been planned through a well defined protocol.
- Triamcinolone Oromucosal paste 0.1% w/w was the first batch produced, Over the period of time, manufactured 02 more steroidal products (Clobetasol 0.05% & Mometasone Furoate 0.1% w/w) in the dedicated area.
- Facility is proposed to convert into General product manufacturing, hence Decontamination study shall be planned to avoid any risk of residual Steroids which might be the chance of contamination of General products.
- To mitigate the risk of Steroidal contamination in the General Products; it was decided to perform Risk Assessment, followed by Decontamination of the facility.
- The Technical Assessment was performed with Risk Based Approach, to provide the complete information of the **Difficult to Clean Surfaces,** considering product contact parts, air flow Pattern, vulnerable locations identified in the facility, Instruments, Equipment and Systems. This Technical Assessment shall help to design a comprehensive protocol for 'Steroidal Decontamination' of the facility.

6.0 CATEGORIZATION ON THE BASIS OF RISK ASSESSMENT:

6.1 Critical Observations:

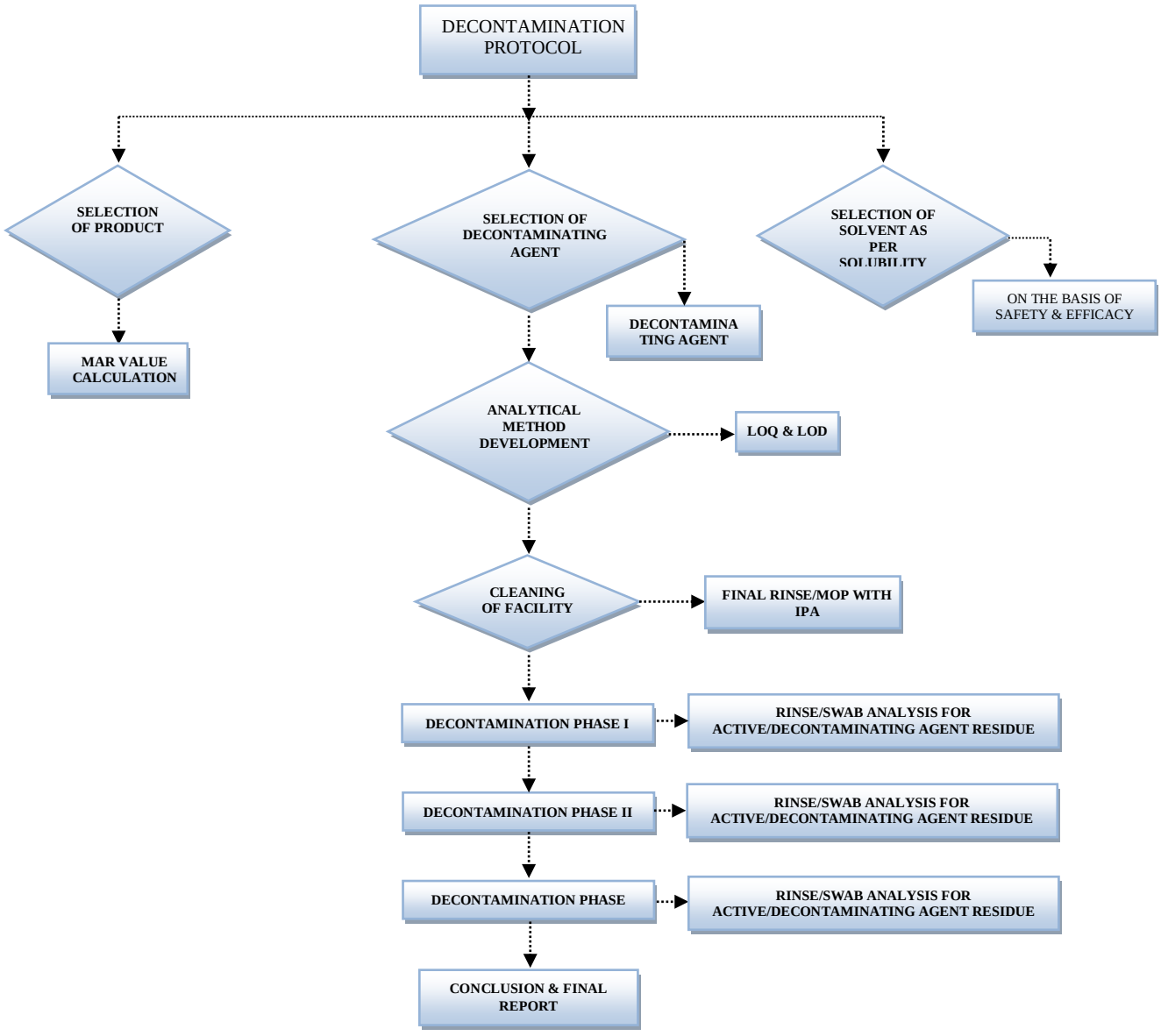
- The Steroids and General products were being manufactured in the same building, which poses high risk of contamination. Although the block is dedicated for Steroids, the peripheral area of the block shall be completely evaluated for the presence of the Steroidal traces.
- **High Risk Zone:** Dispensing, Manufacturing, Holding & Filling area to be decontaminated.
- The Return air risers have 10 micron filters. However, the risers and ducting shall be thoroughly cleaned using vacuum cleaner. All the HEPA filters shall be replaced after cleaning of the risers and ducting.
- There is a dedicated area for Pre Filter Cleaning. The area is getting exposed to the Steroids and other API powders. This poses High Risk of contamination and shall be decontaminated to avoid contamination.
- Though the sampling activity is performed under LAF the complete sampling area shall be considered for the decontamination.
- The HDPE pallets, the balances which were at the time of receiving of Steroids are still to be used for general products to be decontaminated.
- The dynamic pass should be decontaminated.
- The LAF used for sampling shall be decontaminated prior to use for general products.
- The Walls, Ceiling and the flooring in the Dispensing area, Manufacturing area, Washing area, Holding area & Filling area to be decontaminated.
- The equipment parts such as change parts, dismantled parts and the fixed parts poses High Risk of contamination in the subsequent product, hence to be decontaminated as per the procedure.
- The electrical and mechanical panel area of the Dispensing, Manufacturing, Holding & Filling section are also opened. There can be RM powder on the panel inner side of the door, on the flat SS surfaces of the panel area. The pneumatic silicone tubing, motor pulley chain, short electrical wirings to be decontaminated as they are the integral part of the system.

