

www.pharmaerudition.org

ISSN: 2249-3875



# International Journal of Pharmaceutical Erudition

Research for Present and Next Generation

**FEB. 2018**

Vol: 07 Issue:04  
(81-98)





## Research Paper

# PROCESS VALIDATION OF MANUFACTURING OF CALCITRIOL OINTMENT

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Assurance of product quality is derived from careful attention to a number of factors including selection of quality parts and materials, adequate product and process design, control of the process, and in-process and end-product testing. Process validation establishes the flexibility and constraints in the manufacturing process controls in the attainment of desirable attributes in the drug products while preventing undesirable properties. This is an important concept, since it serves to support the underlying definition of validation, which is a systematic approach for identifying, measuring, evaluating, documenting, and re-evaluating a series of critical steps in the manufacturing process that require control to ensure a reproducible final product.

**Key words:** Process validation , calcitriol, ointment, validation

## INTRODUCTION

“The validation is taken up for the generation of sufficient data there by establishing documentary evidence that the products manufactured at commercial scale, meet all quality attribution in consistent manner”.

Once the concept of being able to predict process performance to met user requirements evolved, FDA regulatory official established that there was a legal basis for requiring process validation. The ultimate legal authority is section 501(a)(2)(B) of the FD&C Act, which states that a drug is deemed to be adulterated if the method used in, or the facilities or controls used for, its manufacture, processing packing, or holding do not conform to or were not operated or administrated in conformity with cGMP. Assurance must be given that the drug would meet the requirement of the act as to safety and would have the identity and strength and meet the quality and purity characteristic that it purport or

was represented to possess. That section of the act sets the premise for process validation requirement for both finished pharmaceuticals and active pharmaceutical ingredients, because active pharmaceutical ingredients are also deemed to be drugs under the act.

The cGMP regulations for finish pharmaceuticals, 21 CFR 210 and 211, were promulgated to enforce the requirements of the act. Although these regulations do not include a definition for process validation, the requirement is implicit in the language of 21 CFR 211.100, which states; "There shall be written procedures for production and process control designed to assure that the drug products have the identity, strength, quality, and purity they purport or are represented to possess."

Validation of nonsterile semisolid ointment have several properties ,such as homogenization, mixing, viscosity, uniformity content, minimum fill,



free from microorganism and extremely high standards of purity and quality.

In pharmaceutical industry several approaches have been exists validation of manufacturing area and equipments for different dosage forms. According to current Good manufacturing practice the type of formulation which the manufacture is going to produce require stringency in terms of area, equipment and facilities. In present scenario the manufacture should produce all the document related to facility, equipment and their validation. Information generated during the development stage should thus be used to identify and evaluate the critical pharmaceutical parameters which may need to be examined and possibly controlled in order to ensure batch to batch responsibility. In order to define these critical parameters it may be necessary to challenge the process by making deliberate change to demonstrate the robustness of the process and define the limit of tolerance. Such parameters will very depending upon the nature of the product, the composition and the proposed method of manufacture, the choice of the method should be properly justified in the context of the development data obtained.

### **Type of Process Validation**

#### **Prospective Validation**

In Prospective Validation, the validation protocol is executed before the process is put into commercial use. During the product development phase the production process should be broken down into individual steps. Each step should be evaluated on

the basis of experience or theoretical considerations to determine the critical parameters that may affect the quality of the finished product. A series of experiments should be designed to determine the criticality of these factors. Each experiment should be planned and documented fully in an authorized protocol. All equipment, production environment and the analytical testing methods to be used should have been fully validated. Master batch documents can be prepared only after the critical parameters of the process have been identified and machine settings, component specifications and environmental conditions have been determined.

#### **Concurrent Validation**

Concurrent validation may be the practical approach under certain circumstances.

Examples of these may be when:

- A previously validated process is being transferred to a third party contract manufacturer or to another manufacturing site.
- The product is a different strength of a previously validated product with the same ratio of active/inactive ingredients.
- The number of lots evaluated under the Retrospective Validation were not sufficient to obtain a high degree of assurance demonstrating that the process is fully under control.
- The number of batches produced are limited (e.g. orphan drugs).
- Process with low production volume per batch ( e.g. radiopharmaceuticals, anticancer).
- Process of manufacturing urgently needed



drugs due to shortage (or absence) of supply.

It is important in these cases however, that the systems and equipment to be used have been fully validated previously. The justification for conducting concurrent validation must be documented and the protocol must be approved by the Validation Team. A report should be prepared and approved prior to the sale of each batch and a final report should be prepared and approved after the completion of all concurrent batches. It is generally considered acceptable that a minimum of three consecutive batches within the finally agreed parameters, giving the product the desired quality would constitute a proper validation of the process.

