Section A-Research paper
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Formulation Development & Evaluation of Vitamin D3 Nanoparticles

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ABSTRACT:
The number of poorly soluble drugs has significantly increased during the past ten years. The existing marketed product for Vitamin D3 is available in tablets, soft gelatin capsules, capsules, sachets, liquid, and powder in a sachet dosage form. In all these existing products, the overages are added upto 50%. The major reason for the addition of overages is the degradation of Vitamin D3. The present pharmaceutical composition and a process for preparing the same without any overages. Vitamin D3 capsules for the 2000IU are prepared by Self-Nano Emulsifying Drug Delivery System technology when the hydrophilic solvent system comes in contact with the hydrophobic precursor. Poloxamer 188, Sorbic acid, and BHT are the integral ingredients used for the stabilization of Vit.D3. A developed formulation is stable in accelerated, intermediate, and long-term stability. The role of antioxidant and preservative has an integral role in the stabilization of Vitamin.D3 formulation. The prepared formulation when dissolved in water leads to the formation of a transparent solution containing the drug particles less than 100nm, which is readily absorbed in the body fluid. The stability data and extrapolation reveals that Vit.D3 formulation is stable in the Accelerated stability testing, Intermediate Stability testing and long term stability testing conditions as per ICH guidelines for various attributes like weight variation, disintegration assay, and dissolution.

KEYWORDS:
Self-Nano emulsifying drug delivery systems (SNEDDS), Nanoparticle, Stable, Cholecalciferol, free from overages.

INTRODUCTION:

Vit.D (Vitamin D) is a fat-soluble nutrient that regulates the homeostasis of calcium, and phosphorus as well as cell proliferation, differentiation, apoptosis, immune regulation, genome stability, and neurogenesis. Naturally, Vit.D is available in two different chemical forms Vit.D3 (Cholecalciferol) and Vit.D2 (ergocalciferol). Amongst these, Vit.D3 which is also called "Sunshine Vitamin" gets synthesized under the skin on exposure to ultraviolet light, whereas Fish oil and various types of mushrooms are rich sources of Vit.D2 (ergocalciferol). As per the various pharmacological investigations, more than 90% requirement of Vit. D is fulfilled by Vit. D3 produced under the skin and nearly 10% from food sources[1,2,3,4,5,6,7]. Consequently, low exposure to sunlight in countries where exposure to UV (Ultraviolet) rays is a less and sedentary lifestyle, the use of sunscreen lotions as cosmetics are the leading causes of "Hypovitaminosis D" or Vit.D deficiency/insufficiency. It is estimated that, globally one billion people have Vit.D deficiency leading to bone metabolic disorders, cardiovascular diseases, and diabetes as well as a risk factor for neuropsychiatric disorders and autoimmune diseases[8,9,10]. Hypovitaminosis D is mainly observed in children, pregnant women, and the elderly. In the Indian subcontinent (both rural and urban populations),

approximately 70 to 100% of the population suffers from Hypovitaminosis Dets[11,12, 13]. The report by "International Osteoporosis Foundation" indicates the prevalence of hypovitaminosis D in 78% of the population living in India. In addition, the maximum deficiency was observed in females (70%) than males[14,15,16,17,18.] Prevalence of hypovitaminosis D with a rate of 80.9% was observed in women with age over 80 years in the European continent. In the American continent, Hypovitaminosis D is prevalent in 42% of adults, 82% of black individuals, and 69% of the Hispanic population[19,20,21]. The Dietary Reference Intakes (DRIs) of WHO indicate that, as per age and gender the daily intake of Vit.D may vary from 400 to 800 IU per day[22]. Accordingly, various pharmaceutical dosage forms ranging from the conventional tablet and soft gelatin capsule to Novel Drug Delivery Systems (NDDS) such as Solid Lipid Nanoparticles, Nano-emulsion, Self-Emulsifying Drug Delivery System, Solid Dispersion powders are available in the market[23]. Though the capsule dosage forms are well accepted by consumers, but vehicles used in the capsule dosage forms such as Polyethylene glycol, Tween, and the like are hydrophilic in nature, increasing the degradation of Vit.D. Consequently, dosage forms are prepared with the addition of overages up to 50% which in turn increases the cost and chances of toxicity due to Hypervitaminosis D. Recent market analysis on Vit.D dosage form showed that the powder sachets of Vit.D is also favorable among the consumers of all ages. However, the powdered dosage forms failed to provide uniform distribution of Vit.D in water due to the formation of lumps, which in turn decreases systemic absorption and increases fecal excretion of Vit.D[24]. EP 2201937A1 discloses a multi-particulate pharmaceutical delivery system of Vit.D comprising an inert core, an inner layer comprising Vit.D an emulsifier and an antioxidant, and an outer protective layer. US4929610 discusses pharmaceutical compositions wherein a pharmaceutical carrier is combined with a mixture of hydroxylated derivatives of Vit.D. In these compositions active substances are dissolved in a pharmaceutically usable solvent such as alcohol, propylene glycol, glycerin or polyethylene glycol, and surface-active agents are added therein. These mixtures are then filled in hard or soft gelatin capsules. WO03/059358 discloses an oil composition comprising 25-hydroxy Vit.D3 dissolved in the oil in an amount between 5% and 50% by weight of the total weight of the oil composition. This oil composition is then provided in the form of an emulsion, microencapsulated oil, or a feed premix[25,26,27].

