

**Relative oral bioavailability of three formulations of vitamin D<sub>3</sub>: an open-label, three-treatment study****Krishnakumar M. Nandgaye<sup>1\*</sup>, Rajaram S. Samant<sup>2</sup>, Santoshi B. Kadam<sup>1</sup>, Prashant J. Palkar<sup>1</sup>**

<sup>1</sup>Department of Medical Services, <sup>2</sup>CEO and Chief Mentor, Akumentis Healthcare Ltd., G-Corp Tech Park, Kasarvadavali, Near Hypercity, Ghodbunder Road, Thane (West), Maharashtra, India

**Received:** 26 October 2018**Accepted:** 29 November 2018**\*Correspondence to:**

Krishnakumar M. Nandgaye,  
Email: [krishnan@myakumentis.com](mailto:krishnan@myakumentis.com)

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**ABSTRACT**

**Background:** Supplementation of vitamin D<sub>2</sub> or vitamin D<sub>3</sub> is recommended for vitamin D deficiency. Weekly supplementation of 60,000 IU of vitamin D<sub>3</sub> increases serum 25(OH) D to optimal values. Various marketed forms of vitamin D<sub>3</sub> include tablets, capsule, granules and oral solution. The main objective of this study is to compare the relative bioavailability of vitamin D<sub>3</sub> oral solution with vitamin D<sub>3</sub> tablet and capsule.

**Methods:** This is an open-label, randomized, single-dose, three-treatment study to compare the relative bioavailability of vitamin D<sub>3</sub> oral solution with capsule and tablet. Subjects (n=70) were supplemented with single dose of one of these formulations and their blood sample were assessed for C<sub>max</sub>, AUC<sub>0-28d</sub> and T<sub>max</sub>.

**Results:** The logarithmic transformed data of pharmacokinetic parameters were analyzed for 90% Confidence Intervals (CI) using ANOVA. The mean (90% CI) values of vitamin D<sub>3</sub> oral solution against tablet for the ratio of C<sub>max</sub> and AUC<sub>0-28d</sub> were 113.00 (105.32-121.23) and 105.54 (97.95-113.72) respectively. The mean (90% CI) values of vitamin D<sub>3</sub> oral solution against capsule for the ratio of C<sub>max</sub> and AUC<sub>0-28d</sub> were 115.02 (106.38 - 124.37) and 112.33 (104.44 - 120.81) respectively. These values were within the bioequivalence range of 80-125%.

**Conclusions:** It is concluded that vitamin D<sub>3</sub> Oral Solution formulated with nanotechnology is bioequivalent to vitamin D<sub>3</sub> tablet and capsule. However, oral solution of vitamin D<sub>3</sub> shows higher C<sub>max</sub> and AUC when compared to tablet and capsule formulations.

**Keywords:** Nanotechnology, Oral solution, Relative bioavailability, Vitamin D<sub>3</sub>

**INTRODUCTION**

The primary role of vitamin D has been considered to have a role in calcium homeostasis in the body which is essential for bone mineralization.<sup>1</sup> Multiple published evidences confirm that apart from its well-known function in calcium-phosphate homeostasis, vitamin D also exerts many non-calcemic actions in various tissues and systems. Vitamin D deficiency has been linked with significant complications such as cardiovascular events, obesity, metabolic syndrome, type 2 diabetes, various types of cancer, immune disorders, increased mortality and adverse pregnancy outcomes.<sup>2-4</sup> The Endocrine Society Clinical

Practice Guideline on evaluation, treatment and prevention of vitamin D deficiency (published July 2011) defines vitamin D deficiency as 25(OH)D level below 20ng/ml (50 nmol/l), vitamin D insufficiency as 25(OH)D level at 21-29ng/ml and sufficiency, if the 25(OH)D level is above 30ng/ml.<sup>5</sup> Currently, vitamin D deficiency is considered a public health problem worldwide and its prevalence rises along with latitude, aging, sedentary lifestyle and limited sunlight exposure due to staying indoors or using sunscreen products.<sup>6</sup> Almost 50% of the world population is suffering from vitamin D insufficiency.<sup>7</sup> Vitamin D is a secosteroid hormone that is made in the skin upon exposure of the skin to UV-B radiation.<sup>8</sup> Vitamin D is also obtained in the diet primarily from vitamin D fortified

foods or by the use of vitamin D supplements.<sup>9</sup> Treatment with either vitamin D<sub>2</sub> (ergocalciferol) or vitamin D<sub>3</sub> (cholecalciferol) is recommended for deficient patients.<sup>7</sup> Vitamin D has poor bioavailability, which significantly reduces its efficacy as disease-combating agent.<sup>10</sup>