### Retrospective Validation

In many establishments, processes that are stable and in routine use have not undergone a formally documented validation process. Historical data may be utilized to provide necessary documentary evidence that the processes are validated.

The steps involved in this type of validation still require the preparation of a protocol, the reporting of the results of the data review, leading to a conclusion and recommendation.

Retrospective validation is only acceptable for well established detailed processes that include operational limits for each critical step of the process and will be inappropriate where there have been recent changes in the formulation of the product, operating procedures, equipment and facility.

Some of the essential elements for Retrospective Validation are:

- Batches manufactured for a defined period (minimum of 10 last consecutive batches).
- Number of lots released per year.
- Batch size/strength/manufacturer/year/period.
- Master manufacturing/packaging documents.
- Current specifications for active materials/finished products.
- List of process deviations, corrective actions and changes to manufacturing documents.
- Data for stability testing for several batches.
- Trend analyses including those for quality related complaints.

### Process Re-Validation

Re-validation is usually performed to the confirmation of initial validation for a periodic review. Re-validation provides the evidence that changes in a process and /or the process environment that are introduced do not adversely affect process characteristics and product quality. Documentation requirements will be the same as for the initial validation of the process. Re-validation becomes necessary in certain situations.

The following are examples of some of the planned or unplanned changes that may require re-validation:

- Changes in raw materials (physical properties such as density, viscosity, particle size distribution, and moisture, etc., that may affect the process or product).
- Changes in the source of active raw material manufacturer.
- Changes in packaging material (primary container/closure system).



- Changes in the process (e.g., mixing time, drying temperatures and batch size).
- Changes in the equipment (e.g. addition of automatic detection system). Changes of equipment which involve the replacement of equipment on a “like for like” basis would not normally require a re-validation except that this new equipment must be qualified.
- Changes in the plant/facility.
- Variations revealed by trend analysis (e.g. process drifts). A decision not to perform re-validation studies must be fully justified and documented.

## DRUG PROFILE

### Drug Substance

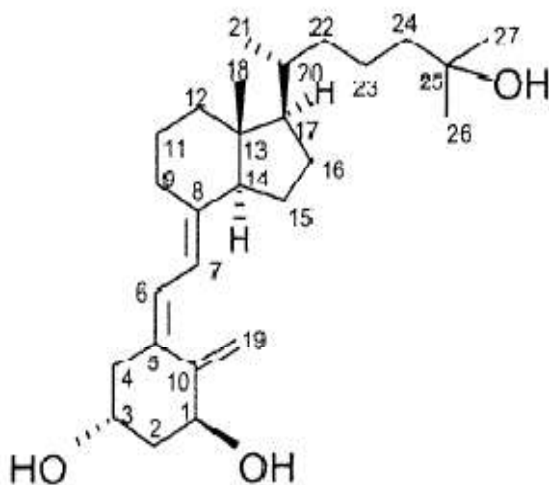
Proper name: Calcitriol

Chemical name: (5Z, 7E)-9, 10-secocholesta-5, 7, 10(19)-triene-1 $\alpha$ , 3 $\beta$ , 25-triol (1 $\alpha$ , 3 $\beta$ , 5Z, 7E)-9, 10-secocholesta-5, 7 10(19)-triene-1, 3, 25-triol

Molecular formula: C<sub>27</sub>H<sub>44</sub>O<sub>3</sub>

Molecular mass: 416.6

Structural formula



**Physicochemical properties:** White to almost white crystalline powder. Air, heat, and light sensitive. Calcitriol is practically insoluble in water, freely soluble in alcohol, and soluble in fatty oils.

### Mechanism of Action

Calcitriol (1 $\alpha$ -25-dihydroxyvitamin D<sub>3</sub>) is the naturally occurring and biologically active metabolite of vitamin D<sub>3</sub>, primarily produced in the skin by exposure to the ultraviolet rays of the sun. Vitamin D<sub>3</sub> must be metabolically activated in the liver and the kidney before it is fully active at target tissues as calcitriol.

Calcitriol primarily regulates systemic calcium and phosphate homeostasis by effects on the gastrointestinal tract, bone, and kidney. The mechanism of action of calcitriol in the treatment of psoriasis has not been established.