Though various attempts have been made in the past none of the dosage forms are stable over the shelf life neither the processes are robust. Therefore, there is an unmet need to develop a novel and robust composition of Vit.D.

Disadvantages of existing Vit.D marketed formulation:

- Dosage form is available with overages upto 50%.
- Most of the dosage is in liquid dosage form which contains hydrophilic excipients, due to Vit.D getting degraded at increased temperature. The price of the bottle pack is higher compared to conventional tablets and capsules and sachets dosage forms.
- As the particle size of Vit.D is increase, the absorption of the drug is decreased, which leads to fecal loss of Vit.D.[28].
- Due to overages upto 50%, chances of toxicities are increased in patients.
- This dosage form is degraded at a rate of 8 to 12% per month.
- This formulation contains hydrophilic excipients at increased Temp. (Temperature), Vit.D gets degraded, hence need to add overages upto 50%.
- The frequency of dosage is increased to get desired therapeutic effect.
- Most of the existing Vit.D dosage form fails in stability conditions as per ICH guidelines.
- During dispersing/ dissolving the contents of dosage form get stuck to the wall of glass or on the surface of a spoon, in that also some amount of drug gets wasted (mainly seen in sachets or dispersible dosage form)
- Soft gelatin dosage form is not accepted by some vegetarian populations[29,30].

Advantages of Innovative formulations:

- Claim dosage form without overages.
- Claim dosage form is in a dry state with preservatives and antioxidants.
- After dissolving in water the particles of the drug are converted into nanoparticles, which are directly absorbed into the systemic circulation.
- Toxic effect is reduced due to no overages.
- Claim dosage form is in a dry state with preservatives and antioxidants.
- The frequency of dosage form is decreased to get desired therapeutic effects.
- Claim dosage form is most stable compared to the existing dosage available on the market.
- Claim dosage form is stable in all the stability conditions as per ICH guidelines. (Accelerated, intermediate, and long-term conditions)
- The claim dosage form can be available at an economical/current market price.
- The claim dosage form can be taken with water, milk, and fruit juice.

- The vegetarian (HPMC) capsule shell, MUPS, and sachet are used for filling the drug pellets[31].

MATERIALS AND METHODS:

A gift sample Vit.D3 (Cholecalciferol) received from M/s. MSN Labs, Sugar globules 30-40# (Mesh) from M/s. Pharm- A- spheres, Vit.E polyethylene glycol succinate (TPGS) from M/s. BASF, Poloxamer 188 from M/s. Spectrum, Sorbic acid from M/s. Merck & HPMC E-5 from M/s.Shin-Etsu.

Method for Preparation of Vitamin D3 Nanoparticles:

Vit.D3 capsules for the 2000IU is prepared by Self-Nano emulsifying Drug Delivery System (SNEDDS) technology when the hydrophilic solvent system comes in contact with the hydrophobic precursor. Poloxamer 188, Sorbic acid, and BHT are the integral ingredients used for the stabilization of Vit.D3[32].

Step I: Preparation of Seal coat: In this step, the seal coat is prepared by dissolving the binder and antioxidant into a solvent mixture (solvent ratio of 70:30 to 60:40). The non-limiting examples of the solvents for seal coat include IPA (Isopropyl Alcohol), MDC (Dichloromethane), methanol, and acetone.