- Glassware used for the sampling and analysis of Steroids shall be discarded. Example: The Bottles used for Purified Water sample collection. Either dedicated or disposable sampling apparatus or containers shall be considered.
- The Risk Assessment shall be performed before decontamination to exactly understand the critical areas to be focused.
- Gowning to be decontaminated separately.

6.2 Major Observations:

- **Medium Risk Zone:** Air lock, Change rooms, & Service floor Area to be decontaminated.
- The drainage points shall be provided attention by regular cleaning.
- The water samples shall be tested extensively to check the presence of the Steroidal contamination.
- The reference standards, working standards of Steroids may be still available in the QC Laboratory. They shall be kept under lock and key.

7.0 FLOW CHART



8.0 ACTIVITY CHART FOR DECONTAMINATION:

- **MAR Value calculation:** On potency basis worst case molecule to be selected & further MAR value to be calculated.

- **Solubility Study:** Solubility study to be performed for final rinsing and moping purpose.

- **Sampling Plan:** Sampling locations and sampling method shall be determined based on the design of the Facility, Size and number of equipments, Product Contact surfaces and other vulnerable surfaces, HVAC Systems etc. Sampling Techniques like Swab or Rinse shall also be specified for each location considering above aspects. The sampling plan **(Annexure I)** shall be finalized with a Risk Based Approach**.**

- **Analytical Method Development:** Suitable Analytical Method shall be developed and Validated on HPLC for detection of low traces of residual Steroids.

- **Recovery Studies and Simulations of Decontamination:** There shall be scientific justification of the methodology for the Decontamination. There shall be sound scientific rationale for the acceptance criteria. Validation of decontamination procedure shall be performed by Simulation Study in the laboratory along with Recovery studies.

- **Initial Cleaning of the facility:** Cleaning of the Equipment and Facility including HVAC System as per the Validated Procedures (**Annexure II).**

- **Decontamination Phase:**
  Phase I Decontamination: To perform the Phase I Decontamination to remove the Steroid traces from the facility by Decontamination Procedure. To perform Swab and Rinse sampling followed by Testing using validated Analytical Method for the Detection of residue after cleaning.
  Phase II Decontamination: To perform the Phase II Decontamination to remove remaining Steroid traces from the facility by validated Decontamination Procedure. To perform Swab and Rinse sampling followed by Testing using validated Analytical Method for the Detection of residue after cleaning.
  Phase III Decontamination: To perform the Phase III Decontamination to remove remaining Steroidal traces from the facility by validated Decontamination Procedure.
  To perform Swab and Rinse sampling followed by Testing using validated Analytical Method for the Detection of residue after cleaning.

- **Decontamination Report with Conclusion:** If the Phase III results complies as per acceptance criteria Final Decontamination Report shall be prepared by compiling all the activities performed and results obtained and facility shall be declared as **Decontaminated.**

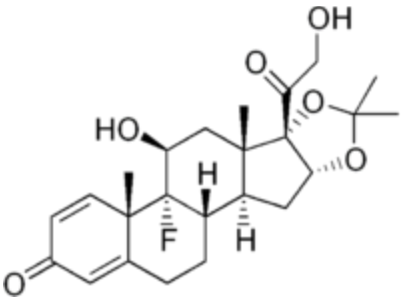
9.0 METHODOLOGY

9.1 SELECTION OF PRODUCT ON THE BASIS OF MAR VALUE:

For selection of worst case molecule from the list of all available (Manufacture in the facility) APIs, matrix has been prepared (Annexure IV & V).

Triamcinolone has been selected for decontamination purpose due to its lowest claim (0.025% or 0.25 mg) & lowest MAR value ie; 89.28 mg. If it is possible to decontaminate the lowest claim active upto lowest MAR value, then the upper claim actives will be cleaned automatically.

CHEMICAL STRUCTURE DETAILS:

PRODUCT NAME	TRIAMCINOLONE ACETONIDE IP
Synonyms	(4a*S*,4b*R*,5*S*,6a*S*,6b*S*,9a*R*,10a*S*,10b*S*)-4b-fluoro-6b-glycoloyl-5-hydroxy-4a,6a,8,8-tetramethyl-4a,4b,5,6,6a,6b,9a,10,10a,10b,11,12-dodecahydro-2*H*-naphtho[2',1':4,5]indeno[1,2-*d*][1,3]dioxol-2-one
Molecular formula	$C_{24}H_{31}FO_6$
Molecular weight	434.50 g·mol^{-1}
Category	Corticosteroid
Molecular structure	
Hazard symbols	

9.2 SIMULATION STUDY FOR STEROIDS DECOMPOSITION, DECONTAMINATING AGENT CONCENTRATION AS WELL AS CONTACT TIME:

- The objective of this experiments is to optimize the effective concentration and contact time of de contaminating agent (Sodiy.
- The experiments shall be initiated with the concentration of 2% & 5% Sodium Hypochlorite Solution. The suitable concentration will be considered based on the results.

(Sodium Hypochlorite solution)

Experiment 1:

Chemical & reagents used:

- **2% v/v Sodium Hypochlorite Solution:** Take 5 ml of 10% Sodium Hypochlorite Solution & dilute upto 25 ml with Purified water.
- **5% v/v Sodium Hypochlorite Solution:** Take 5 ml of 10% Sodium Hypochlorite Solution & dilute upto 10 ml with Purified water.

1: For Triamcinolone Acetonide

- **Step 1:** Take 30 mg of Triamcinolone Acetonide & dilute into 100 ml of diluent (90:10 of Methanol & Water). Take 5 ml of the above & make up to 100 ml with same diluent.
- **Step 2:** Take 5 ml of sample & mix 5 ml of 2% Sodium Hypochlorite Solution.
- **Step 3:** Take 5 ml of sample & mix 5 ml of 5% Sodium Hypochlorite Solution.
- **Step 4:** Run the sample on HPLC as per methodology (9.6.4).

2: For Clobetasol Propionate

- **Step 1:** Take 100 mg of Clobetasol Propionate & dilute into 100 ml of diluent (Methanol). Take 5 ml of the above & make up to 100 ml with same diluent.
- **Step 2:** Take 5 ml of sample & mix 5 ml of 2% Sodium Hypochlorite Solution.
- **Step 3:** Take 5 ml of sample & mix 5 ml of 5% Sodium Hypochlorite Solution.
- **Step 4:** Run the sample on HPLC as per methodology (9.6.4).

