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# FORMULATION AND *IN-VITRO* CHARACTERIZATION OF CALCITRIOL SOFT GELATIN CAPSULE

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## ABSTRACT

The scenario of pharmaceutical drug delivery is rapidly changing conventional pharmaceutical dosage forms are being replaced by new drug delivery systems. These new drug delivery systems are having edge over conventional ones in terms of many biopharmaceutical parameters. The basic of formulation of such a soft gelatine capsules should be to produce the smallest possible capsule consistent with the maximum stability, therapeutic effectiveness, and manufacture efficiency. Calcitriol Soft gelatin capsule formulation strategies are to develop proper shell formula with respect to gelatin/plasticizer combination, to develop a proper fill composition i.e. development of a clear solution of Calcitriol yet it is small enough to be encapsulated easily, to study the effect of various parameters on rate of release, to check the release rate of drug from shell by rapture test. These parameters are crucial to the physical stability of the capsule during manufacture, drying and on storage. The technologically important factors to be born in mind are temperature, viscosity and surface activity of the fill material and, in the case of suspensions, the particle size of the suspended drug.

**KEYWORDS**: Calcitriol, Soft gelatin capsule, stability, biopharmaceutical parameters, plasticizer.

## INTRODUCTION

Poor aqueous solubility has been identified as the single largest physicochemical challenge for the oral absorption of compounds and almost inevitably leads to their lower oral bioavailability from the conventional dose forms.[1] Traditional approaches to enhance the absorption of a compound relate to improving its solubility and rate of dissolution in the GIT fluids. These approaches include using a form of the compound with optimum aqueous solubility, for example, salt form, amorphous form, prodrug form, nanosizing or employing a vehicle in which the compound is soluble and remain solubilized upon contact with the GIT aqueous environment. The least complex way to present a compound to the GIT for absorption is to administer the compound as a solution or solubilized form, thereby removing any dissolution rate-limiting step in the absorption process.[2]

The formulation of drugs into soft gelatin capsules has gained popularity throughout the past decade due to the many advantages of this dosage form. The bioavailability of hydrophobic drugs can be significantly increased when formulated into soft gelatin capsules. Many problems associated with tableting, including poor compaction and lack of content or weight uniformity, can be eliminated when a drug is incorporated into this dosage form. Improved stability of drugs that are highly susceptible to oxidation can be achieved when formulated into a soft gelatin capsule.[3,4]

A softgel shell formulation typically consists of a film forming material, such as gelatin, water dispersible or soluble plasticizer(s), and water. The formulation may also contain other minor additives, such as opacifiers, colorants, flavors, sweeteners, and preservatives. Softgels may also be coated with a variety of polymers for certain targeted enteral delivery applications. Bloom strength, also known as jelly strength, is expressed as the weight in grams that, when applied with a 12.7mm diameter plastic plunger, will produce a depression exactly 4mm deep in a jelly containing 6.67% w/w of gelatin in water matured for 16–18 hr at $10^0$C. Bloom strength of gelatin used in a softgel shell may vary from 150 to 250 g, with the higher the bloom strength, the more physically stable is the resulting softgel shell. As the cost of a softgel product is related directly to the bloom strength of gelatin used, gelatin of higher bloom strength is usually reserved only when necessary to improve the physical stability of a softgel product or for large size softgels which require greater structural strength during manufacture. Viscosity determination is performed on a 6.67% w/w concentration of

gelatin in water at $60^0$C and usually ranges between 25 and 45 mill poise. Iron levels present in the gelatin raw material is derived mainly from the water used in its production and should not exceed 15 ppm as higher levels may potentially result in the color reactions with other softgel components. Gelatin is an excellent growth medium for many bacteria and thus requires considerable care during its manufacture and handling to avoid contamination.[5-7]

The first step in the developing a solution containing softgel is to determine the solubility of the drugs in a range of pharmaceutically acceptable solvents. After the solubility's are measured, the solvents are then selected on the basis of their regulatory acceptability and known compatibility with softgel dosage forms. Solvents that provide adequate solubility of the drug can be selected, though it may be necessary to combine them to achieve the desired in vitro or in vivo characteristics and to ensure good physical stability. In addition to characterizing the in vitro and in vivo performance of the preliminary formulation, it is important to evaluate the drug solubility in the mixtures for the physical stability under accelerated conditions, chemical stability under accelerated conditions, excipient compatibility. For softgels containing suspension fills, the solubility of the drug in a range of pharmaceutically acceptable solvents is also measured and excipients in which the drug shows little or no solubility are then selected. These formulations generally require viscosity enhancers in order to provide adequate suspending characteristics for the drug during processing. This is vital in maintaining drug homogeneity during manufacture. The type and level of viscosity enhancer is optimized to provide the best manufacturability.

Because of the migration of components (water, plasticizer, drugs, etc.) within the softgel both during and following encapsulation, formulation of the fill material must be conducted concurrently with formulation of the shell for maximum product quality. Without this simultaneous development, it is not uncommon for problems to arise. The formulation for soft gelatin capsules involves liquid rather than powder technology. Materials are generally formulated to produce the smallest possible capsule consistent with the maximum stability, therapeutic effectiveness, and manufacturing efficiency. The liquids are limited to those that do not have an adverse effect on the gelatin walls. The pH of the liquid can be between 2.5 and 7.5. Liquids with pH < 2.5 would tend to cause leakage by hydrolysis of the gelatin. Both liquids with pH > 7.5 and aldehyde decrease shell solubility by tanning the gelatin. All liquids used for filling must flow by gravity at a temperature of $35^o$C or less. The viscosity of

fills can range from mobile liquids to thick suspensions or pastes. The sealing temperature of gelatin films is 37-40$^0$C.