Step II: Seal coating of sugar pellets: This step is very crucial in sealing the hygroscopicity of the sugar pellets and thereby improving the shelf life of the composition. Therefore, the seal coat so prepared in Step I is sprayed onto the sugar pellet at a bed Temp. ranging from 31°C to 33°C and at a spraying speed of 1 to 3gm/min.

Step III: Preparation of drug solution: The Vit.D and TPGS is dissolved into the solvent at a ratio of 70:30 to 60:40 at a speed of 500 RPM for 20min to obtain a drug solution. The non-limiting examples of the solvents for seal coat include isopropyl alcohol, dichloromethane, methanol, and acetone. The binder, preservative, and antioxidant are then mixed with the drug solution to obtain a drug coating solution.

Step IV: Preparation of Vit. Pellets: The drug coating solution prepared in Step III is then sprayed on to seal coated pellets at speed of 1 to 3gm/min to obtain the Vitamin pellets. In this step, the dew point is maintained in the range of 12 to18[33,34,35].

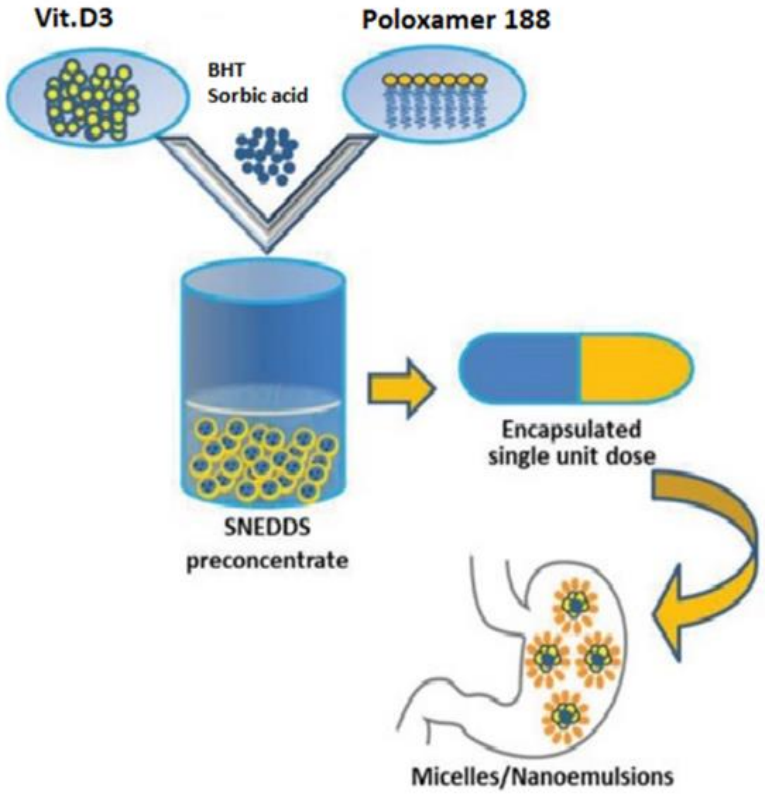


Fig. 1 Vitamin D3 Precursor formulation

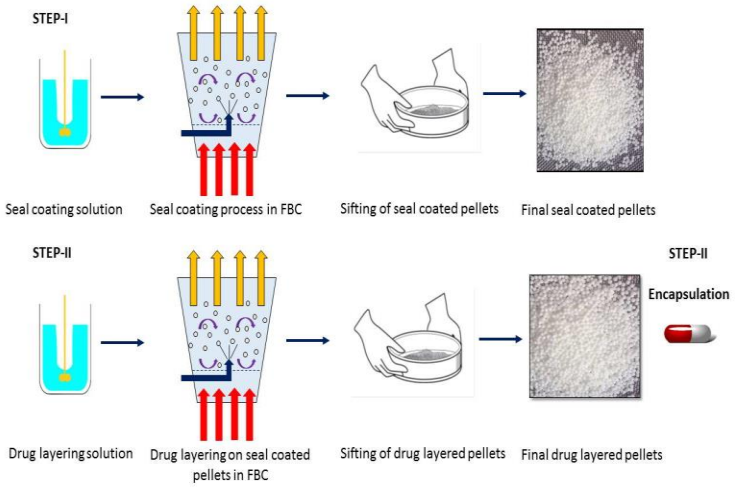


Fig 2 Seal coating and Drug layering step in the formulation of Vit.D3 pellets.