3: For Mometasone Furoate

- **Step 1:** Take 20 mg of Mometasone Furoate & dilute into 100 ml of diluent (90:10 of Methanol & Water). Take 5 ml of the above & make up to 50 ml with same diluent.
- **Step 2:** Take 5 ml of sample & mix 5 ml of 2% Sodium Hypochlorite Solution.
- **Step 3:** Take 5 ml of sample & mix 5 ml of 5% Sodium Hypochlorite Solution.
- **Step 4:** Run the sample on HPLC as per methodology (9.6.4).

CONTACT TIME STUDY: Triamcinolone/Mometasone

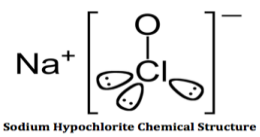
Variables	2% NaOCl Concentration	5% NaOCl Concentration
Contact Time (In Minutes)	2%	5%
15	Result Mention in report	Result Mention in report
30	Result Mention in report	Result Mention in report
60	Result Mention in report	Result Mention in report
90	Result Mention in report	Result Mention in report
120	Result Mention in report	Result Mention in report

Clobetasole:

Variables	2% NaOCl Concentration	5% NaOCl Concentration
Contact Time (In Minutes)	**2%**	**5%**
15	Result Mention in report	Result Mention in report
30	Result Mention in report	Result Mention in report
60	Result Mention in report	Result Mention in report
90	Result Mention in report	Result Mention in report
120	Result Mention in report	Result Mention in report

- Sample shall be withdrawn at 15, 30, 60, 90 & 120 minutes to quantify the residual concentration of Steroids for the effectiveness of decontaminating agent & contact time/exposure time.
- Based on the efficacy & effectiveness of decontaminating agent on all 03 Steroids (Triamcinolone/Clobetasol/Mometasone), exposure time & concentration shall be established for decontamination process.

SODIUM HYPOCHLORITE



Sodium Hypochlorite Chemical Structure

IUPAC NAME

Sodium hypochlorite

Other names

- Antiformin
- Bleach
- Chloride of soda

In dilution:

- Carrel-Dakin solution
- Modified Dakin's solution
- Surgical chlorinated soda solution

PROPERTIES	
Chemical formula	NaClO
Molar mass	74.442 g/mol
Appearance	greenish-yellow solid (Pentahydrate)
Odor	chlorine-like and sweetish
Density	1.11 g/cm^3
Melting point	18°C (64 °F; 291 K) Pentahydrate
Boiling point	101°C (214°F; 374 K) (decomposes)
Solubility in water	29.3 g/100mL (0°C)
Acidity (pK_a)	>7

9.3 SELECTION OF EQUIPMENTS: Equipment has been selected on the basis of its usage such as contact parts (Annexure III).

9.4 SELECTION OF SURFACE AREA:

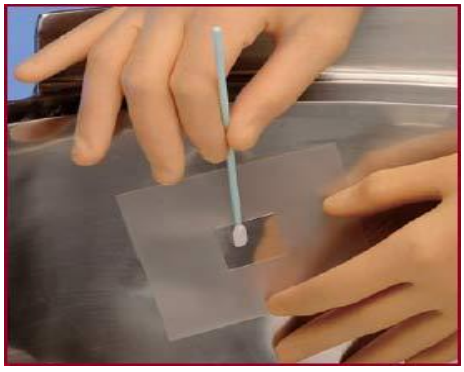
- Walls, Floors, Container, Vessel, Sampling and Dispensing accessories, Weighing Balance and Inner/outer surface of LAF, Return Riser, Supply Air of AHU's and Drain shall be mopped with Sodium Hypochlorite N% Solution (Annexure I).
- All containers shall be mopped with Sodium Hypochlorite N% solution.
- All the relevant User points of water system shall be mopped with Sodium Hypochlorite N% solution in respective areas.
- All the relevant drains of manufacturing area, QC area and warehouse shall be considered for sampling (Swab).

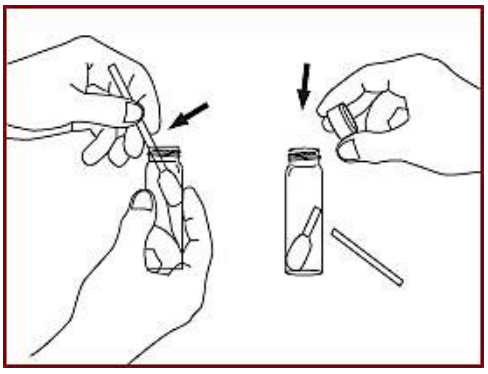
9.5 SELECTION OF SAMPLING METHOD (Swab & Rinse):

9.5.1 SWAB SAMPLING METHOD

- After visual inspection is found satisfactory swab sampling shall be carried out.
- Swab sample from inner and outer surface shall be considered from **"Hard to clean"** location of equipments as per sampling plan**.**
- Swab sample shall be collected as per template having dimension of 5cm x 5cm (25 cm^2).
- Do not rub the surface into to & fro motion.
- Put the swab into a clean tube and transfer to the quality control laboratory in a dry state.
- Swab samples for detection of residual Steroids should be collected from different locations as mentioned in sampling plan. Wherever required, sampling plan shall be supported by diagram.

Figure of Swab Stick





9.5.2 RINSE SAMPLING METHOD (Indirect sampling):

- Equipment or facility shall be cleaned as per Validated Cleaning Procedure.
- After visual inspection is found satisfactory rinse sampling shall be carried out.
- The identified equipment surface shall be rinsed and samples shall be collected for analysis. Wherever required, sampling plan shall be supported by diagram.
- In case of containers flush the equipments with predefined volume of Purified water as mentioned in cleaning procedure of equipments) for steroids take rinse sample from bottom discharge.
- Ensure complete surface is covered while rinsing.
- Ensure sufficient contact time to dissolve the residue.

9.6 SOLUBILITY STUDY:

Solubility of all 03 Steroids has been done in following mentioned solvents:

- Isopropyl Alcohol.
- Methanol.
- Methylene Dichloride
- Acetone
- 5M Hcl
- 5M NaOH.

The objective of this experiment is to find out the solubility of the all 03 Steroids in the above mentioned solvents. So after cleaning & moping further rinsing & moping with solvent can be done to remove the Steroids. On the basis of Experimental plan & solubility data (Annexure VI) it has been concluded that all these 03 Steroids having some solubility in Isopropyl Alcohol, hence on the basis of safety, efficacy & solubility IPA can be used for rinsing/moping after the cleaning process.