Oral dosage forms like tablet, capsule and oral solution have different absorption rate. The efficiency of oral absorption of conventional Vitamin D<sub>3</sub> is approximately 50%.<sup>11</sup> In general, the availability for absorption of a drug is more in oral solutions comparing to the capsule and tablet respectively.<sup>12</sup> Bioavailability of vitamin D can be increased by nanotechnology in an effective way.<sup>10</sup> The International Organization for Standardization (ISO) has defined nanoparticle as a “nano-object with all three external dimensions in the nanoscale” where nanoscale is defined as the size range from approximately 1-100nm (ISO, 2008).<sup>13</sup> Despite the availability of vitamin D<sub>3</sub> oral solution in the market, none of the studies has been conducted which compares bioavailability of vitamin D<sub>3</sub> oral solution with tablet and capsule together. The main objective of this study is to compare the relative bioavailability of vitamin D<sub>3</sub> oral solution formulated with nanotechnology with conventional vitamin D<sub>3</sub> tablet and capsule.

## METHODS

The study was conducted in accordance with the IEC approved protocol and clinical research guidelines established by the basic principles defined in the ICH-GCP guidelines, Schedule Y (as amended) of Central Drugs Standard Control Organization (CDSCO) 2005; Ethical Guidelines for Biomedical Research on Human Participants, ICMR (Indian Council of Medical Research; 2006), Declaration of Helsinki (64<sup>th</sup> WMA General Assembly, Fortaleza, Brazil, October 2013), ICH (Step 5) 'Guidance on Good Clinical Practice', OECD principles of Good Laboratory Practice and all other applicable regulatory requirements.

This was an open-label, balanced, randomized, three-treatment, single-period, parallel, oral relative bioavailability study. The single-dose oral bioavailability of the test formulation, Hi-D™ 5mL shot containing vitamin D<sub>3</sub> 60000IU (Akumentis Healthcare Limited, Mumbai, India) was compared with two reference products, D<sub>3</sub> Must® Tablets containing vitamin D<sub>3</sub> 60000 IU (Mankind Limited, India) and Uprise D<sub>3</sub>® Soft Gelatin Capsules containing vitamin D<sub>3</sub> 60000 IU (Alkem Limited, India). A total of 72 healthy, adult, male subjects were dosed in three successive groups under fasting conditions. Each group comprises 24 subjects.

### Inclusion criteria

Healthy, non-smoking, non-alcoholic male human subjects aged between 18 and 45 years, subjects with a BMI between 18.50 - 24.90kg/m<sup>2</sup> and body weight not less than 50.00kg, subject willing to limit direct sunlight

exposure at least 10 days before the first dose and throughout the study, subjects willing to apply sunscreen (at least 45 SPF) if anticipating exposure to direct sunlight for >1 hour, subjects ready to avoid most dairy products, vitamin D<sub>3</sub>-fortified foods, and foods known to be high in vitamin D<sub>3</sub>, subjects with negative urine screen result for drugs of abuse (including amphetamines, barbiturates, benzodiazepines, marijuana, cocaine, and morphine) and subjects willing to adhere to the protocol requirements and to provide written informed consent.

### Exclusion criteria

Known hypersensitivity to vitamin D or any of its analogues and derivatives, use of any prescribed medication (including herbal remedies) during two weeks before the start of the study or OTC medicinal products during the week prior to study initiation, subject who has received active vitamin D<sub>3</sub> compounds or a high dose of vitamin D<sub>3</sub> (>5000IU) within 30 days before study entry, subject with a 25(OH)D level <15ng/mL at screening, subjects with major illness during the 90 days before screening and subjects with abnormal diet patterns (for any reason) during the four weeks preceding the study, including fasting, high protein diets etc.

The subjects, who were eligible when assessed against the inclusion and exclusion criteria for the study, were randomly assigned to the products. Randomization was carried out using the PROC PLAN procedure of SAS® (SAS Institute Inc., U.S.A.) version 9.4 in blocks such that the design was balanced.

### Treatment

After an overnight fast of at least 10.00 hours, the study drug Hi-D 5mL oral Shot (Oral Solution) containing Vitamin D<sub>3</sub> 60000 IU (Test Product - T) or D<sub>3</sub> Must Tablets containing Vitamin D<sub>3</sub> 60000 IU (Reference Product 1 - R1) or Uprise D<sub>3</sub> Soft Gelatin Capsules containing Vitamin D<sub>3</sub> 60000 IU (Reference Product 2 - R2) was administered (allocated as per the randomization schedule) to subjects orally while in a sitting position with approximately 240mL water at ambient temperature. This activity was followed by a thorough mouth check to assess the compliance to dosing. The subjects were instructed not to chew or crush the tablet or capsule but to swallow it as a whole. Dosing was done under subdued light in the morning. Primary endpoints were C<sub>max</sub>, AUC<sub>0-28D</sub> and secondary parameter was T<sub>max</sub>.