Table 1 Optimization batches for Vit.D3 Seal coating for 2000IU by changing binding concentration.

Ingredients	**Batch No.**				
	OPT 2001	OPT 2002	OPT 2003	OPT 2004	OPT 2005
Sugar globules 30-40#	83.809	83.809	83.809	83.809	83.809
BHT	0.043	0.043	0.043	0.043	0.043
Co-povidone VA-64	2.200	3.080	4.147	6.600	8.800
IPA 70%	53.338	53.338	53.338	53.338	53.338
DCM 30%	26.271	26.271	26.271	26.271	26.271
Total	165.662	166.542	167.608	170.062	172.262
Loss of Solvent	79.61	79.610	79.610	79.61	79.610
Total wt. of seal coated pellets	86.052	86.932	88.000	90.452	92.652

Table 2 Forced Degradation studies for Seal Coating pellets

Batch No.	**Temp.**	**Condition**	**Days**	**Physical condition (Initial)**	**Physical condition (Final)**
OPT2001 (2.5%)	40°C/90%RH	Opened (pin hole bottle)	7 Days	White to off-colored free-flowing pellets.	Sticky, doublets and triplites were found. Pellets were not free-flowing
		Closed (Bottle)			
OPT2002 (3.5%)		Opened (pinhole bottle)		White to off-colored free-flowing pellets.	Sticky, doublets and triplites were found. Pellets were not free-flowing
		Closed (Bottle)			
OPT2003 (5.0%)		Opened (pinhole bottle)		White to off-colored free-flowing pellets.	White to off-colored free-flowing pellets.
		Closed (Bottle)			
OPT2004 (7.5%)		Opened (pinhole bottle)		White to off-colored free-flowing pellets.	White to off-colored free-flowing pellets.
		Closed (Bottle)			
OPT2005 (10%)		Opened (pinhole bottle)		White to off-colored free-flowing pellets.	White to off-colored free-flowing pellets.

Table 3 Optimization Batches for Drug layering formula for Vit.D3 capsules with Co-Povidone VA-64

Ingredients	**Batch No.**				
	OPT 2001	**OPT 2002**	**OPT 2003**	**OPT 2004**	**OPT 2005**
Total seal coated pellets	88.00	88.00	88.00	88.00	88.00
Cholecalciferol (Vit.D3)	0.068	0.068	0.068	0.068	0.068
Vit.E TPGS	0.167	0.167	0.167	0.167	0.167
Poloxamer 188	0.167	0.167	0.167	0.167	0.167
Co-povidone VA 64	0.764	1.582	2.292	3.056	3.820
Sorbic acid	0.008	0.008	0.008	0.008	0.008
BHT	0.008	0.008	0.008	0.008	0.008
IPA 70%	25.460	25.460	25.460	25.460	25.460
DCM 30%	12.540	12.540	12.540	12.540	12.540
Total	127.182	127.946	128.710	129.474	130.238
Loss of Solvent	38.00	38.000	38.000	38.000	38.000
Total wt. of seal coated pellets	89.182	89.946	90.710	91.474	92.238
% Dispersion	5%	5%	5%	5%	5%
Capsule size	3	3	3	3	3

Table 4 Optimization Batches for Drug layering formula by change Poloxamer 188 concentration

Ingredients	Batch No.				
	OPT 2001	**OPT 2002**	**OPT 2003**	**OPT 2004**	**OPT 2005**
Total seal coated pellets	88.00	88.00	88.00	88.00	88.00
Vit D3	0.068	0.068	0.068	0.068	0.068
Vit.E TPGS	0.167	0.167	0.167	0.167	0.167
Poloxamer 188	0.084	0.167	0.334	0.251	0.335
Co-povidone VA 64	1.582	1.582	1.582	1.582	1.582
Sorbic acid	0.008	0.008	0.008	0.008	0.008
BHT	0.008	0.008	0.008	0.008	0.008
IPA 70%	25.460	25.460	25.460	25.460	25.460
DCM 30%	12.540	12.540	12.540	12.540	12.540
Total	127.917	128.000	128.167	128.084	128.168
Loss of Solvent	38.000	38.000	38.000	38.000	38.000
Total wt. of seal coated pellets	89.917	90.000	90.167	90.084	90.168
% Dispersion	5%	5%	5%	5%	5%
Capsule size	3	3	3	3	3

Table 5 Optimization Batches for Drug layering formula by change in Co-Povidone VA-64 conc.