Drugs that are not sufficiently soluble in the solvent or combinations of solvent can be formulated into suspensions and encapsulated. The particle size of insoluble drugs should be 80 mesh or finer for maximum suspension homogeneity and capsulation equipment requirements. Examples of suspending agents include paraffin wax, beeswax, and hydrogenated vegetable oil for oily vehicles, and solid glycerol esters (such as higher molecular weight PEG) for non oily vehicles. Surfactants, such as polysorbates, are often added to the dispersion to promote wetting of the ingredients and/or dispersion of the fill in vivo. In general, many different materials may be encapsulated; however, limitation does exist for some compounds due high solubility in water and/or inherent chemical reactivity and the resultant effect on the shell. These compounds include strong acids and alkalis and their salts, as well as ammonium salts. Some compounds, such as aldehydes, can react with gelatin, causing cross linking and resulting in a product that lacks bioavailability. In addition, any substance (such as aspirin) that is unstable in the presence of moisture may also exhibit unacceptable chemical stability in soft gels.

Compared to lipophilic solutions, fill compositions with hydrophilic components are more challenging to encapsulate, since they are prone to interact with the shell. The most critical period for diffusional exchanges between shell and fill is the manufacturing process, since the moisture content of the initial shells before drying is around 40% and the equilibrium moisture level is only reached after several days. Thus, during manufacture and drying, hydrophilic components of the fill may migrate rapidly into the shell and vice versa, thereby changing the initial composition of both, the shell and the fill.[8-13]

## MATERIALS AND METHODS

### Materials

Calcitriol was obtained as gift sample from Formosa, Taiwan. Butylated Hydroxyanisole, Butylated Hydroxytoluene, Merck, Mumbai. Gelatin Type A, Gelatin Type B and other ingredients, Alkem Mumbai, India, Other reagents and solvents used were of analytical grade.

## METHODS

### 1. Gel mass preparation

Dispensed required quantities of all excipents as per batch formula. Titanium dioxide was passes through colloidal mill for 15 min with water. In part quntity of water color solution was prepared. Gelatin, Glycerin, sorbitol, Titanium dioxide dispersion, Colour solution and water were added to geatin prepartion tank. Water quantity for gel mass preparation in every batch was taken as 60% of total wt. of Gelatin, Glycerin,Sorbitol, Methyl paraben, Propyl paraben, $TiO_2$, Colours. Some of this water gets evaporated during gel mass preparation. So finally at the time of gel mass unloading in gelatin tanks; 37% - 43% water remains in Gel mass which is further utilised for encapsulation purpose.

**Gelatin Preparation Tank (250 Lit.)**

Initial mixing: 10 Min.25 RPM

Vaccum: 650-700mmof Hg

Temperature: 50-60$^0$ C

**Mixing for Gel mass preparation**

Time: 2 Hr Temperature 60-65$^0$ C at Rpm 25-26

**Deareation**

Time 20 min Temperature: 60-65$^0$C

RPM: 25-26 Vacuum 650-700 mmHg

After physically observing gel mass for air bubbled or agglomerates, gel mass was unloaded in gelatin holding tank. Color solution was mixed with gel mass in holding tank. Color solution vessel was rinsed with water mixture was added to gel mass. Gel mass was stored in holding tank at 45°-55° C.

### 2. Medicament Preparation

**API Potency Calculation: Assay = 102.7** ( Practically assay values of pure drug can't be above 100%, so values above 100% are considered to be 100% for calculation purpose.).

$$\text{Practical wt.} = \frac{\text{Theoretical wt.} \times 100}{\text{Assay}}$$

Sodium lamp, Nitrogen flushing mechanical stirrer are used at the time of preparation of medicament. Dissolve Calcitriol, BHA, BHT in Medium chain Triglyceride with continues stirring till a clear solution obtained. (Time 30-45min).

**Processing Parameters**

**Table 1: Processing Parameters of Calcitriol Soft Gelatin Capsule Manufacturing.**

| Parameter | Observed Value |
|---|---|
| Prepared Gelatin | **52 Kg** |
| Equipment used | **250 Lt Gelatin Reactor** |
| RPM | **25-26** |
| Temperature | **60°-70° C** |
| Process Time | **120 min** |
| Vacuum | **650-70 mm Hg** |
| Vacuum Applied Time | **20 min** |
| Area Maintained | **40% RH** |
| Die | **4 minim, round shape** |
| Machine Speed | **2-5 rpm** |
| Gelatin Tank Temperature | **66° C** |
| Spreader Box (Right) | **62° C** |
| Spreader Box (Left) | **61° C** |
| Duct Temperature | **10.4° C** |
| Segment Temperature | **44.6° C** |
| Ribbon Thickness | **0.9 mm** |
| Area Maintained | **43% RH** |
| Tunnel Drying | **20-30 min** |
| Polishing | **Pan coater using dusting** |
| Final Drying | **18-22 % RH, 48hr** |

## 3. Manufacturing Process

The gelatin mass is feed by gravity to a metering devices (spreader box) which control the flow of the mass onto air cooled (13-14$^{O}$C) rotating drum. Gelatin ribbon of controlled ($\pm$10%) thickness are formed. The wet shell thickness may vary from0.022-0.045inch, but for most capsule, it is between 0.025-0.032 inch. Thicker shells are used on product requiring greater structural strength. Product cost is directly proportional to shell thickness. The ribbons are fed through mineral oils lubricated bath over guide rolls and then down between the wedge and the die rolls. The material (oils) to be encapsulated flows by gravity displacements pump. The pump accurately meters the material through the leads and wedge and into the gelatin ribbons betweens the die rolls. The bottom of the wedge contains small orifices lined up with the die pocket of the die rolls. The capsule is about half sealed when the pressure of

the pumped material force the gelatin into the die pocket, where the capsule are simultaneously filled shaped hermetically sealed and cut from the gelatin ribbon. The sealing of the capsule is achieved by mechanical pressure on the die rolls and the heating (37-40$^0$C) of the ribbons by the wedge. During the manufacturing, capsule sample are taken periodically for seal thickness and fill weight checks. The sealed thickness are measured with the help Vanier caliper and change in ribbon thickness, heat or die pressure are made necessary. Filled weight are checked by simply weighing the whole fresh capsule. Immediately after manufacturing the capsule are automatically conveyed through a naphtha wash unit to remove the mineral oils lubricated and subjected to a preliminary drying room which remove a 60-70% water with 20-30% RH. Capsule at equilibrium with 20-30%RH at 21-24$^0$C are consider dry and shell of such capsule contained 6-12% water depending on the gelatin formula used. After drying the capsule are transited to the inspection departments an held until released by the quality controls departments.