### Pharmacokinetics

The systemic exposure of calcitriol ointment 3  $\mu$ g/g was assessed in 23 patients with chronic plaque psoriasis over 21 days with application to 35% body surface area. At Day 21, the geometric mean plasma concentration values of C<sub>max</sub> increased by 36% over baseline and the geometric mean value of AUC(0-12hr) increased by 44%. There was no correlation between the observed elevated calcitriol levels and the pharmacodynamic parameters of serum albumin adjusted calcium, serum phosphorus, urinary calcium and urinary phosphorus.

### Dosage Forms, Composition and Packaging

Calcitriol ointment 3 $\mu$ g/g is available in collapsible aluminium tubes coated internally with an epoxy-



phenolic resin and fitted with a white high density polyethylene or polypropylene screw cap. Tubes contain 5 g or 60 g of ointment.

Calcitriol is a white, translucent, ointment containing 3 µg/g (0.0003% w/w) of calcitriol. Other components of the ointment are vitamin E (dl-α tocopherol) added as an antioxidant, mineral oil, and white petrolatum.

### Dosage and Administration

- Calcitriol is for TOPICAL USE ONLY and not for oral, ophthalmic or intravaginal use.
- There is no clinical trial experience with the use of calcitriol in children and there is limited experience with the use of in the elderly.
- Calcitriol should be used in pregnant or nursing women only if the benefit versus risk is favorable.
- Calcitriol is contraindicated in patients with severe renal impairment or end-stage renal disease.
- Calcitriol is not recommended in patients with mild to moderate renal impairment or in patients with liver dysfunction.

### Indications and Clinical Use

Calcitriol ointment 3 µg/g is indicated for:

- Topical treatment of mild to moderate plaque type psoriasis (psoriasis vulgaris) with up to 35% body surface area involvement.

geriatrics (> 65 years of age):

Clinical studies of Calcitriol ointment did not include sufficient numbers of subjects 65 years and older to determine whether they respond differently from younger subjects. See Warnings and Precautions.

pediatrics (< 18 years of age):

Calcitriol is not recommended for pediatric use. Safety and effectiveness in pediatric patients have not been established.

### Contraindications

- Patients who are hypersensitive to this drug or to any ingredient in the formulation or component of the container. For a complete listing, see the Dosage Forms, Composition and Packaging section of the product monograph.
- NOT FOR OPHTHALMIC or INTERNAL USE.
- Patients with hypercalcemia and patients known to suffer from abnormal calcium metabolism.
- Patients on systemic treatment of calcium homeostasis.
- Patients with severe renal impairment or end-stage renal disease.

### Administration

The affected area should be washed and dried gently. The ointment should be applied and rubbed in gently until the medication is no longer visible. The treated area should not be bandaged or occluded in any way.

Hands should be washed thoroughly with soap and water after each application.

After satisfactory improvement has occurred, the drug should be discontinued. If recurrence takes place after discontinuation, the treatment may be reinstated.

### Storage and Stability

Store at room temperature (15° - 30° C)

### Process Validation Protocol For Calcitriol Ointment

Protocol approval:





Signing of this Approval page of Validation protocol No. VP/PV/PD1/040 indicates agreement with the process Validation approach described in this document. If modification to the process Validation become necessary, an addendum will be prepared and approved.

#### Responsibility:

##### ➤ Quality Assurance:

Quality assurance will be responsible for Preparation, training and approval of protocol, review of the data compiled, review of deviations (if any), monitoring the process as per the process parameters and for withdrawal of validation samples in co-ordination with production. Review of

Equipment qualification, facility qualification and utility validations reports cGMP compliance during manufacturing process, review and evaluation of the data/results generated during validation. Preparation of Process validation summary report, its review and approval.

##### ➤ Production

Production will be responsible for Training of personnel for unit operation and related documentation. Executing the batches as per the Batch production record and execution of Process Validation Protocol. Compilation of data related to manufacturing area and furnishing the same for review. Review of protocol and summary report.

**Table 1:Product detail**

<b>Product name</b>	<b>Sorvate ointment</b>
generic name	calcitriol ointment 0.0003.% w/w
shelf life	18 months
label claim	composition: each gm contains : calcitriol ip                            3mcg ointment base                             q.s.
batch size	250kg
overages	calcitriol ip 5.0%
market	domestic
packing	20gm : 20 gm to be filled in aluminium collapsible tube. one such tube to be packed in a carton along with leaflet. 15 such cartons are to be wrapped in a bop reams. 24 such packs are to be packed in 4x2x3 layers in a 5 ply shipper with "c" taping at to and bottom. total quantity in a box is 360x20g

**Table 2.0 Raw Material detail**

Item code	Ingredient	Specification	Unit	Std. Qty.
RW 1006	WHITE PETROLATUM	USP	Kg	
RL 1020	LIQUID PARAFFIN	BP	Kg	
RV 1008	VITAMIN –E-ACETATE	IP	Kg	
1301	CALCITRIOL*	IP	Mg	



### ➤ Quality Control

Quality control will be responsible for: Raw material and packing material analysis in process and finished product samples analysis as per the sampling plan. Collection and review of in process test data and Finished Product analysis data. Submission of data/results to QA for review and evaluation.