Parameters	Limit	Batch No.				
		OPT 2006	OPT 2007	OPT 2008	OPT 2009	OPT20010
Description	White cap and yellow body-colored capsule containing white to off white pellets					
Weight of empty capsule size " 3 "	46 to 50	48.833	48.716	49.239	48.543	48.669
Weight of pellets	85 to 95	90.138	90.102	90.217	90.369	90.639
Weight of filled capsules	130 to 146	138.80	137.98	138.36	138.92	138.96
Lock length (mm)	15.25-16.50	15.45	15.85	15.56	15.57	15.65
Disintegration time (Minutes)	NMT 15	3	4	3	4	3
Assay	90 to 110	99.99	100.16	100.16	100.38	100.45
Dissolution	NLT 80% in 30 min.	99.93	100.12	92.86	86.78	81.34

CHARACTERIZATION:

1. Assay: By infrared Absorption: The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the standard preparation, obtained in the assay.
2. Loss on drying: Drying in the vacuum at 80°C for 4 hrs: it loses NMT 0.5% of its weight.
3. Specific rotation: + 105° to + 112°
4. Residual Solvents: IPA, DCM, Acetone, and Methanol were found to be within the limit.
5. Packaging and storage: Preserve hermetically under nitrogen, in a cool place, and protected from light.

Assay Estimation: Weigh and transfer an accurately weighed portion of the powder, equivalent to about 20 µg of Cholecalciferol to a container having a polytheized screw cap. Add 8 mL of dimethyl sulfoxide and 12ml of hexane, and shake for 45 min. on a wrist action shaker with tubes in a bath maintained at 60. Centrifuge for 10 min.. Withdrawn the hexane layer using pipette and transfer to an evaporation flask. Add 12 mL of n-hexane to the dimethyl sulfoxide layer, mix on a vortex mixer for 5 min., and again withdraw the hexane layer using a Pipette and add to the evaporation flask. Repeat this extraction with three additional 12-mL portions of n-hexane, adding the hexane extracts to the evaporation flask. Evaporate the combined hexane extracts in a vacuum at room temperature to dryness. Dissolve the residue in a known volume of n-hexane, and dilute quantitatively with n-hexane to obtain a solution having a concentration of about 2 µg per ml.

Mobile phase— Prepare a filtered and degassed mixture of n-hexane and IPA.

Chromatographic system the liquid chromatograph is equipped with a 265-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L8. Flow-1 ml/min. Chromatograph the System suitability preparation, and record the peak responses as directed for Procedure: the resolution, R, between the Vit.D form present and its corresponding precursor is not less than 10. Inject standard solution & record the chromatogram. The % RSD for replicate injections should not be more than 3%

Procedure: separately injects equal volumes (about 100 µL) of the Standard preparation and the assay preparation into the chromatograph, record the chromatograms, and measure the responses for the Vit.D peaks. Calculate the quantity, in µg, of ergocalciferol ($C_{28}H_{44}O$) or cholecalciferol ($C_{27}H_{44}O$) in the capsule by the formula:

1.09CD (rU)

in which 1.09 is a correction factor to account for the average amount of previtamin D present in the formulation, C is the concentration, in µg per mL, USP Cholecalciferol RS in the Standard preparation; D is the dilution factor, in

mL, used to prepare the Assay preparation; and rU is the peak heights for cholecalciferol obtained from the Assay preparation and the Standard preparation, respectively