**Table 2: Trials For Calcitriol Soft Gelatine Capsle 0.25 mcg, 0.5 mcg**

| **Batch No.** | **F01** | | **F02** | |
|---|---|---|---|---|
| **Medicament** | | | | |
| **Ingredient** | **mg/Capsule** | **% w/w** | **mg/Capsule** | **% w/w** |
| **Calcitriol** | 0.0005 | 0.00031 | 0.00025 | 0.00016 |
| **Medium Chain Triglycerides** | 159.99950 | 99.99969 | 159.99984 | 99.99984 |
| **BHA** | | | | |
| **BHT** | | | | |
| **Total** | **160.00** | 100.00 | **160.00** | 100.00 |
| **Gel Mass** | | | | |
| **Ingredient** | **mg/Capsule** | **% w/w dry basis** | **mg/Capsule** | **% w/w dry basis** |
| **Gelatin (Type A)** | 65.51 | 65.51 | 65.58 | 65.58 |
| **Gelatin (Type B)** | | | | |
| **Liquid Sorbitol** | 15.00 | 15.00 | 15.00 | 15.00 |
| **Glycerin** | 18.33 | 18.33 | 18.36 | 18.36 |
| **Methyl Paraben** | | | | |
| **Propyl Paraben** | | | | |
| **Titanium Dioxide** | 0.90 | 0.90 | 0.90 | 0.90 |
| **FD&C Yellow # 6** | 0.10 | 0.10 | 0.16 | 0.16 |
| **FD&C Red # 3** | 0.16 | 0.16 | 0.00 | 0.00 |
| **Water** | q.s. | | q.s. | |
| **Total** | **100.00** | **100.00** | **100.00** | **100.00** |
| **Capsule Wt.** | **260.00** | -- | **260.00** | -- |
| **Description** | **Light Red, round** | | **Light Orange, round** | |
| **Remark** | **Type A Gelatin With Preservative & Antioxidant** | | | |

| Batch No. | F03 | | F04 | |
|---|---|---|---|---|
| **Medicament** | | | | |
| **Ingredient** | **mg/Capsule** | **% w/w** | **mg/Capsule** | **% w/w** |
| **Calcitriol** | 0.0005 | 0.00031 | 0.00025 | 0.00016 |
| **Medium Chain Triglycerides** | 159.99950 | 99.99969 | 159.99984 | 99.99984 |
| **BHA** | | | | |
| **BHT** | | | | |
| **Total** | **160.00** | 100.00 | **160.00** | 100.00 |
| **Gel Mass** | | | | |
| **Ingredient** | **mg/Capsule** | **% w/w dry basis** | **mg/Capsule** | **% w/w dry basis** |
| **Gelatin (Type A)** | | | | |
| **Gelatin (Type B)** | 65.51 | 65.51 | 65.58 | 65.58 |
| **Liquid Sorbitol** | 15.00 | 15.00 | 15.00 | 15.00 |
| **Glycerin** | 18.33 | 18.33 | 18.36 | 18.36 |
| **Methyl Paraben** | | | | |
| **Propyl Paraben** | | | | |
| **Titanium Dioxide** | 0.90 | 0.90 | 0.90 | 0.90 |
| **FD&C Yellow # 6** | 0.10 | 0.10 | 0.16 | 0.16 |
| **FD&C Red # 3** | 0.16 | 0.16 | 0.00 | 0.00 |
| **Water** | q.s. | | q.s. | |
| **Total** | **100.00** | **100.00** | **100.00** | **100.00** |
| **Capsule Wt.** | **260.00** | -- | **260.00** | -- |
| **Description** | **Light Red, round** | | **Light Orange, round** | |
| **Remark** | **Type A Gelatin With Preservative & Antioxidant** | | | |

| Batch No. | F05 | | F06 | |
|---|---|---|---|---|
| **Medicament** | | | | |
| **Ingredient** | **mg/Capsule** | **% w/w** | **mg/Capsule** | **% w/w** |
| **Calcitriol** | 0.0005 | 0.0003 | 0.00025 | 0.00016 |
| **Medium Chain Triglycerides** | 159.9995 | 99.9997 | 159.99984 | 99.99984 |
| **BHA** | | | | |
| **BHT** | | | | |
| **Total** | **160.00** | 100.00 | **160.00** | 100.00 |
| **Gel Mass** | | | | |
| **Ingredient** | **mg/Capsule** | **% w/w dry basis** | **mg/Capsule** | **% w/w dry basis** |
| **Gelatin (Type A)** | 65.31 | 65.31 | 65.38 | 65.38 |
| **Gelatin (Type B)** | | | | |
| **Liquid Sorbitol** | 15.00 | 15.00 | 15.00 | 15.00 |
| **Glycerin** | 18.33 | 18.33 | 18.36 | 18.36 |
| **Methyl Paraben** | 0.18 | 0.18 | 0.18 | 0.18 |
| **Propyl Paraben** | 0.02 | 0.02 | 0.02 | 0.02 |
| **Titanium Dioxide** | 0.90 | 0.90 | 0.90 | 0.90 |

| | | | | |
|---|---|---|---|---|
| **FD&C Yellow # 6** | 0.10 | 0.10 | 0.16 | 0.16 |
| **FD&C Red # 3** | 0.16 | 0.16 | 0.00 | 0.00 |
| **Water** | q.s. | | q.s. | |
| **Total** | **100.00** | **100.00** | **100.00** | **100.00** |
| **Capsule Wt.** | **260.00** | -- | **260.00** | -- |
| **Description** | **Light Red, round** | | **Light Orange, round** | |
| **Remark** | **Type A Gelatin With Preservative & Antioxidant** | | | |