### ➤ Engineering.

Engineering will be responsible for qualification and calibration of all the processing equipment/instrument before the start of Process validation batches.

To maintain the system to provide required environmental conditions and other utilities for manufacturing of the batches.

#### Reference Document :

- BMR (Batch manufacturing record)
- BPR (Batch processing record)
- FPS (Finish product specification)

SFS ( semi-finish product specification)

Raw Material functions for Bulk manufacturing for 250kg (Table 2)

5% overages of Calcitriol are added in formulation

**Table 3: Raw material qualification**

Item code	Ingredient	Specification	Qualification
RW 1006	WHITE PETROLATUM	USP	Qualified
RL 1020	LIQUID PARAFFIN	BP	Qualified
RV 1008	VITAMIN –E-ACETATE	IP	Qualified
RC 1301	CALCITRIOL*	IP	Qualified

to compensate the loss during process as the label claim is very less.

Standard quantity of Calcitriol includes 5% overages.

\*Calcitriol quantity is to be dispensed in Milligrams

Calculation :

If % Assay is more than 100% then Assay is to be taken as 100% in the calculation

Note : Calculation of Calcitriol , Vitamin E Acetate & their Compensation with white petrolatum to be done manually it is not calculated through SAP .

Quantity of Calcitriol in Gram

$$A = \frac{0.0003 \times 100 \times \text{Batch Size in kg} \times 10^5 \times 1000}{\% \text{Assay of calcitriol} \times 100 \times 100}$$

On as such basis

$$\text{Quantity of Calcitriol in MG} = A \times 1000$$

$$\text{Quantity of Calcitriol in KG} = A / 1000$$

**Quantity of Vitamin E Acetate in Kg**

$$B = \frac{0.05 \times 100 \times \text{Batch Size in kg} \times 100 \times 1000}{\% \text{Assay of Vitamin E Acetate} \times 100 \times 100}$$

On as such basis:-

Quantity of White Petrolatum in kg

$$C = 2370500 - [A(\text{qty. in kg}) + B]$$





### **Oleaginous phase preparation**

- All the qualified ingredient were taken and placed into stainless steel steam- jacketed vessel- 01.
- Set the temperature of vessel 70°C to 75°C.
- All the ingredient was melt and mixed.
- The oil phase was transferred by pump to the ointment manufacturing vessel for homogenization.

### **Preparation and addition of calcitriol phase**

- Calcitriol phase was prepared into the stainless steel tank.
- Environmental condition made by switch off the light and was dark room because of calcitriol is light sensitive substance.
- Accurate quantity of calcitriol was taken prescribed in specification.
- Solution was prepared in suitable solvent DMSO.
- The temperture was 46°C during the addition of calcitriol into solvent.
- The solution was Stirrered by switch on the stirrer.
- Stirrer was done for 15 minutes..
- After stopping stirring a clear solution was obtained.

### **Homogenization operation**

- The ointment that required further treatment were then pumped to proper homogenization.
- Homogenization was take place into ointment manufacturing vessel.
- Homogenization was done for 25 minutes.
- Uniform dispersion of calcitriol ,as well as reduction of the size of the fatty aggregates

attained by passage of warm(30°C - 40°C) ointment in homogenizer.

### **Mixing and cooling operation**

- Mixing of ointment was take place in manufacturing vessel
- Mixing of ointment was done on temperature 35°C to 45°C because at this temperature intimate mixing of ointment occurred.
- Continuous mixing was done, mixing speed was 25 RPM.
- The phase mixing temperature lowered to prevent premature crystallization or congealing of its component.
- Temperature dropped 34°C to 30°C.

### **Final mixing operation**

- Final mixing was taken into ointment manufacturing vessel.
- Final mixing was done by creating vacuum.
- Vacuum was created 400 to 600 mm of Hg.
- Mixing speed was 25 RPM.
- Mixing was done for 30 minutes.
- Ointment was pumped to filling.