9.7 ANALYTICAL METHOD FOR DECONTAMINATION OF STEROIDS:

- **For Triamcinolone acetonide:**

S.No.	Chemicals/Reagents/Filters	Grade
1.	Water	Purified
2.	Acetonitrile	HPLC
3.	Methanol	HPLC
4	0.45 µm PVDF Pre-filter (Cat No. SYVG0602MNXX104)	-

Chromatographic conditions:

Mode	: HPLC
Column	: Inertsil ODS3, 250 mm x 4.6 mm, 5µm or Equivalent.
Column oven Temperature	: 25°C
Sampler temperature	: 10°C
Flow rate	: 1.0 ml/min
Detector	: UV 254nm
Injection size	: 20 µL
Run time	: 15 minutes
Retention time	: About 6 minute
Diluent	: Pre-filtered and degassed Methanol: Water in ratio 90:10 v/v

Mobile phase:

Prepare pre-filtered and degassed mixture of Water: Acetonitrile: Methanol (40:40:20) v/v

Triamcinolone Acetonide Solution:

Weigh accurately about 30 mg of Triamcinolone Acetonide and transfer into a 100 ml volumetric flask. Add 70 ml of methanol. Sonicate for about 5 minute, make up the volume with methanol and mix well.

Pipette out 5 ml of Triamcinolone Acetonide Standard stock solution, and transfer into 100 ml volumetric flask. Make up the volume with diluent and mix well. Filter through 0.45µm PVDF pre-filter.

(Concentration: 15 mcg/ml)

Procedure:

Equilibrate the HPLC column with mobile phase. Inject separately 20 µL of diluent as a blank, solution (Six replicates), into the chromatograph as per injection sequence given below. Record the chromatogram and measure the peak response of Triamcinolone Acetonide.

Injection sequence:

S.No.	Solution	No of Injections
1	Blank (Diluent)	1
2	Triamcinolone acetonide solution (6 replicates)	6
3	Triamcinolone acetonide solution with diluent	1
4	Triamcinolone acetonide sol with 2% Sod Hypo Sol at initial	1
5	Triamcinolone acetonide sol with 2% Sod Hypo Sol after 15 minute	1
6	Triamcinolone acetonide sol with 2% Sod Hypo Sol after 30 minute	1
7	Triamcinolone acetonide sol with 2% Sod Hypo Sol after 60 minute	1
8	Triamcinolone acetonide sol with 2% Sod Hypo Sol after 90 minute	1
9	Triamcinolone acetonide sol with 2% Sod Hypo Sol after 120 minute	1
10	Triamcinolone acetonide sol with 5% Sod Hypo Sol at initial	1
11	Triamcinolone acetonide sol with 5% Sod Hypo Sol after 15 minute	1
12	Triamcinolone acetonide sol with 5% Sod Hypo Sol after 30 minute	1
13	Triamcinolone acetonide sol with 5% Sod Hypo Sol after 60 minute	1
14	Triamcinolone acetonide sol with 5% Sod Hypo Sol after 90 minute	1
15	Triamcinolone acetonide sol with 5% Sod Hypo Sol after 120 minute	1
16	Std BKT	1

System Suitability:

i) % RSD of peak area due Triamcinolone Acetonide obtained from five replicate injections of Triamcinolone solution chromatograms should not be more than 2.

ii) The tailing factor for Triamcinolone Acetonide peak obtained Triamcinolone solution chromatogram should not be more than 2.0.

iii) The theoretical plates for Triamcinolone Acetonide peak obtained Triamcinolone solution chromatogram should not be less than 2000.

Calculations:

$$\% \text{ of Triamcinolone Acetonide} = \frac{AT \times WS \times 5 \times 5 \times 100 \times 100 \times 10 \times P}{AS \times 100 \times 100 \times 10 \times WT \times 5 \times 5 \times 100} \times 100$$

Where,

AT = Peak area due to Triamcinolone Acetonide in sample solution chromatogram.
AS = Average peak area due to Triamcinolone Acetonide in six replicates of mix standard solution chromatograms.
WS = Weight of Triamcinolone Acetonide working standard taken in mg.
P = % Potency of Triamcinolone Acetonide working standard on as is basis.
LC = Label Claim of Triamcinolone Acetonide in % w/w (0.10)
WT = Weight of sample taken in mg

- **ESTIMATION OF MOMETASONE FUROATE: By HPLC**

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with base deactivated endcapped octadecylsilane bonded to porous silica (5 pm) (such as Hypersil BDS C18) or equivalent
- column temperature: 60°
- mobile phase: a mixture of 45 volumes of water and 55 volumes of methanol,
- flow rate: 1 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume: 20 µl.
- methanol 80 per cent(80volume methanol :20 volume purified water)

Mometasone Furoate Solution: Weigh accurately 20 mg & transfer in 100 ml volumetric flask, add 50 ml methanol (80 per cent), shake to dissolve and make the volume 100 ml with methanol (80 per cent). Further dilute 5 ml to 50 ml with methanol (80 per cent).

(Concentration: 20 mcg/ml)

Procedure: Separately inject 20 µl of Mometasone Furoate solution (five replicate) into liquid chromatography and record the chromatograms. Measure the responses for major peak areas of Mometasone Furoate solution.

S.No.	Description	No. of Injections
1	Blank	01
2	Mometasone standard Solution (6 replicates)	06
3	Mometasone standard solution with diluent	01
4	Mometasone standard sol with 2% Sod Hypo Sol at initial	01
5	Mometasone standard sol with 2% Sod Hypo Sol after 15 minute	01
6	Mometasone standard sol with 2% Sod Hypo Sol after 30 minute	01
7	Mometasone standard sol with 2% Sod Hypo Sol after 60 minute	01
8	Mometasone standard sol with 2% Sod Hypo Sol after 90 minute	01
9	Mometasone standard sol with 2% Sod Hypo Sol after 120 minute	01
10	Mometasone standard sol with 5% Sod Hypo Sol at initial	01
11	Mometasone standard sol with 5% Sod Hypo Sol after 15 minute	01
12	Mometasone standard sol with 5% Sod Hypo Sol after 30 minute	01
13	Mometasone standard sol with 5% Sod Hypo Sol after 60 minute	01
14	Mometasone standard sol with 5% Sod Hypo Sol after 90 minute	01
15	Mometasone standard sol with 5% Sod Hypo Sol after 120 minute	01
16	Bracketing Standard	01

System suitability: The test is not valid unless the tailing factor is not more than 2.The column efficiency in not less than 1000 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Calculation : Mometasone Furoate

$$\% \text{ of Mometasone} = \frac{AT \times WS \times 5 \times 5 \times 100 \times 50 \times 10 \times P}{AS \times 100 \times 50 \times 10 \times WT \times 5 \times 5 \times 100}$$

Where

At = Average area of test preparation Mometasone Furoate
As = Average area of standard preparation of Mometasone Furoate
Ws = Weight of standard Mometasone Furoate
Wt = Weight of test sample
P =Purity of Mometasone Furoate working standard.