| **Batch No.** | **F07** | | **F08** | |
|---|---|---|---|---|
| **Medicament** | | | | |
| **Ingredient** | **mg/Capsule** | **% w/w** | **mg/Capsule** | **% w/w** |
| **Calcitriol** | 0.0005 | 0.00031 | 0.00025 | 0.00016 |
| **Medium Chain Triglycerides** | 159.99950 | 99.99969 | 159.99984 | 99.99984 |
| **BHA** | | | | |
| **BHT** | | | | |
| **Total** | **160.00** | 100.00 | **160.00** | 100.00 |
| **Gel Mass** | | | | |
| **Ingredient** | **mg/Capsule** | **% w/w dry basis** | **mg/Capsule** | **% w/w dry basis** |
| **Gelatin (Type A)** | | | | |
| **Gelatin (Type B)** | 65.31 | 65.31 | 65.38 | 65.38 |
| **Liquid Sorbitol** | 15.00 | 15.00 | 15.00 | 15.00 |
| **Glycerin** | 18.33 | 18.33 | 18.36 | 18.36 |
| **Methyl Paraben** | 0.18 | 0.18 | 0.18 | 0.18 |
| **Propyl Paraben** | 0.02 | 0.02 | 0.02 | 0.02 |
| **Titanium Dioxide** | 0.90 | 0.90 | 0.90 | 0.90 |
| **FD&C Yellow # 6** | 0.10 | 0.10 | 0.16 | 0.16 |
| **FD&C Red # 3** | 0.16 | 0.16 | 0.00 | 0.00 |
| **Water** | q.s. | | q.s. | |
| **Total** | **100.00** | **100.00** | **100.00** | **100.00** |
| **Capsule Wt.** | **260.00** | -- | **260.00** | -- |
| **Description** | **Light Red, round** | | **Light Orange, round** | |
| **Remark** | **Type A Gelatin With Preservative & Antioxidant** | | | |

| **Batch No.** | **F09** | | **F10** | |
|---|---|---|---|---|
| **Medicament** | | | | |
| **Ingredient** | **mg/Capsule** | **% w/w** | **mg/Capsule** | **% w/w** |
| **Calcitriol** | 0.0005 | 0.00031 | 0.00025 | 0.00016 |
| **Medium Chain Triglycerides** | 159.6795 | 99.79969 | 159.67975 | 99.79984 |
| **BHA** | 0.16 | 0.10 | 0.16 | 0.10 |
| **BHT** | 0.16 | 0.10 | 0.16 | 0.10 |
| **Total** | **160.00** | 100.00 | **160.00** | 100.00 |
| **Gel Mass** | | | | |

| Ingredient | mg/Capsule | % w/w dry basis | mg/Capsule | % w/w dry basis |
|---|---|---|---|---|
| Gelatin (Type A) | 65.31 | 65.31 | 65.38 | 65.38 |
| Gelatin (Type B) | | | | |
| Liquid Sorbitol | 15.00 | 15.00 | 15.00 | 15.00 |
| Glycerin | 18.33 | 18.33 | 18.36 | 18.36 |
| Methyl Paraben | 0.18 | 0.18 | 0.18 | 0.18 |
| Propyl Paraben | 0.02 | 0.02 | 0.02 | 0.02 |
| Titanium Dioxide | 0.90 | 0.90 | 0.90 | 0.90 |
| FD&C Yellow # 6 | 0.10 | 0.10 | 0.16 | 0.16 |
| FD&C Red # 3 | 0.16 | 0.16 | 0.00 | 0.00 |
| Water | q.s. | | q.s. | |
| Total | **100.00** | **100.00** | **100.00** | **100.00** |
| Capsule Wt. | **260.00** | -- | **260.00** | -- |
| Description | **Light Red, round** | | **Light Orange, round** | |
| Remark | **Type A Gelatin With Preservative & Antioxidant** | | | |

| Batch No. | F11 | | F12 | |
|---|---|---|---|---|
| Medicament | | | | |
| Ingredient | mg/Capsule | % w/w | mg/Capsule | % w/w |
| Calcitriol | 0.0005 | 0.00031 | 0.00025 | 0.00016 |
| Medium Chain Triglycerides | 159.6795 | 99.79969 | 159.67975 | 99.79984 |
| BHA | 0.16 | 0.10 | 0.16 | 0.10 |
| BHT | 0.16 | 0.10 | 0.16 | 0.10 |
| Total | **160.00** | 100.00 | **160.00** | 100.00 |
| Gel Mass | | | | |
| Ingredient | mg/Capsule | % w/w dry basis | mg/Capsule | % w/w dry basis |
| Gelatin (Type A) | | | | |
| Gelatin (Type B) | 65.31 | 65.31 | 65.38 | 65.38 |
| Liquid Sorbitol | 15.00 | 15.00 | 15.00 | 15.00 |
| Glycerin | 18.33 | 18.33 | 18.36 | 18.36 |
| Methyl Paraben | 0.18 | 0.18 | 0.18 | 0.18 |
| Propyl Paraben | 0.02 | 0.02 | 0.02 | 0.02 |
| Titanium Dioxide | 0.90 | 0.90 | 0.90 | 0.90 |
| FD&C Yellow # 6 | 0.10 | 0.10 | 0.16 | 0.16 |
| FD&C Red # 3 | 0.16 | 0.16 | 0.00 | 0.00 |
| Water | q.s. | | q.s. | |
| Total | **100.00** | **100.00** | **100.00** | **100.00** |
| Capsule Wt. | **260.00** | -- | **260.00** | -- |
| Description | **Light Red, round** | | **Light Orange, round** | |
| Remark | **Type A Gelatin With Preservative & Antioxidant** | | | |