### Statistical analysis

Descriptive statistics (geometric mean, arithmetic mean, median, and standard deviation, coefficient of variation, minimum and maximum) were computed and reported for primary and secondary pharmacokinetic parameters for 25(OH)D. Statistical analysis was performed using SAS® version 9.3. Bioequivalence was evaluated by means of statistical analysis of variance (ANOVA) with 90%

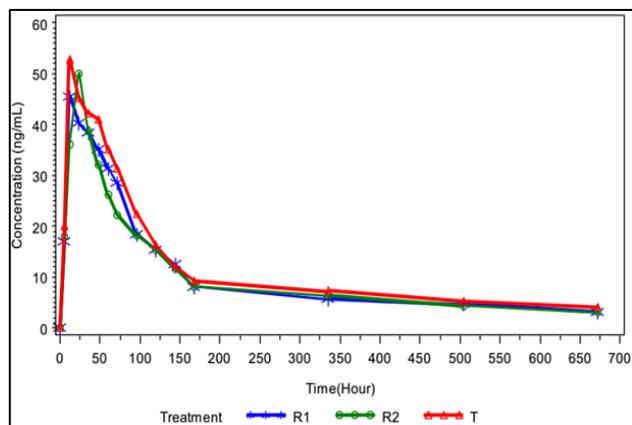
confidence intervals (CI) of the test/reference ratio with logarithm-transformed data. The bioequivalence acceptance criteria required that the 90% CI should be contained within the acceptance interval of 80-125%.

**RESULTS**

Overall demographic characteristics of all subjects are given in Table 1.

**Table 1: Overall demographic profile of all subjects (N = 72).**

Variable	Profile	Percentage		
Race	Asian	100.00%		
	Others	0.00%		
Gender	Male	100.00%		
	Female	0.00%		
Diet	Non-Vegetarian	95.83%		
	Vegetarian	5.56%		
Smoking status	Non-smokers	100.00%		
	Smokers	0.00%		
Alcohol consumption	Non alcoholics	100.00%		
	Alcoholics	0.00%		
	<b>Mean</b>	<b>SD</b>	<b>Min</b>	<b>Max</b>
Age (yr)	26.9	5.31	19	44
Height (cm)	168.5	5.50	156	179
Weight (kg)	61.2750	6.275	51.1	80.08
BMI	21.533	1.9694	18.68	26.4



**Figure 1: Linear plot of mean serum concentration of baseline corrected 25-hydroxy vitamin D vs time for test product (T), reference product 1 (R1) and reference product 2 (R2) (N=72).**

Values of pharmacokinetic parameters (Table 2) for  $C_{max}$  (40.017 ng/ml vs 35.414ng/ml) and  $AUC_{0-28d}$  (4898.528 ng.hr/ml vs 4641.275 ng.hr/ml) of Test and R1 were noted respectively which indicates that the values of  $C_{max}$  and  $AUC_{0-28d}$  were higher in case of oral solution compared to tablet. Similarly, values of pharmacokinetic parameters for  $C_{max}$  (40.017ng/ml vs 34.791ng/ml) and  $AUC_{0-28d}$  (4898.528ng.hr/ml vs 4361.000 ng.hr/ml) of Test and R2 were noted respectively which indicated that the values of

$C_{max}$  and  $AUC_{0-28d}$  higher were in case of oral solution as compared to capsule.

Pharmacokinetic parameter for  $T_{max}$  was found to be 15.500 hr, 19.500hr and 19.000hr for Test, R1 and R2 respectively. Linear plot of mean serum concentration of baseline corrected 25-hydroxy vitamin D Vs time for test product (T), reference product 1 (R1) and Reference product 2 (R2) is given in Figure 1.

**Table 2: Descriptive statistics of formulation means for 25-hydroxy vitamin D obtained by a non-compartmental model (N = 72).**

Pharmacokinetic Parameters (Units)	Mean ± SD		
	Test product [T]	Reference product [R1]	Reference product [R2]
$C_{max}$ (ng/mL)	40.017	35.414	34.791
$AUC_{0-28d}$ (ng.hr/mL)	4898.528	4641.275	4361.000
$T_{max}$ (hr)	15.500	19.500	19.000

The logarithmic transformed data of pharmacokinetic parameters were analyzed for 90% Confidence intervals (CI) using ANOVA. The mean (90% CI) values for Test and R1 of  $C_{max}$  were 113.00 (105.32-121.23) and of  $AUC_{0-28d}$  were 105.54 (97.95-113.72) given in Table 3. The mean (90% CI) values for T and R2 of  $C_{max}$  were 115.02 (106.38-124.37) and of  $AUC_{0-28d}$  were 112.33 (104.44-120.81) indicated in Table 4.