Dissolution Procedure: Dissolution test carried out using 6 capsules. Dissolution testing carried out using USP type 2 (Paddle) apparatus at 75 RPM using 0.3 % SDS in water as dissolution medium using 500 ml dissolution media. Methanol used as diluent. Standard Preparation: -Transfer about 20mg(18-22mg) of Cholecalciferol Working Standard (WS) (40,000IU/mg), accurately weighed, to a 100ml of volumetric flask. Dissolve in and dilute to volume with diluent, mix well. Pipette 5ml of this solution into a 100ml of volumetric flask and dilute to volume with diluent, mix well (about 400 IU/ml of Cholecalciferol). Sample Preparation: Shake well and determine the weight per ml of sample. Shake well and accurately weigh transfer 10gm (9.0 to 11.0gm) (Eq. to 120000 IU of Cholecalciferol) sample into 50ml of volumetric flask. Add 30ml of diluent and vortex it for 1 min. sonicates for about 30ml of diluent to volume and vortex it for 1 min, sonicated for about 30 min. with intermittent shaking. Dilute to volume with diluent, mix well and centrifuge at 4000rpm for 5 min.to get clear supernatant. Pipette 4ml of clear solution into 25ml of volumetric flask and dilute to volume with diluent and mix well. Procedure: Set up the chromatographic system as described under instrumental conditions. Determine the system precision by injecting five replicate injections should not be more than 5.0%. The retention time of the Cholecalciferol peak is about 8.5min. The number of theoretical plates should not be less than 2000 and the tailing factor should not be more than 2.0 Prepare samples, make injections and calculate mean area counts for each sample. Stability Studies for Cholecalciferol Capsule 2000IU in PVC-PVDC Blister pack (250/60gsm) for Accelerated, Intermediate, and Long-Term conditions. Stability Studies for Cholecalciferol Capsule 2000IU in PVC-PVDC Blister pack (250/60gsm) for Accelerated, Intermediate, and Long-Term conditions.

Table 6 Instrumental Condition: Use a suitable HPLC with the following conditions.

Column	**Luna C18, (250MM X 4.6 MM, 5µm) (Phenomenex, USA).**
Detector	265nm
Flow rate	1.0 ml/min
Column Temperature	40°C
Sample temperature	15°C
Injection volume	20µl
Run Time	20 min

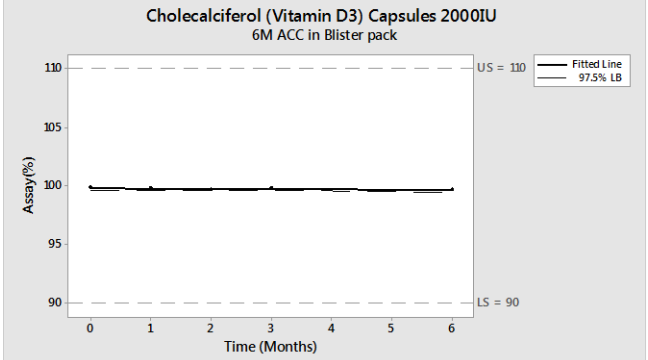
RESULTS:

Table 7 Result of optimization Batches for Drug layering formula by in change Poloxamer 188 conc.

Parameters	**Limit**	**Batch No.**				
		OPT 2011	**OPT 2012**	**OPT 2013**	**OPT 2014**	**OPT 20015**
Description	White cap and yellow body-colored capsule containing white to off white pellets					
Weight of empty capsule size " 3 "	46 -50	48.768	48.456	49.249	48.847	48.369
Weight of pellets	85-95	90.128	90.107	90.546	90.669	90.639
Weight of filled capsules	130-146	138.80	137.98	138.36	138.92	138.96
Lock length	15.25- 16.50	15.45	15.85	15.56	15.57	15.65
Disintegration time (min)	NMT 15	3	4	3	4	3
Assay	90 to 110	99.99	100.16	100.16	100.38	100.45
Dissolution	NLT 80% in 30 min..	76.65	98.56	98.76	99.10	99.12

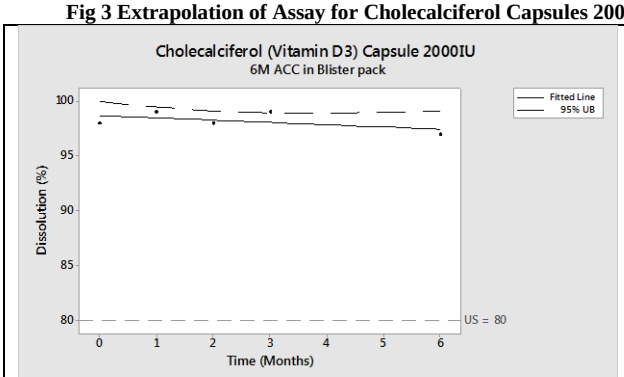
Table 8 Stability Studies for Cholecalciferol Capsule 2000IU in PVC-PVDC Blister pack (250/60gsm) for Accelerated, Intermediate, and Long-Term conditions