### **Filling and packaging operation**

- Aluminum collapsible tubes was used for filling the ointment.
- Tube filling, cramping & sealing machine was used to filling the ointment into tubes Automatic carton packaging machine were used to packaging tubes to carton.
- 20 gm to be filled in aluminum collapsible tube.
- One such tube to be packed in a carton along with leaflet.
- 15 such carton were to be wrapped in a bop



creams.

➤ 24 such packs were to be packed in 4\*2\*3 layers in a 5 ply shipper with "c" taping at top and bottom.

➤ Total quantity in a box is 360\*20 gm.

### Procedure for Analytical Test

#### Descriptions

A printed carton containing leaflet and a printed aluminum collapsible tube containing White to off white ointment.

#### Identification:

The principal peak in the chromatogram obtained with the sample preparation corresponds to the peak in the chromatogram obtained with the standard preparation in the Assay.

The retention time of the principal peak in the chromatogram of standard preparation due to calcitriol = **16.747**.

The retention time of the principal peak in the

principal peak in the chromatogram of sample preparation due to Calcitriol = **16.683**.

#### Minimum Fill:

Select a sample of 10 filled tubes, and remove any labeling that might be altered in weight during the removal of the tube contents. Thoroughly clean and dry the tubes from outside by a suitable means, and weigh individually. Quantitatively remove the contents from each tube, cutting the latter open and washing with a suitable solvent, if necessary, taking care to retain the closure and other parts of each tube. Dry, and again weigh each empty tube together with its corresponding parts. The difference between the two weights is net weight of the contents of the tube. Calculate the content of each tube and also calculate the average minimum fill. The average net content of the 10 tubes is not less than the labeled amount, and the net content of any single tube is not less than 90 % of the labeled amount.

**Table 4.0 Minimum fill**

No.	Weight of filled tube (g)	Weight of empty tube (gm)	Weight of content (gm)
1	23.4	3.3	20.1
2	23.5	3.3	20.2
3	23.5	3.3	20.2
4	23.4	3.3	20.1
5	23.4	3.3	20.1
6	23.4	3.3	20.1
7	23.4	3.3	20.1
8	23.4	3.3	20.1
9	23.3	3.3	20.0
10	23.4	3.3	20.1
Average Net Content (g)			20.15
Individual Net Content (g) min			20.0
Individual Net Content (g) max			20.2

**pH:**

Take 2.5 g of sample into a 100 ml clean and dry beaker, add to it 50 ml of water, heat on a water bath maintained at about 60°C to 70°C. Cool to room temperature, centrifuge at 3000 rpm for 10 minutes, and take the pH of supernatant water extract on a suitable pH meter.

Sample temperature = **25°C**

Observed pH = **6.67**

**Viscosity:**

Instrument : Brookfield Cap 2000 +

RPM : 80

Temp : 25°C

Time : 30 sec.

Procedure : Take about 250mg of sample and place it on the pane, run the instrument as per the above Parameters, record the viscosity in poise.

RPM: 80

Time: 30 seconds

Apparatus: Brookfield cab 2000+

Temp : 25

Weight of sample taken : 265.4

Viscosity = 5,484 pascal

**Assay of Calcitriol :***Reagent & Chemicals*

1. Methanol HPLC grade
2. Acetonitrile HPLC grade
3. Tris Buffer –  
[Tris(Hydroxymethylaminomethane) Gr Grade
4. Orthophosphoric Acid HPLC grade
5. Water Milli Q
6. Calcitriol working standard.

**Chromatographic Conditions**

Column : Inertsil ODS -3V, 250mm x4.6 mm, 5 micron

Wavelength : 265 nm.

Flow Rate : 1.0 ml/min.

Injection Volume : 75  $\mu$  L

Rum Time : 55 Minutes

Buffer: prepare 0.1% Tris Buffer [Tris (Hydroxymethyl) aminomethane]

pH : 6.67

Diluent : Buffer and Acetonitrile in to the ratio of 50:50% v/v.

Mobile Phase A : Mix Buffer : Acetonitrile : Methanol in the ratio 300:600:100

Mobile phase BL mixed Buffer. Acetonitrile in the ratio 200:800

Weighed 2.39 mg of Calcitriol working standard in a 200ml volumetric flask, added 10ml of Tetrahydrofuran and added 200ml of diluent.

Diluted (ml of above solution to ) ml volumetric flask with diluent and mix.

*Preparation of sample solution:*

Weight 5.4120gm sample and transferred it into a 50ml volumetric flask.

*System suitability:*

Inject the standard solution in to the chromatogram and record chromatogram.