- **ESTIMATION OF CLOBETASOL PROPIONATE: By HPLC**

Equipment Required:

HPLC
Electronic Balance
Ultrasonic cleaner
Glass wares required:
Volumetric Flask
Bulb Pipette
Beaker

Reagent Required:
Dipotassium Hydrogen Phosphate
O-Phosphoric Acid.
Acetonitrile (HPLC grade)
Methanol (HPLC grade)
Water

Working standard:
Terbinafine Hydrochloride BP
Clobetasol Propionate BP

Chromatographic Conditions:

Column	**:** C18 (ODS-3V), 150 cm x 4.6 mm, 5-µ (Inertsil GL Sciences Inc.)
Flow rate	**:** 2.0 ml/min
Wave length	**:** 240 nm
Injection Volume	**:** 50 µl
Column temperature	**:** 40°C

Procedure:
Buffer : Weight accurately 1.74 gm Dipotassium Hydrogen Phosphate in 900 ml water and dissolve and adjusted to pH 7.5 with phosphoric acid and make up the volume up to 1000 ml.

Mobile Phase (A): Acetonitrile

Mobile Phase (B): A degassed and filtered 1.74 g/l solution of dipotassium hydrogen phosphate previously adjusted to pH 7.5 with phosphoric acid.

Mobile Phase (D): Methanol

Run the instrument as per gradient program

Time	Flow	%A	% B	%C	%D
0.01	2.0	45.0	40.0	0.0	15.0
7.00	2.0	45.0	40.0	0.0	15.0
7.01	2.0	70.0	20.0	0.0	10.0
18.00	2.0	70.0	20.0	0.0	10.0
18.01	2.0	45.0	40.0	0.0	15.0
21.0	2.0	45.0	40.0	0.0	15.0

Clobetasol Propionate Solution: Weigh accurately 100 mg of Clobetasol Propionate and transfer in to a 100 ml volumetric flask, add 50 ml of Methanol shake to dissolve and dilute to 100 ml with Methanol. Dilute 5 ml to 100 ml with Methanol. Dilute 10 ml of solution in 50 ml with Methanol.

(Concentration : Clobetasol Propionate 10 mcg/ml)

Procedure: Separately inject 50 µl of standard preparation and test preparation into liquid chromatography and record the chromatograms. Measure the responses for major peak areas of standard & test preparation from percent potency of working standard used.

S.No.	Description	No. of Injections
1	Blank	01
2	Clobetasol Propionate Solution (5 replicates)	05
3	Clobetasol Propionate solution with diluents	01
4	Clobetasol Propionate with 2% Sod Hypo Sol at initial	01
5	Clobetasol Propionate with 2% Sod Hypo Sol at 40 minute	01
6	Clobetasol Propionate with 2% Sod Hypo Sol after 60 minute	01
7	Clobetasol Propionate with 2% Sod Hypo Sol after 120 minute	01
8	Clobetasol Propionate with 5% Sod Hypo Sol at initial	01
9	Clobetasol Propionate with 5% Sod Hypo Sol at 40 minute	01
10	Clobetasol Propionate with 5% Sod Hypo Sol after 60 minute	01
11	Clobetasol Propionate with 5% Sod Hypo Sol after 120 minute	01
12	Bracketing Standard	01

System suitability: The test is not valid unless the tailing factor is not more than 2.0.The column efficiency in not less than 1000 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

For Clobetasol Propionate

$$= \frac{At}{As} \times \frac{Ws}{100} \times \frac{5}{100} \times \frac{10}{50} \times \frac{100}{Wt} \times \frac{100}{5} \times \frac{50}{10} \times \frac{P}{100} \times 100 = \% \text{ W/W}$$

Where

At = Average area of test preparation Clobetasol Propionate
As = Average area of standard preparation of Clobetasol Propionate
Ws = Weight of standard Clobetasol Propionate
Wt = Weight of test sample
P =Purity of Clobetasol Propionate standard.

9.8 RECOVERY STUDY LOQ DETERMINATION:

- Based on MAR value & solubility, Triamcinolone has been considered for worst case product and analytical method validation for the LOQ to be performed with Triamcinolone.
- Recovey study for Swab shall be performed with Triamcinolone on below mentioned surfaces:

Swab recovery for residual Steroid shall be calculated on different surfaces by spiking the surfaces with known quantity of Steroid. To identify different Product Contact Surfaces eg. **1. SS 316 L Sheet** **2. Teflon Sheet** **3. GI Sheet** **4. Alupan sheet**	To prepare the solution of (Solution A) in the suitable solvent as per establishment of LOQ. To spray 1 ml of Solution A on 100 sq. cm surface of individual type as identified above. To allow it to dry completely. To swab the surface using Swab stick as per validated procedure. To dissolve the swab in 10 ml of the suitable solvent. To analyze the swab as per validated analytical procedure. To calculate the percentage recovery (NLT 70 %).

Note: Recovery sample concentration shall be established based on the LOQ.
Acceptance Criteria: Minimum 70 % Recovery is acceptable.
The swab technique is suitable for the easy to swab plain surface preferably 100 sq. cm.

9.9 TRACES OF SODIUM HYPOCHLORITE (AFTER FINAL CLEANING WITH PURIFIED WATER):

- **SWAB SAMPLE:**

Take swab sample in a 20 ml volumetric flask and add 10 ml of purified water mix well and Transfer sample in a glass-Stopperd flask containing a solution of 300 of potassium iodide in 100 ml of water, add 20 ml of dilute acetic acid and titrate the liberated iodine with 0.01 M Sodium Thiosulphate using starch solution, added towards the end of the titration, as indicator. 1 ml of 0.01 M Sodium Thiosulphate is equivalent to 0.003546 g of available chlorine.