## 4. Evaluation of Soft Gel Capsule

### Loss on drying (In House Development)

Soft gelatin capsules were cut along the seam by a sharp blade. Dispose off the medicament in it and then the capsules shells were dipped in petroleum ether to clean the shell (to remove any residual of the medicament). Soft gelatin capsule shells were than wiped with kimtex cloth. Initial weight of the shells was noted in petridish (weight taken should be at least 1gm). Then the petridish was placed in hot air oven at $105^0$C for 3 hrs. After 3 hrs again the weight of petridish was taken and L.O.D was calculated as

$$\text{Loss on drying (\%w/w)} = \frac{\text{Loss of weight of capsule shell after 3 hrs of drying (gram)}}{\text{Initial weight (gram)}} \times 100$$

### Weight variation (As per USP)

Gross weight of 20 capsules each in mg and weight of 20 empty shells each in mg was taken and the average fill weight was calculated using the following formula:

$$\text{Average fill weight of capsule (mg.)} = \frac{\text{(Gross weight of capsule in mg –Weight of empty shell in mg)}\ \textit{total of 20 capsules}}{20}$$

Relative Standard deviation values are in bracket of Average wt. value for each batch.

### Viscosity of Gel Mass Preparation

### Viscometer

Brookfield Viscosimeter, Fill the tube with the exact amount of gel mass (adjusted to 20.0 ± 0.1 ) as specified by the manufacturer. Adjust the meniscus of the column of liquid in the capillary tube to the level of the top graduation line with the aid of either pressure or suction. Open both the filling and capillary tubes in order to permit the liquid to flow into the reservoir against atmospheric pressure.[Note—Failure to open either of these tubes will yield false values.].

### The gel ribbon thickness

The gel ribbon thickness of all batches was measured by using caliper gauge. Then mean thickness was calculated.

**Disintegration Test (As per USP)**

6 capsules were introduced into the basket rack assembly of disintegration test apparatus and discs were added to each tube. The temperature of water was maintained at 37ºC±2ºC. The time in minutes at which last, of the 6, capsules disintegrated.

**5. Evaluation of Final product**

**Fill Weight (Weight variation): (As per USP)**

Weight of 20 capsules was noted down and the average weight of capsules was calculated by using the following formula:

**Content uniformity (Assay)**

It is carried out as per European pharmacopoeia. Carry out the assay as rapidly as possible avoiding exposure to light and air. Test Solution: Dissolve 1.ooo mg the substance to be examined without heating in 10.0 ml of the mobile phase.

**Reference solution: a)**: Dissolve 1.000 mg of Calcitriol CRS without heating in mobile.

**Phase Reference solutions: b)**: Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase. Reference solution's (c) Keep 2 ml of reference solution (a) for 30 min at $80^0$c.

The chromatographic procedure may be carried out using a column 0.25m long and 4.6 mm in internal diameter packed with octylsilyl silica gel for chromatography R1 (5 micro m),as mobile phase at flow rate 1.0 ml/min, a mixture of 450 volume of a solution containing 1.0g/1 of tris aminomethane R adjusted to pH 7.0 to 7.5 with a phosphoric acid R and 550 v0lume of acetonitrile R, as detector a spectrophotometer set at 230 nm, a loop injector. Maintaining the temperature of the column at 40 $^0$C and Calculate the percentage contents of Calcitriol. Relative Standard deviation (RSD) values are in bracket of Average Assay value for each batch.

**Disintegration Test: (As per USP)**

Six capsules were introduced into the basket rack assembly of disintegration test apparatus and discs were added to each tube. The temperature of water was maintained at 37ºC±2ºC. The time in minutes at which last, of the 6, capsules disintegrated completely except fragments from the capsule shell was noted down. Media: Purified water.

**Loss on drying**

Instrument**:** Hot air oven, Soft gelatin capsules were cut along the seam by a sharp blade. Dispose off the medicament in it and then the capsules shells were dipped in petroleum ether or Isopropyl alchohol to clean the shell (to remove any residual of the medicament). Soft gelatin capsule shells were than wiped with lint free cloth. Initial weight of the shells was noted in petridish (weight taken should be atleast 1gm). Then the petridish was placed in hot air oven at $105^0$C for 3 hrs. After 3 hrs again the weight of petridish was taken and L.O.D was calculated as.

**Loss of weight of capsule shell after**

$$\text{Loss on drying (\%w/w)} = \frac{\text{3 hrs of drying (gram)}}{\text{Initial weight (gram).}} \times 100$$

**Shell and Seam thickness**

Instrument**:** Vernier caliper, The shell thickness and seam thickness of all batches of soft gelatin capsule was measured by using Vernier caliper and the mean thickness was calculated.

**Rupture Test for Soft Shell Capsules**

Medium: water; 500 mL, Apparatus - Use Apparatus 2 as paddle, operating at 50 rpm. Time: 15 minutes. Place 1 capsule in each vessel, and allow the capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the capsules, and record the time taken for each capsule shell to rupture.

| Drug Name | Dosage Form | USP Apparatus | Speed (RPM) | Medium | Volume (ml) |
|---|---|---|---|---|---|
| Calcitriol | Soft gelatin Capsule | II (Paddle) | 50 | Water | 500 |

**Stability Studies (best fill formulations)**

In any rational design and evaluation of dosage forms for drugs, the stability of the active component must be a major criterion in determining their acceptance or rejection. Stability of a drug can be defined as the ability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications. Stability of

a drug can be defined as the time from the date of manufacture and the packaging of the formulation, until its chemical or biological activity is not less than a predetermined level of labeled potency and its physical characteristics have not changed appreciably or deleteriously. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as a temperature, humidity and light, enabling recommended storage conditions, re-test periods and shelf-lives. Generally, the observation of the rate at which the product degrades under normal room temperature requires a long time. To avoid this undesirable delay, the principles of accelerated stability studies are adopted. ICH specifies storage conditions.