Batch No.	**Condition**	**Month**	**Description**	**Wt. of caps. (mg)**	**D.T (MM: SS)**	**Assay**	**% DR**
28840023	40°C/75% RH	0	White cap and yellow body-colored capsule.	91.780	2.25	99.87	98
28840023		3		92.213	2.45	99.36	99
28840023		6		92.860	2.33	98.54	97
28840023	30°C/65% RH	3		90.436	2.16	99.30	98
28840023		6		92.456	2.20	98.76	99
28840023	25°C/60% RH	3		92.347	2.43	98.43	98
28840023		6		93.456	2.20	99.87	99



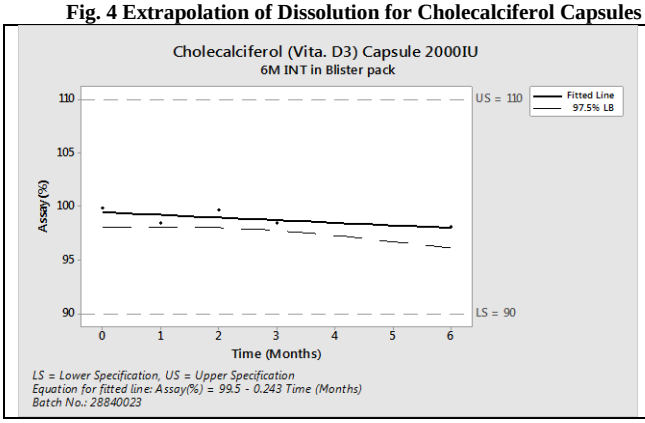
Shelf Life Estimation
Lower spec limit = 90 Upper spec limit = 110
Shelf life = time period in which you can be 95% confident that at least 50% of response is within spec limits
Shelf life = *
The mean response slope is not significantly different from zero. No shelf life estimate is available.

Fig 3 Extrapolation of Assay for Cholecalciferol Capsules 2000IU For ACC Stability Studies



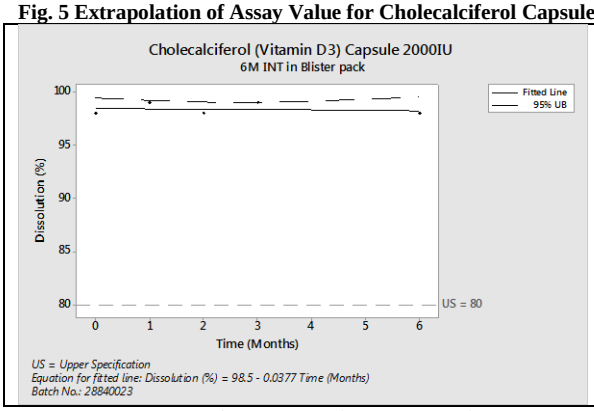
Shelf Life Estimation
Upper spec limit = 80
Shelf life = time period in which you can be 95% confident that at least 50% of response is below upper spec limit
Shelf life = *
The mean response at time 0 is not significantly smaller than the upper specification limit.
No shelf life estimate is available.

Fig. 4 Extrapolation of Dissolution for Cholecalciferol Capsules 2000IU for ACC Stability Studies



Shelf Life Estimation
Lower spec limit = 90 Upper spec limit = 110
Shelf life = time period in which you can be 95% confident that at least 50% of response is within spec limits
Shelf life = *
The mean response slope is not significantly different from zero. No shelf-life estimate is available.

Fig. 5 Extrapolation of Assay Value for Cholecalciferol Capsules 2000IU for INT Stability Studies



Shelf Life Estimation
Upper spec limit = 80
Shelf life = time period in which you can be 95% confident that at least 50% of response is below upper spec limit
Shelf life = *
The mean response at time 0 is not significantly smaller than the upper specification limit.
No shelf life estimate is available.

Fig. 6 Extrapolation of Dissolution for Cholecalciferol Capsules 2000IU for INT Stability Studies

Figure 3 is an extrapolation for 6 month accelerated stability condition, revealing the information that there is no significant impact of temperature and humidity on the assay value of drug layered pellets. The dosage form is found to be stable in PVC/PVDC blister packs. The assay value of not deflected by any external factors because it is in a dry state in presence of preservatives, antioxidants, and stabilizers. The formulation passes in accelerated stability conditions as per ICH guidelines.