% of Sodium Hypochlorite =	**Burette Reading**	**X**	**0.0003546**	**X**	**Normality of 0.01M Sodium Thiosulphate**	**X**	**100**
	0.01			**x**	**20 ml**		

- **RINSE SAMPLE:**

Transfer 20 ml of Rinse sample in a glass-Stopperd flask containing a solution of 300 mg of potassium iodide in 100 ml of water, add 2 ml of dilute acetic acid and titrate the liberated iodine with 0.01 M Sodium Thiosulphate using starch solution, added towards the end of the titration, as indicator.

1 ml of 0.01 M Sodium Thiosulphate is equivalent to 0.0003546 g of available chlorine.

10.0 DECONTAMINATION PROCEDURE:

10.1 Decontamination of AHU's:

- **Pre Cleaning:** Clean the Panels, Doors and Filters of AHUs as per the Cleaning SOP & finally mop with IPA.
- Clean the Ducting using vacuum cleaner then mop with 2% Extran MA02 followed by moping with purified water using long handled wipers/mop & finally moping with IPA .
- Clean and Sanitize the Service Floor where the AHUs are installed.
- **Filters Replacement:** Remove the existing Filters. Replace the Pre filters and HEPA Filters as per the validated SOP after decontamination.
- **Decontamination:** Take the Sodium Hypochlorite solution of desired concentration in the suitable spray gun and uniformly spray on the internal and external surface.
- Ensure that the entire surface is covered by the spray.
- Allow the Sodium Hypochlorite solution to remain on the surface for the validated contact time.
- **Post Cleaning:** Clean the surface with Purified Water and Wipe out with lint free cloth (Clean Room Wipes) to remove the Sodium Hypochlorite residues on the surface.
- Test for the Sodium Hypochlorite residues on the surface.
- Collect the swab & rinse sample as applicable.
- **Sanitization:** Sanitize the AHU surface as per the validated procedure.
- Record the activity with Start Time and End Time.

10.2 Decontamination of Walls, View Panels, Doors and False Ceiling:

- **Pre Cleaning:** Clean the Walls, View Panels, Doors and False Ceiling as per the Cleaning SOP finally mop with IPA.
- **Decontamination:** Take the Sodium Hypochlorite solution of desired **validated** concentration in the suitable spray gun and uniformly spray on the surface. Sodium Hypochlorite solution shall be prepared taking necessary precautions
- Ensure that the entire surface is covered by the spray.
- Allow the Sodium Hypochlorite solution to remain on the surface for the **validated** contact time.
- **Post Cleaning:** Clean the surface with WFI and Wipe out with lint free cloth (Clean Room Wipes) to remove the Sodium Hypochlorite residues on the surface. **Observe for any impact on the surface.**
- Test for the Sodium Hypochlorite residues on the surface. Test pH of the Rinse shall be between 5-7 pH.
- Collect the swab and rinse sample as applicable.
- **Sanitization:** Sanitize the surface as per the validated procedure
- Record the activity with Start Time and End Time.

10.3 Decontamination of LAF:

- Switch off the blower of LAF.
- **Pre Cleaning:** Clean the LAF as per the Cleaning SOP & finally mop with IPA.
- **Remove the Filters: Decontamination:** Take the Sodium hypochlorite solution of desired concentration in the suitable spray gun and uniformly spray on the internal and external surface.
- Ensure that the entire surface is covered by the spray.'

- Allow the Sodium Hypochlorite solution on the surface for the validated contact time.
- **Post Cleaning:** Clean the surface with Purified Water and Wipe out with lint free cloth (Clean Room Wipes) to remove the Sodium Hypochlorite Solution on the surface.
- Test for the Sodium Hypochlorite residues on the surface.
- Collect the swab and rinse sample as applicable.
- **Sanitization:** Wipe the external surface of pre filter grill with lint free cloth moistened with IPA. (To be filtered through 0.2 micron filter as per SOP).
- Wipe the outer body of LAF hood with lint free cloth moistened with IPA.
- Install the new Filters as per SOP.
- Start the blower of LAF station after cleaning.
- Record the activity with Start Time and End Time.

10.4 Decontamination of Equipment Surface (Applicable to all Equipment):

- **Pre Cleaning:** Wash and Clean all the Equipment as per the Cleaning SOP & finally mop with IPA.
- **Decontamination:** Take the Sodium Hypochlorite solution of desired concentration in the suitable spray gun and uniformly spray on the internal and external surface.
- Ensure that the entire surface of equipment is covered by the spray.
- Allow the Sodium Hypochlorite solution on the surface for the validated contact time.
- **Post Cleaning:** Clean the surface with Purified Water and Wipe out with lint free cloth (Clean Room Wipes) to remove the Sodium Hypochlorite solution on the surface.
- Test for the Sodium Hypochlorite solution residues on the surface.
- Collect the swab and rinse sample as applicable.
- **Sanitization:** Wipe the external surface of Equipment with lint free cloth moistened with IPA.
- Record the activity with Start Time and End Time.

10.5 Decontamination of Equipment Change Parts

- **Pre Cleaning:** Wash and Clean all the change parts as per the Cleaning SOP & finally dipped in IPA.
- **Decontamination:** Take the Sodium Hypochlorite solution of desired concentration in the suitable spray gun and uniformly spray on the surface.
- Ensure that the entire surface of Change Parts is covered by the spray. Wherever necessary dip the change parts in the Sodium Hypochlorite solution of the desired concentration.
- Allow the Sodium Hypochlorite solution on the surface for the validated contact time.
- **Post Cleaning:** Clean the surface with Purified Water and Wipe out with lint free cloth (Clean Room Wipes) to remove the Sodium Hypochlorite solution on the surface.
- Test for the Sodium Hypochlorite residues on the surface.
- Collect the swab and rinse sample as applicable.
- **Sanitization:** Wipe the Change Parts with lint free cloth moistened with IPA.
- Record the activity with Start Time and End Time.

10.6 Decontamination of Instruments

- **Pre Cleaning:** Clean all the Instrument as per Cleaning SOP e.g. Balance & finally mop with IPA.
- **Decontamination:** Take the Sodium Hypochlorite solution of desired concentration and apply uniformly on the internal and External surface using a lint free cloth.