- Long-Term Testing: 25°C±2°C /60%RH±5%, 30°C±2°C /65%RH±5%
- Accelerated Testing: 40°C±2°C /75%RH±5%

Stability studies were carried out at 25°C/60%RH, 30°C/65%RH, 40°C/75%RH.

## RESULT AND DISCUSSIONS

**Table 3: Loss on Drying (%w/w) at Various Stages of Drying**.

| Condition/Drying | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| **Gel mass** | 39.25 | 38.02 | 40.22 | 39.25 | 42.12 | 40.61 |
| **Capsules Initial** | 35.5 | 35.6 | 35.8 | 36.02 | 36.31 | 35.46 |
| **Capsules 12 hours** | 18.5 | 17.02 | 18.62 | 18.86 | 18.73 | 19.06 |
| **Capsules 24 hours** | 15.4 | 16.33 | 15.59 | 15.44 | 15.34 | 16.82 |
| **Capsules 36 hours** | 10.22 | 11.04 | 9.82 | 10.77 | 9.89 | 11.44 |

| Condition/Drying | F7 | F8 | F9 | F10 | F11 | F12 |
|---|---|---|---|---|---|---|
| **Gel mass** | 40.13 | 40.94 | 38.85 | 40.24 | 39.24 | 42.41 |
| **Capsules Initial** | 36.38 | 38.52 | 34.43 | 37.01 | 33.43 | 37.26 |
| **Capsules 12 hours** | 19.38 | 19.94 | 17.25 | 19.85 | 15.85 | 20.86 |
| **Capsules 24 hours** | 16.28 | 19.25 | 14.22 | 16.43 | 12.46 | 18.62 |
| **Capsules 36 hours** | 11.10 | 10.58 | 9.28 | 9.85 | 11.68 | 10.17 |

After final drying of the soft gelatine capsules, L.O.D. of all of its appeared in the specified range. It also validated the drying time to be 48 hrs. LOD of soft gel capsule maintained in the level by drying at 105°C in a particular time 3 hours interval and optimize drying time for achieve LOD in particular limit. Initial LOD of the batch is near about 37-43 %w/w and after 36 Hr it was observe that the %w/w. we will maintain at ranges between 8-12 % w/w. for

finished product & 10-22% for stability conditions which are the limit specified and batch no F9 are obtained in this range.

**Table 4: Fill Weight & Capsule Shell Weight; At the Time of Manufacturing**

| Batch No | Avg Initaial Weight(mg) | Avg. Shell Weight(mg) | Avg. Fill Weight(mg) |
|---|---|---|---|
| **F1** | 320(3.28) | 158(2.18) | 162(3.58) |
| **F2** | 315(4.99) | 159(3.89) | 156(5.29) |
| **F3** | 318(2.30) | 160(1.19) | 158(2.60) |
| **F4** | 322(4.02) | 163(3.20) | 159(2.45) |
| **F5** | 315(5.73) | 159(4.91) | 156(4.16) |
| **F6** | 310(3.04) | 152(2.22) | 158(1.47) |
| **F7** | 328(3.12) | 166(2.98) | 162(1.99) |
| **F8** | 312(4.83) | 152(4.69) | 160(3.70) |
| **F9** | 322(2.14) | 162(2.00) | 160(1.01) |
| **F10** | 318(3.87) | 158(3.19) | 160(3.21) |
| **F11** | 329(5.88) | 160(4.90) | 169(4.92) |
| **F12** | 328(2.89) | 163(2.21) | 165(2.23) |

From the above table it was found that fill weight of the soft gel capsule is in a specific limit. Soft gelatin capsule weight was ranging 320 mg±5% (304-336 mg) for capsule before drying Target wt – 160mg/Capsule fill contents and the shell weight. Which is less than 5 % indicates that the variation in the weight of the Capsule is within standard official limits. No weight variation was observed, as the medicaments characteristics were maintained through the development process.

**Table 5: Average Weights of Capsules(Final Product) from each Batch.**

| Batch No. | Average Weight (mg.) |
|---|---|
| **F1** | 258.57 |
| **F2** | 261.22 |
| **F3** | 255.86 |
| **F4** | 260.65 |
| **F5** | 262.22 |
| **F6** | 256.89 |
| **F7** | 262.11 |
| **F8** | 260.1 |
| **F9** | 260.14 |
| **F10** | 261.19 |
| **F11** | 262.53 |
| **F12** | 262.61 |

Note: Average capsule weight of batch F9 and F10 was found to be 260.10 mg and 260.19 resp. which is in a specific limit.

**Table 6: Viscosities of Various Batches are as Follows.**

| Batch No. | Viscosity (cps) |
|---|---|
| **F1** | 17500 |
| **F2** | 20000 |
| **F3** | 18000 |
| **F4** | 16900 |
| **F5** | 18900 |
| **F6** | 19700 |
| **F7** | 20000 |
| **F8** | 18200 |
| **F9** | 19300 |
| **F10** | 19800 |
| **F11** | 18700 |
| **F12** | 18300 |

The viscosity of Gel mass is determine by using a Brookfield Viscometer and observed the viscosity in a ranges 16900-19300cps.

**Table 7: Gel Ribbon Thickness of Different Batch.**

| Batch No. | Right Ribbon Thickness (mm) | Left Ribbon Thickness (mm) |
|---|---|---|
| **F1** | 0.92 | 0.92 |
| **F2** | 0.92 | 0.92 |
| **F3** | 0.88 | 0.88 |
| **F4** | 0.96 | 0.96 |
| **F5** | 0.92 | 0.92 |
| **F6** | 0.89 | 0.89 |
| **F7** | 0.93 | 0.93 |
| **F8** | 0.91 | 0.91 |
| **F9** | 0.9 | 0.91 |
| **F10** | 0.9 | 0.9 |
| **F11** | 0.91 | 0.89 |
| **F12** | 0.92 | 0.91 |

Thickness of Soft Gelatin Capsule was observed by Vernier Caliper. The results obtained did not show any measurable deviation thickness of Capsule. Capsule Gel ribbon should be maintained at 0.87-0.93 in ranges.