Figure 4 is an extrapolation for 6 month accelerated stability condition, revealing the information that there is no significant impact of temperature and humidity on the dissolution rate of drug layered pellets. The dosage form is found to be stable in PVC/PVDC blister packs. The dissolution value is found to be in the specification range, during all the stability conditions. The formulation passes in accelerated stability conditions in dissolution criteria as per ICH guidelines.

Figure 5 is an extrapolation for 6 month accelerated stability condition, revealing the information that there is no significant impact of temperature and humidity on the assay value of drug layered pellets. The dosage form is found to be stable in PVC/PVDC blister packs. The assay value of not deflected by any external factors because it is in a dry state in presence of preservatives and stabilizers. The formulation passes in accelerated stability conditions as per ICH guidelines.

Figure 6 is an extrapolation for 6 month accelerated stability condition, revealing the information that there is no significant impact of temperature and humidity on the dissolution rate of drug layered pellets. The dosage form is found to be stable in PVC/PVDC blister packs. The dissolution value is found to be in the given range, during all the stability conditions. The formulation passes in accelerated stability conditions in dissolution criteria as per ICH guidelines.

DISCUSSION:

The prepared Vit.D3 pellets are prepared without adding any overages to avoid the toxic effect of Vit. D3. The dosage form is in a dry state and the role of preservatives and antioxidants in an optimum ratio plays important in the stabilization of formulation. After adding the drug pellets to a glassful of water the transparent solution is formed and the particle size is less than 100nm which is directly absorbed into the systemic circulation. The Vit.D3 drug layered nanoparticles were developed and optimized to form the soluble system. Analytical methods were developed for the estimation of Vit.D3 in drug-layered pellets. The nanoparticle formulation was characterized for particle size, P.D.I, Zeta-potential, and in vitro drug release studies, which reveals the formation of Vitamin D3 nanoparticles with release profile. The stability data and extrapolation reveal that Vit.D3 formulation is stable in the ACC, INT, and LT conditions as per ICH guidelines for various attributes like assay, and dissolution. The following significant conclusions were obtained from the above result:

- The formulation contains drug pellets without any overages.
- The drug particle size of less than 100nm reveals Vit.D3 fastest absorption in the body fluid.
- In vitro drug release studies reveal the formation of Vitamin D3 nanoparticles with a release profile.
- The stability data and extrapolation reveal that Vit.D3 formulation is stable in the ACC, INT, and LT conditions as per ICH guidelines.
- Vit.D3 formulation is the most stable compared to the existing dosage available on the market.

CONCLUSION:

The current marketed Vit. D3 formulation is available with overages of vit. D3. By avoiding or deleting overages of Vit. D3 from the developed formulation i.e. Vit. D3 stabilized nanoparticle the dose accuracy is maintained.

ABBREVIATIONS:

Table 7 List of Abbreviations

Abbreviation	Meaning	Abbreviation	Meaning
Vit. D	Vitamin D	RPM	Revolutions per minute
Vit.D2	Vitamin D2	Min	Minutes
Vit.D3	Vitamin D3	BHA	Butylated Hydroxyanisole
SNEDDS	Self-Nano Emulsifying Drug Delivery System	Wt.	Weight
UV	Ultraviolet	Hrs	Hours
%	Percentage	NMT	Not More Than
WHO	World Health Organization	NLT	Not Less Than
IU	International Unit	mm	Millimetre
Temp	Temperature	Conc.	Concentration
ICH:	International Council for Harmonisation	µg	Microgram
HPMC	Hydroxypropyl methylcellulose	mL	Millilitre
MUPS	The Multiple-Unit Pellet System	nm	Nanometer
#	Mesh	cm	Centimeter

Abbreviation	Meaning	Abbreviation	Meaning
M/s	Messrs	µL	Microliter
Eq:	Equivalent	USP	United States Pharmacopeia
mg	Milligram	HPCL	High Performance Liquid Chromatography
BHT	Butylated Hydroxytoluene	Gsm	Grams Per Square Metre
IPA	Isopropyl Alcohol	ACC	Accelerated Stability Testing
MDC	Dichloromethane	INT	Intermediate Stability Study
°C	Degree Celsius	LT	Long Term Stability Study
TPGS:	Tocopheryl polyethylene glycol succinate	RS	Reference Standard

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

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