- Ensure that the entire surface of Instrument is covered.
- Allow the Sodium Hypochlorite solution on the surface for the validated contact time.
- **Post Cleaning:** Clean the surface with WFI and Wipe out with lint free cloth (Clean Room Wipes) to remove the Sodium Hypochlorite on the surface.
- Test for the Sodium Hypochlorite residues on the surface.
- Collect the swab and rinse sample as applicable.
- **Sanitization:** Wipe the surface of the instruments with lint free cloth moistened with IPA.
- Record the activity with Start Time and End Time.

10.7 Decontamination of the Utility Lines

- Perform the decontamination of the Utility lines and Pendents exposed in the manufacturing block
- **Pre Cleaning:** Clean all the Instrument as per Cleaning SOP & finally mop with IPA.
- **Decontamination:** Take the Sodium Hypochlorite solution of desired concentration and apply uniformly on the surface using a lint free cloth.
- Ensure that the entire surface of the utility lines and pendents are covered.
- Allow the Sodium Hypochlorite solution on the surface for the validated contact time.
- **Post Cleaning:** Clean the surface with WFI and Wipe out with lint free cloth (Clean Room Wipes) to remove the Sodium Hypochlorite on the surface.
- Test for the Sodium Hypochlorite residues on the surface
- Collect the swab and rinse sample as applicable.
- **Sanitization:** Wipe the surface of the utility lines and pendents with lint free cloth moistened with IPA.
- Record the activity with Start Time and End Time.

10.8 Decontamination of the drainages:

- **Pre Cleaning:** Clean all the Drain points as per Cleaning SOP & finally drain with IPA.
- **Decontamination:** Take the Sodium Hypochlorite solution of N% concentration and pour in the drain. Keep the same for minimum 2 hrs.
- **Post Cleaning:** Drain with sufficient PW.
- **Sanitization:** Pour the sufficient volume of validated disinfectant and hold it overnight.

11.0 ACCEPTANCE CRITERIA:

All the samples of Swab and Rinse shall be analyzed for residual steroids & residue of decontaminating agent as per the validated analytical Method.

Acceptance criteria for Steroids:	Below Then Detection Limit (LOD).
Acceptance criteria for Decontaminating Agent:	Should be absent.
Acceptance criteria for pH in rinse sample:	5.0-7.0

12.0 DEVIATIONS:

All Protocol Deviation, Non conformances and out of specification results obtained shall be investigated in Accordance with corresponding SOPs and documents.

13.0 CONCLUSION:

Based on the outcome of the data all result swab/rinse shall be compiled in the form of study Report.

14.0 REFERENCES:

- Indian Pharmacopoeia 2014.
- Quality Assurance of Pharmaceuticals. A compendium of guidelines and related materials, Volume 2, Second Updated Edition. Good manufacturing practices and inspection. Geneva, World Health Organization, 2007.
- World Health Organization WHO Technical Report Series, No. 957, 2010 Annex 3 WHO good manufacturing practices for pharmaceutical products containing hazardous substances.

15.0 ABBREVIATIONS:

ppm	:	Parts per million
HPLC	:	High Performance Liquid Chromatography
ml	:	milliliter
mg	:	milligram
LOD	:	Limit of Detection
LOQ	:	Limit of Quantification
API	:	Active Pharmaceutical Ingredient
M	:	Molar
µ	:	Micron
µl	:	Microliter
WS	:	Working Standard
QA	:	Quality Assurance
QC	:	Quality control
cm	:	Centimeter
SOP	:	Standard Operating Procedure

16.0 ANNEXURES:

ANNEXURE - I

SAMPLING LOCATIONS FOR DECONTAMINATION OF STEROIDS

S.No.	Name of equipment	Sampling of location	No. of sampling for Steroidal Residual analysis	No. of Sampling for Decontaminating agent residue	Sampling location no.
EQUIPMENT DECONTAMINATION LOCATION:					
1.	Oil base mixture vessel	Inner surface	1	1	Location-01
		Equipment lid	1	1	Location-02
2.	Water base melting vessel	Equipment lid	1	1	Location-03
		Stirrer small blades	1	1	Location-04
		Inner surface	1	1	Location-05
3.	Multi mixture equipment	Hopper contact surface	1	1	Location-06
		Teflon scraper	1	1	Location-07
		Discharge valve	1	1	Location-08
4.	Dispensing tools & transfer equipment	S.S container surface	1	1	Location-09
		Ladle	1	1	Location-10
5.	Holding vessel	Holding tank contact surface	1	1	Location-11
		Holding tank outlet	1	1	Location-12
6.	Tube filling sealing machine hopper	Hopper lid	1	1	Location-13
		Hopper curved (Near chute) area	1	1	Location-14
7.	Tube filling sealing machine filling piston	Piston-1	1	1	Location-15
8.	Tube filling & sealing machine Nozzle	Nozzle-1	1	1	Location-16
9.	Bulk transfer ling (holding tank to filling machine hopper)	S.S pipe after circulation pump	1	1	Location-17
		Hose pipe after piston	1	1	Location-18
10.	Tube filling sealing machine S.S container	Container contact surface	1	1	Location-19
DISPENSING AREA DECONTAMINATION LOCATION:					
11.	Wall	At corner	1	1	Location-20
12.	Ceiling	At corner	1	1	Location-21
13.	Epoxy Floor	At corner	1	1	Location-22
14.	Pellets	At bottom	1	1	Location-23
15.	Laminar Air flow	At corner	1	1	Location-24
16.	Dynamic Pass box	Back	1	1	Location-25