**Table 8: Disintegration Time at the Time of Manufacturing.**

| | Time (Min:Sec) (Total content out) | | | | | |
|---|---|---|---|---|---|---|
| **Capsule** | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** |
| **1** | 6:52 | 6:13 | 6:15 | 6:06 | 6:44 | 5:24 |
| **2** | 6:45 | 6:19 | 5:56 | 5:57 | 6:38 | 6:54 |
| **3** | 6:39 | 5:49 | 5:34 | 6:55 | 5:46 | 5:19 |
| **4** | 5:23 | 4:03 | 6:58 | 7:11 | 6:03 | 6:16 |
| **5** | 4:41 | 3:12 | 6:14 | 5:19 | 6:47 | 7:13 |
| **6** | 5:14 | 6:36 | 6:52 | 6:15 | 7:29 | 6:01 |
| **Avg. DT** | **5:55** | **5:22** | **6:18** | **6:17** | **6:34** | **6:11** |

| | Time (Min:Sec) (Total content out) | | | | | |
|---|---|---|---|---|---|---|
| **Capsule** | **F7** | **F8** | **F9** | **F10** | **F11** | **F12** |
| **1** | 7:16 | 6:37 | 6:46 | 6:09 | 6:48 | 6:46 |
| **2** | 6:11 | 7:49 | 7:04 | 6:25 | 7:50 | 7:13 |
| **3** | 6:54 | 6:22 | 5:36 | 5:49 | 7:23 | 5:45 |
| **4** | 7:42 | 7:56 | 6:29 | 6:53 | 5:58 | 7:06 |
| **5** | 7:12 | 7:09 | 6:57 | 6:19 | 6:19 | 6:48 |
| **6** | 6:56 | 6:54 | 6:09 | 6:13 | 6:25 | 7:14 |
| **Avg. DT** | **7:01** | **7:07** | **6:30** | **6:18** | **6:47** | **6:48** |

Disintegration test was carried out in Electro lab (ED-2AL). Disintegration time for 6 Capsule was found to be 5-7 min indicating that disintegration time within the specification limit.

**Table 9: Content Uniformity of Various Batches.**

| Batch No. | Assay % w/w (RSD) |
|---|---|
| **F1** | 96.2 (4.3) |
| **F2** | 95.7 (3.1) |
| **F3** | 96.9 (4.9) |
| **F4** | 97.3 (2.8) |
| **F5** | 98.1 (3.8) |
| **F6** | 100.2 (4.2) |
| **F7** | 101.5 (4.5) |
| **F8** | 99.7 (5.1) |
| **F9** | 100.3 (2.7) |
| **F10** | 100.7 (1.5) |
| **F11** | 99.9 (3.8) |
| **F12** | 101.9 (2.6) |

Content uniformity by Assay Method is use to determine them and it was observed that in ranges 98-101 %w/w.

**Table 10: Disintegration Time of Final Product.**

| | Time (Min:Sec) (Total content out) | | | | | |
|---|---|---|---|---|---|---|
| **Capsule** | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** |
| **1** | 8:52 | 8:13 | 7:15 | 8:06 | 6:44 | 5:24 |
| **2** | 7:35 | 6:19 | 7:56 | 7:57 | 7:38 | 6:54 |
| **3** | 8:39 | 7:49 | 7:34 | 6:55 | 7:46 | 5:19 |
| **4** | 8:23 | 8:03 | 6:58 | 8:11 | 8:03 | 6:16 |
| **5** | 7:41 | 8:12 | 6:14 | 8:19 | 6:47 | 7:13 |
| **6** | 8:14 | 7:36 | 6:52 | 7:15 | 8:29 | 6:01 |
| **Avg. DT** | **8:14** | **7:42** | **7:08** | **7:47** | **7:34** | **6:11** |

| | Time (Min:Sec) (Total content out) | | | | | |
|---|---|---|---|---|---|---|
| **Capsule** | **F7** | **F8** | **F9** | **F10** | **F11** | **F12** |
| **1** | 7:16 | 7:37 | 7:06 | 6:09 | 8:48 | 6:46 |
| **2** | 8:11 | 7:49 | 6:44 | 7:25 | 7:50 | 7:13 |
| **3** | 6:54 | 6:22 | 7:36 | 7:49 | 7:23 | 5:45 |
| **4** | 7:42 | 7:56 | 7:29 | 7:53 | 5:58 | 7:06 |
| **5** | 7:12 | 8:09 | 6:57 | 7:19 | 6:19 | 6:48 |
| **6** | 6:56 | 6:54 | 8:09 | 8:13 | 6:25 | 7:14 |
| **Avg. DT** | **7:21** | **7:27** | **7:20** | **7:28** | **7:07** | **6:48** |

Average disintegration time of batch F09 and F10 was found to be 7:28 and 7:07 which is comparable to that of innovator. Disintegration test was carried out in Electro lab (ED-2AL). Disintegration time for 6 Capsule was found to be 6-8 min in each batch indicating that disintegration time within the specification limit**.**

**Table 11: Loss on Drying (%w/w) of Finished Product**.

| **Condition** | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** |
|---|---|---|---|---|---|---|
| LOD (%w/w) | 11.04 | 12.46 | 11.13 | 10.16 | 10.29 | 10.88 |

| **Condition** | **F7** | **F8** | **F9** | **F10** | **F11** | **F12** |
|---|---|---|---|---|---|---|
| LOD (%w/w) | 10.25 | 10.53 | 9.26 | 9.48 | 10.33 | 10.91 |

**L.O.D. of Innovator:** 9.34 %w/w

LOD of soft gel capsule maintained in the level by drying at 105°C in a particular time 3 hours interval and optimize drying time for achieve LOD in particular limit. Initial LOD of the batch is near about 37-43 %w/w and after 36 Hr it was observe that the %w/w. we will maintain at ranges between 8-12 % w/w.for finished product & 10-22% for stability conditions which are the limit specified and batch no F9 and F10 are obtained in this range.