1. Theoretical plates for calcitriol peak is 4900.
2. The tailing factor is 1.152.
3. The relative standard deviation for six replicate injection of standard solution is 0.321%.
4. Area count of calcitriol peak in the chromatogram of sample solution **59388**.
5. Average area count of calcitriol peak in the



chromatogram of the standard solution **51364**.

6. Percent potency of working standard on as such basis **9951**.

7. Label claim of calcitriol in %(LC) **0.0003**.

Calculation: (given in formula 1)

$$\begin{aligned} \% \text{ Assay} &= \frac{\%w/w}{0.0003} \times 100 \\ &= 105.85 \end{aligned}$$

(NOTE): Limit between 100 to 110 % of labeled amount

### Content of vitamin E acetate(by HPLC method)

#### Reagent & Chemicals

Methanol HPLC grade

Acetonitrile HPLC grade

Absolute ethanol AR grade

Pyridine HPLC grade

Vitamin E acetate WS

Chromatographic Conditions

Column : Inertsil ODS -3V, 250mm x4.6 mm, 5 micron

Wavelength : 285 nm.

Flow Rate : 1.0 ml/min.

Injection Volume : 20  $\mu$  L

Rum Time : 20 Minutes

Mobile phase

Buffer water : Pyridine in ratio **980:20:30**

Buffer weighed **73.38 gm** of sodium perchlorate in methanol

Diluent tetrahydrofurane : ethanol **750:650 %v/v**

$$\text{Formula 1} = \frac{\text{Sample area}}{\text{Std area}} \times \frac{\text{wt of Std in mg}}{200} \times \frac{5.0}{200} \times \frac{50}{\text{wt. of spl in mg}} \times \frac{\% \text{ Potency of Std}}{100} \times 100$$

Calculation

$$\text{Formula} = \frac{\text{Sample area}}{\text{Std area}} \times \frac{\text{wt of Std in mg}}{200} \times \frac{5.0}{200} \times \frac{50}{\text{wt. of spl}} \times \frac{\% \text{ Potency of Std}}{100} \times 100$$

*Preparation of standard solution:*

Weight **43.4mg** of vitamin E acetate working standard in a **100ml** of volumetric flask added **70ml** of diluent. Diluted **5ml** of above solution to **100ml** volumetric flask with diluent and mix.

*Preparation of sample solution :*

Weight **3.9126gm** sample and transferd into a **100ml** volumetric flask

*System suitability:*

Inject the standard solution in to the chromatogram and record chromatogram.

8. Theoretical plates for calcitriol peak is **3274**.

9. The tailing factor is **0.986**.

10. The relative standard deviation for six replicate injection of standard solution is **0.136**.

11. Area count of vitamin E acetate peak in the chromatogram of sample solution **86899**.

12. Average area count of vitamin E acetate peak in the chromatogram of the standard solution **99923**.

13. Percent potency of working standard on as such basis **97.30**.



$$\begin{aligned} \% \text{ Assay} &= \frac{\%w/w}{0.05} \times 100 \\ &= \mathbf{93.86\%} \end{aligned}$$

NOTE: Limit between 80.0 to 120.0% of amount I;e; 0.05%

### Microbial enumeration test

Dissolve or suspend 10.0g of the sample in suffered sodium chloride-peptone solution pH 7.0 or in Fluid Casein Digest-Soya Lecithin Polysorbate 20 medium to make 100ml. – (Solution-A).

a) Total Viable count :

Bacterial count and Yeasts and moulds count(By pour plate method)

Using Petri dishes 9 cm in diameter, add to each dish 1 ml of the sample prepared as described above and add 15 ml to 20 ml of a

liquefied agar medium suitable for the cultivation of bacteria (Casein soya bean digest agar), and 15 ml to 20 ml of a liquefied agar medium suitable for the cultivation of fungi (Sabouraud-dextrose agar) at not more than 45°C . Prepare for each medium at least two Petri dishes. Incubate the plates at 30°C to 35°C (20°C to 25°C for 5-7 days for fungi) for 3-5 days, unless a reliable count is obtained in a shorter time. Select the plates showing the highest number of colonies less than 250 (50 colonies for fungi). Take the arithmetic average of the counts and calculate the number of colony-forming units per gram.

Tests for specified micro-organisms:

**Pseudomonas aeruginosa:** Sample preparation and pre-incubation: Use 10 ml of solution A (or the

**Table 5: Microbial enumeration test**

Plate No.	No. of colonies	Mean	Positive control (Suspension Valid up to )	Negative control	Observed by	Date
1	02	02		No Growth		
2	02					

Result: Bacterial count **20 cfu/gm/ml**

ii) Total Yeasts and Moulds Count:

1 ml Soln A is plated in duplicate with Sabouraud Dextrose Agar and Incubated at 20-25°C for 5-7 days.