S.No.	Name of equipment	Sampling of location	No. of sampling for Steroidal Residual analysis	No. of Sampling for Decontaminating agent residue	Sampling location no.
17.	Balance	Top	1	1	Location-26
18.	Return Riser	At front side	1	1	Location-27
MANUFACTURING AREA DECONTAMINATION LOCATIONS:					
19.	Wall	At corner	1	1	Location-28
20.	Ceiling	At corner	1	1	Location-29
21.	Epoxy Floor	At corner	1	1	Location-30
22.	Utility	Purified Water user point	1	1	Location-31
23.	Drain	Inlet	1	1	Location-32
24.	Balance	Top	1	1	Location-33
25.	Dynamic Pass Box	Back	1	1	Location-34
26.	Return Riser	At front side	1	1	Location-35
HOLDING TANK AREA DECONTAMINATION LOCATIONS:					
27.	Wall	At corner	1	1	Location-36
28.	Ceiling	At corner	1	1	Location-37
29.	Epoxy Floor	At corner	1	1	Location-38
30.	Light fixture	At the edges	1	1	Location-39
31.	Return Riser	At front side	1	1	Location-40
WASHING AREA DECONTAMINATION LOCATIONS:					
32.	Wall	At corner	1	1	Location-41
33.	Ceiling corners	At corner	1	1	Location-42
34.	Epoxy Floor	At corner	1	1	Location-43
35.	Door corners	At the edge near wall	1	1	Location-44
36.	Utility	Purified Water user point	1	1	Location-45
37.	Drain	At inlet	1	1	Location-46
38.	Return Riser	At front side	1	1	Location-47
FILLING AREA DECONTAMINATION LOCATIONS:					
39.	Wall	At corner	1	1	Location-48
40.	Ceiling corners	At corner	1	1	Location-49
41.	Epoxy Floor	At corner	1	1	Location-50
42.	Utility	Purified Water	1	1	Location-51
43.	Drain	At inlet	1	1	Location-52
44.	Return Riser	At the front side	1	1	Location-53
SERVICE FLOOR AREA DECONTAMINATION LOCATIONS:					
45.	Wall	Near Return air	1	1	Location-54
46.	Floor	Near AHU	1	1	Location-55
47.	Ceiling	At corner	1	1	Location-56
48.	AHU Pre-filter	On front side	1	1	Location-57
49.	Bleed damper	On front side	1	1	Location-58

S.No.	Name of equipment	Sampling of location	No. of sampling for Steroidal Residual analysis	No. of Sampling for Decontaminating agent residue	Sampling location no.
50.	**Exhaust damper**	**On front side**	1	1	Location-59
51.	**Duct**	**At corner**	1	1	Location-60
52.	**Garments**	**On surface**	1	1	Location-61

Sampling locations has been selected on the basis of worst case (hard to clean & hard to reach). Sampling location has been divided into following categories: Equipment decontamination location, Dispensing area decontamination locations, Manufacturing area decontamination location, Holding Tank area decontamination location, washing area decontamination location, Filling area decontamination location & Service area decontamination location.

ANNEXURE II

SOP OF CLEANING PROCEDURES

S.No.	NAME	SOP No.
1.	Operation & Cleaning of Multi-mixer vessel	
2.	Operation and Cleaning of Six Head Volumetric Filling Machine	
3.	Cleaning & Sanitization of Transfer Pump, SS Pipes & Hose Pipes	
4.	Cleaning & sanitization of Holding Vessels	
5.	Operation & Cleaning of Static Pass Box	
6.	Procedure of Cleaning of Container & Utensils	
7.	Cleaning of Pellets, Crates, Racks & Trolley	
8.	Operation & Cleaning of Dynamic Pass Box	
9.	Cleaning & Sanitization of Production Core area	
10.	Cleaning & Sanitization of Drains in manufacturing area	

ANNEXURE III

EQUIPMENTS USED FOR STEROIDAL PREPARATIONS

S.No.	Name of Equipment	Equipment ID
1.	Multi Mixer 250 kg with load cell and transfer pump	
2.	Wax Phase Melting Vessel	
3.	Water Phase Melting Vessel	
4.	Holding Vessel	
5.	SS Container	
6.	Balance	
7.	Bulk Transfer Line (Holding to Filling Machine Hopper)	
8.	Tube filling Sealing machine	
9.	RLAF	

ANNEXURE IV

MATRIX ON THE BASIS OF POTENCY & SOLUBILITY ALONG WITH MDD

S.No.	Steroid Active pharmaceutical Ingredients	Category	Strength (%)	Water Solubility	Ease to clean (Stickiness), Nature of molecule (Waxy Solid, Powder, Liquid)	Batch Size (kg)		STD (FTU)	LDD (FTU)	Avg. Wgt. (mg)	MDD = LDD*Avg. wgt. (mg)
						Min.	Max.				
1.	**Triamcinolone Oromucosal paste BP 0.1% w/w**	Corticosteroid	0.025%	Practically Insoluble	Crystalline Powder (Light Sensitive)	200	500	1	4	1000	4000
2.	**Ofloxacin, Terbinafine & Clobetasol Cream**	Glucocorticoid	0.05%	Practically Insoluble	Crystalline Powder (Light Sensitive)	200	500	1	14	1000	14000
3.	**Mometasone Furoate IP 0.1% w/w**	Glucocorticoid	0.1%	Practically Insoluble	Powder	200	500	1	1	1000	1000

ANNEXURE V

MATRIX FOR MAR VALUE

S.No.	Product A	SFG No.	API	STD (mg)	Product B	Triamcinolone Acetonide IP	Clobetasol Propionate IP	Mometasone Furoate IP
					SFG No.	-	-	-
					Batch Size of Product B	500	500	**500**
					MDD	4000	14000	**1000**
1.	**Triamcinolone Oromucosal paste BP 0.1% w/w**	-	**Triamcinolone Acetonide IP**	0.25	MDD (Dose Units x Avg. Wgt.) Product B	NA	89.28	1250
2.	**Ofloxacin, Terbinafine & Clobetasol Cream**	-	**Clobetasol Propionate IP**	0.5		625	NA	2500
3.	**Mometasone Furoate IP 0.1% w/w**	-	**Mometasone Furoate IP**	1		1250	357	NA

Triamcinolone Acetonide has been selected as the worst case on the basis of Potency & its dosage form as it is used as Oromucosal paste while other steroids are topically used.

$$\text{MAR VALUE} = \frac{\text{STD (Product A) x Maximum Batch Size (Product B)}}{\text{Safety Factor (100) x MDD (Product B)}}$$

ANNEXURE VI

SOLUBILITY IN SOLVENTS

S.No.	Steroid	SOLUBILITY					
		IPA	Methanol	MDC	Acetone	5M HCL	5M NaOH
1.	**Triamcinolone Oromucosal BP**	Slightly Soluble	Sparingly Soluble	Slightly Soluble	Slightly Soluble	Insoluble	Insoluble
2.	**Clobetasol**	Soluble	Soluble	Soluble	Freely Soluble	Insoluble	Insoluble
3.	**Mometasone Furoate IP**	Slightly Soluble	Soluble	Soluble	Soluble	Insoluble	Insoluble

As all 03 Steroids are practically insoluble in water, hence dilution has been tried for other solvents also. On the basis of solubility, IPA has been Selected for final cleaning purpose & after that decontamination process to be started.