**Table 12: Shell Thickness and Seam Thickness of Various Batches.**

| Batch No. | Overall Shell Thickness (mm) | (Seam thickness mm) |
|---|---|---|
| **F1** | 0.51 | 0.6 |
| **F2** | 0.55 | 0.54 |
| **F3** | 0.54 | 0.6 |
| **F4** | 0.56 | 0.58 |
| **F5** | 0.51 | 0.55 |
| **F6** | 0.56 | 0.55 |
| **F7** | 0.57 | 0.58 |
| **F8** | 0.55 | 0.59 |
| **F9** | 0.52 | 0.6 |
| **F10** | 0.52 | 0.61 |
| **F11** | 0.53 | 0.59 |
| **F12** | 0.54 | 0.6 |

Overall Shell thickness and Seam thickness of batch F9 & F10 was found to be in accordance with Innovator product shell & seam thickness. Thickness of Soft Gelatin Capsule was observed by Vernier Caliper. The results obtained did not show any measurable deviation thickness of Capsule. Capsule overall shell thickness 0.52-0.55mm and seam thickness 0.54-0.61mm should be obtained.

**Table 13: Rupture Time for All Batches Are as Follows.**

| Batch No. | Rupture time Min: Sec |
|---|---|
| F1 | 1:59 |
| F2 | 2:11 |
| F3 | 1:54 |
| F4 | 3:39 |
| F5 | 2:51 |
| F6 | 1:58 |
| F7 | 2:31 |
| F8 | 3:27 |
| F9 | 2:09 |
| F10 | 2:21 |
| F11 | 2:32 |
| F12 | 2:54 |

Rapture test is important and carried out as per USP and find out the rapture time. It is observed that 1-3.5min all batches should be rapture within this time. It means the entire batch passes the rapture test.

**Table 14: Evaluation of F9 after Different Time Interval at Different Temperature.**

| Conditions | Parameters Evaluated | Storage Conditions | | | | |
|---|---|---|---|---|---|---|
| | | Initial | 15 days open | 1 month | 2 month | 3 month |
| **25°C/60%RH** | Weight gain Shell (%) | 0.59 | 11.60 | 2.64 | 2.88 | 3.59 |
| | Assay | 99.92 | 101.06 | 99.22 | 100.41 | 100.18 |
| | DT | 8:10 | 9:24 | 8:17 | 9:01 | 8:43 |
| | L.O.D. (%w/w) | 9.29 | 15.42 | 12.79 | 11.68 | 11.94 |
| **30°C/65%RH** | Weight gain Shell (%) | 0.46 | 12.91 | 2.65 | 2.48 | 2.62 |
| | Assay | 100.9 | 96.71 | 98.84 | 100.2 | 99.63 |
| | DT(min) | 8:08 | 9:47 | 8:31 | 8:20 | 8:12 |
| | L.O.D. (%w/w) | 9.32 | 14.56 | 10.12 | 9.85 | 9.61 |
| **40°C/75%RH** | Weight gain Shell (%) | 0.61 | 12.58 | 2.88 | 2.19 | 2.96 |
| | Assay | 100.32 | 99.43 | 100.33 | 99.81 | 100.62 |
| | DT | 8:10 | 9:21 | 8:27 | 9:03 | 9:15 |
| | L.O.D. (%w/w) | 9.26 | 14.16 | 9.45 | 9.82 | 9.33 |

**Table 15: Evaluation of F10 after Different Time Interval at Different Temperature.**

| Conditions | Parameters Evaluated | Storage condition | | | | |
|---|---|---|---|---|---|---|
| | | Initial | 15 days open | 1 month | 2 month | 3 month |
| **25°C/60%RH** | Weight gain Shell (%) | 0.49 | 13.62 | 2.29 | 2.91 | 2.51 |
| | Assay | 100.9 | 99.16 | 99.86 | 100.6 | 100.6 |
| | DT | 7:13 | 8:19 | 7:33 | 7:28 | 8:02 |
| | L.O.D. (%w/w) | 9.42 | 14.73 | 11.28 | 11.35 | 11.46 |
| **30°C/65%RH** | Weight gain Shell (%) | 0.42 | 12.70 | 1.93 | 12.36 | 2.00 |
| | Assay | 100.2 | 99.59 | 100.65 | 100.3 | 100.60 |
| | DT | 7:31 | 8:36 | 7:50 | 7:16 | 7:29 |
| | L.O.D. (%w/w) | 9.56 | 14.2 | 10.22 | 9.76 | 9.91 |
| **40°C/75%RH** | Weight gain Shell (%) | 0.58 | 13.9 | 2.26 | 3.59 | 3.45 |
| | Assay | 100.3 | 99.69 | 99.93 | 99.96 | 100.02 |
| | DT | 7:51 | 8:06 | 7:30 | 7:19 | 7:28 |
| | L.O.D. (%w/w) | 9.47 | 13.39 | 9.88 | 9.31 | 9.56 |

The optimized batch was placed in also stability chamber and analyzed in the same way just like a final evaluation parameter. It is found that all parameter is near about to same in comparison with a final product evaluation.

**ACKNOWLEDGEMENT**

In the presents investigation an attempt was made to formulate a capsule formulation containing calcitriol Drug. The study was aimed at developing a stable soft gelatin capsule formulation doing in Rupture test, LOD, Assay identical to innovator product Rocaltrol. Calcitriol soft gelatin capsule was prepared by rotary die process, this method was very useful for the finish product. By comparing data of all of the above batches results of F09 & F10 were satisfactory as well as up to the mark. So batches F09 & F10 were subjected to comparative study with innovator Rocaltrol for parameters. Study results states that Calcitriol soft gelatin capsules 0.5 mcg & 0.25 mcg F09 & F10 respectively were stable and pharmaceutically equivalent to innovator product Rocaltrol. These meet the objective of study for the Development of Calcitriol soft gelatin capsules 0.5 mcg & 0.25 mcg.

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