**Table 6: Microbial enumeration test**

Plate No.	No. of colonies	Mean	Positive control (Suspension Valid up to )	Negative control	Observed by	Date
1	Nil	Nil		No Growth		
2	Nil					

Result: Yeast & Moulds - <10 cfu/gm/ml



-quantity corresponding to 1 g or 1 ml) and inoculate into 100 ml of soyabean casein digest broth, homogenize and incubate at 30°C to for 18 to 24 hours.

Selection and subculture: Subculture on a plate of cetrimide agar and incubate at 30-35 °C for 18 to 24 hours.

Interpretation:

Growth of green colonies indicates the possible presence of *P. aeruginosa*. This is confirmed by identification tests. The product passes the test if colonies of the types describe are not present or if the confirmatory identification tests are negative.

Identification test:

*Oxidase and Pigment Test* (For *Pseudomonas aeruginosa*):

With the aid of an inoculating loop, streak representative suspect colonies from the agar surface of Cetrimide Agar Medium on the agar surfaces of *Pseudomonas Agar* medium for Detection of Fluorescin and *Pseudomonas Agar* Medium for Detection of Pyocyanin contained in Petri dishes. If numerous colonies are to be transferred.

RESULT:

Positive : Greenish fluorescent colonies observed.

Negative : Typical colonies **not observed**.

Sample : Typical colonies **not observed**.

B. *Staphylococcus aureus* :

Sample preparation and pre-incubation :

Use 10 ml of solution A (or the quantity corresponding to 1g or 1 ml) and inoculate into 100 ml of soyabean casein digest broth, homogenize and incubate at 30°C to 35°C for 18 to 24 hours.

Selection and subculture :

Subculture on a plate :

Subculture on a plate of mannitol salt agar an incubate at 30-35°C for 18-72 hrs.

Interpretation:

The possible presence of *S.aureus* is indicated by the growth of yellow/white colonies surrounded by a yellow zone. This is confirmed by identification tests. The product passes the test if colonies of the type described are not present or if the confirmatory identification tests are negative.

Coagulase Test (For *Staphylococcus aureus*):

With the aid of an inoculating loop, transfer representative suspect colonies from the agar surfaces of the Mannitol-Salt Agar Medium to individual tubes, each containing 0.5 ml of mammalian, preferably rabbit or horse plasma with or without suitable additives. Incubate in a water bath at 37°C, examining the tubes at 3 hours and subsequently at suitable intervals up to 24 hours. Test positive and negative controls simultaneously with the unknown samples. If no coagulation in any degree is observed, the sample meets the requirements of the test for absence of *Staphylococcus aureus*.

Result: Positive: Yellow colonies surrounded by Yellow Zones observed.

Negavie : Typical colonies **not observed**.

Sample: Typical colonies **not observed**.

## RESULTS FOR VALIDATION OF CALCITRIOL OINTMENT

**Environmental condition manufacturing**

(a) Temp 21.8°C (b) RH 59%



**Table 7: Equipment Details**

S.No	Equipment Name	Area	Equipment ID
1	Manufacturing plant-I (Oil phase vessel & Manufacturing vessel)	Production	FG 01, FG 02
2	Stirrer	Production	FG 03
3	Jacketed vessel	Production	FG 04
4	Bump pump	Production	FG 05
5	Tube filling, crimping & sealing machine	Production	PG 01, PG 02, PG 03
6	Automatic Carton packing machine	Packaging	PG 04
8	pH meter	QC	QC 106
9	Digital viscometer	QC	QC 075
10	HPLC	QC	QC 064
11	Digital balance	QC	QC 017

**In-process & critical check and critical control point.****Table 8: In-process & critical check point**

S.No.	Process	Parameters	Observation			Comply/n ot comply
			B.No-1	B.No-2	B.No-3	
1	Preparation of Oleaginous phase	70° C to 75° C	72.2° C	72.5° C	72.1° C	Comply
2	<ul style="list-style-type: none"> <li>➤ Preparation of Calcitriol phase</li> <li>Environmental Condition .</li> <li>➤ Stirring time</li> <li>➤ Appearance</li> </ul>	Dark room or yellow room  10to15 min.  Clear solution	Dark room  15 minutes Clear	Dark room  15 minutes Clear	Dark room  15 minutes Clear	Comply
3	Addition of Calcitriol phase into Oleaginous phase	45° C to 48° C	45.5° C	45.4° C	46° C	Comply
4	Homogenization time	20-25 minutes	25 minutes	25 minutes	25 minutes	Comply
5	Mixing & cooling	At 18 to 30 rpm till temp. drops to 30° to 34° C	25 rpm	25 rpm	25 rpm	Comply
6	Final Mixing (400 to 600 mm of Hg )	At 18 to rpm to 30 rpm for 30 minutes	25 rpm 30 minutes	25 rpm 30 minutes	25 rpm 30 minutes	Comply



### Test perform during in-process

**Table 9: After final manufacturing tests criteria**

S. No	Parameters	Acceptance criteria	Observation			Comply/Not comply
			B.No.-1	B.No.- 2	B.No.-3	
1	Description	White to of white ointment	O.K.	O.K.	O.K.	Comply
2	pH	5.0 to 8.0	6.67	6.12	6.32	Comply
3	Viscosity	4.0 to 8.0 poise	5.484 poise	5.212 poise	5.112 poise	Comply
4	Assay	90% to 110%	105.85%	102.84%	105.45%	Comply

### During filling and packaging

**Table 10: During filling and packaging tests criteria**

S.No	Parameters.	Acceptance criteria	Observation			Comply/Not comply
			B.No-1	B.No.-2	B.No.-3	
1	Description	White to off white ointment	O.K.	O.K.	O.K.	Comply
2	pH	5.0 to 8.0	6.10	6.21	6.15	Comply
3	Viscosity	4.0 to 8.0 poise	5.812 poise	5.533 poise	5.451 poise	Comply
4	Antimicrobial test	Should comply as per USP	O.K.	O.K.	O.K.	Comply
5	Assay	90% to 110%	105.1%	103.8%	104.2%	Comply
6	Embossing	Should be legible	O.K.	O.K.	O.K.	Comply
7	Crimping	Should be satisfactory	O.K.	O.K.	O.K.	Comply
8	Minimum fill	20gm to 20.30gm	20.02gm	20.12gm	20.21gm	Comply
9	Coding detail	Should be legible	O.K.	O.K.	O.K.	Comply



## Analytical result for finish product

**Table 11: Finish product tests criteria**

S.No.	Parameters.	Acceptance criteria	Observation			Comply/Not comply
			B.No1	B.No2	B.No3	
1	Description	White to of white ointment	Ok	Ok	Ok	Comply
2	Identification	Principal peak of std calcitriol=16.747	16.683	16.652	16.632	Comply
3	Minimum fill	20gto20.30g	20.121g	20.112g	20.231g	Comply
4	Assay	90%to110%	105.85%	104.23%	104.12%	Comply
5	Contant of Vit-E-Acetate	80%to120%	93.86%	95.12%	95.82%	Comply
6	pH	5.0to8.0	6.12	6.49	6.31	Comply
7	Viscosity	4.0to8.0 poise	5.123 p	5.142 p	5.312 p	Comply

## CONCLUSION

The formal process validation study conducted at own production scale batches(usually three consecutive batches).The information from these studies are available in various guidelines articles and literature. These scheme was submitted in the marketing authorization dossier and should include following information: Short description of the process with a summary of the critical processing steps or critical parameters to be monitored during validation, finished product specification, detail of analytical methods, in-process controls proposed with acceptance criteria, additional testing intended to be carried out, sampling plan where, when and how the samples are taken, details of method for recording and evaluation of results.

On completion of these scheme, a report containing all the information and signed by the appropriate authorized person was generated for examination by the supervisory authority according to regulatory guideline, report include batch analytical data, certificate of analysis, bath production records and report on unusual findings, modification or changes with appropriate rationale, result and conclusions.

The process validation was started at the qualification of equipment. All the equipment was qualified at the time of process validation. Environmental condition monitoring manufacturing area is critical process parameter for process validation. In Environmental monitoring critical parameter like ,temperature, relative humidity are generally monitored. The maximum and minimum



temperature was found to be 21.8°C and 20.8°C respectively in processing area. The maximum and minimum relative humidity was found 59% and 60% respectively in processing area. All the critical parameters were found to be as per acceptance criteria. Hence the product can be successfully manufactured at the commercial scale and the manufacturing process was found to be validated. where the result obtain show significance deviations from those expected, the regulatory authorized need to be informed immediately. In such case corrective action should be proposed and any change proposed in the manufacturing process should receive prior regulatory approval by way of variation.

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