**Table 3: Geometric least squares means, ratios, 90% Confidence Intervals, power and p-values for pharmacokinetic parameters ( $C_{max}$  and  $AUC_{0-28d}$ ) of baseline corrected 25-hydroxy vitamin D (N = 48) (T vs R1).**

Pharmacokinetic parameters (Units)	Geometric mean ratio test/reference (%)	90% Confidence interval (parametric)	
		Lower	Upper
$C_{max}$ (ng/mL)	113.00	105.32	121.23
$AUC_{0-28d}$ (ng.hr/mL)	105.54	97.95	113.72

According to the USFDA and EMA Guidance, in studies to determine bioequivalence after a single dose, for  $C_{max}$  and  $AUC_{0-28d}$  parameters the 90% confidence interval for the ratio of the test and reference products should be contained within the acceptance interval of 80-125%.

As seen in Table 3 and Table 4, the 90% confidence intervals of the differences of least squares means for the Ln-transformed pharmacokinetic parameters  $C_{max}$  and  $AUC_{0-28d}$  of vitamin D<sub>3</sub> oral solution are within the bioequivalence acceptance limits of 80.00 - 125.00% when compared with vitamin D<sub>3</sub> tablet and capsule.

**Table 4: Geometric least squares means, ratios, 90% Confidence Intervals, power and p-values for pharmacokinetic parameters ( $C_{max}$  and  $AUC_{0-28d}$ ) of baseline corrected 25-hydroxy vitamin D (N = 48) (T vs R2).**

Pharmacokinetic parameters (Units)	Geometric mean ratio test/reference (%)	90% Confidence interval (parametric)	
		Lower	Upper
$C_{max}$ (ng/mL)	115.02	106.38	124.37
$AUC_{0-28d}$ (ng.hr/mL)	112.33	104.44	120.81

## DISCUSSION

Vitamin D was firstly defined as a vitamin and now is recognized as a prohormone.<sup>9</sup> It is a precursor to its active and biologically functional metabolite, a lipophilic secosteroid hormone known as calcitriol.<sup>14</sup> Vitamin D plays several roles in the body, influencing bone health as well as serum calcium and phosphate levels. Furthermore, vitamin D may modify immune function, cell proliferation, differentiation and apoptosis.<sup>15</sup> Vitamin D deficiency is a worldwide well-recognized problem with health consequences. In India more than 90% of apparently healthy Indians have subnormal 25(OH)D levels.<sup>5</sup> The status of vitamin D depends on the production of Vitamin D and vitamin D intake through the diet or vitamin D supplements. In India, current recommendations for correction of vitamin D level is by giving 60,000 IU of oral vitamin D on a weekly basis for 8 weeks.<sup>16</sup>

Owing to its fat-soluble nature, dietary vitamin D is absorbed with other dietary fats in the small intestine. The efficient absorption of vitamin D is dependent upon the presence of fat in the lumen, which triggers the release of bile acids and pancreatic lipase. In turn, bile acids initiate the emulsification of lipids, pancreatic lipase hydrolyzes the triglycerides into monoglycerides and free fatty acids, and bile acids support the formation of lipid containing micelles, which diffuse into enterocytes.<sup>9</sup>

It has been observed in studies that nanoparticles of vitamin D improve the pharmacokinetic parameters. Sun et al, studied the advantages of employing nanoparticles of oleoyl alginate ester (OAE) as carriers of oral vitamin D<sub>3</sub>, both in in-vitro and in-vivo studies. The pharmacokinetic findings from their study suggest higher absorption of vitamin D<sub>3</sub> on oral administration, after incorporation into OAE nanoparticles, as compared with conventional vitamin D<sub>3</sub>.<sup>17</sup>

Study of oral dosage form on absorption rate showed that oral solution has highest absorption rate comparing to the other dosage form. The availability for absorption decreases in the order: solution > suspension > powder-filled capsule > compressed tablet > coated tablet.<sup>12</sup> In the present study, authors have compared the relative bioavailability of vitamin D oral solution with the tablet

and capsule. Present study outcomes showed that AUC and  $C_{max}$  of vitamin D<sub>3</sub> oral solution are greater than tablet and capsule. On the basis of present study results, it can be conjectured that vitamin D oral solution can show an increased serum concentration of 25(OH) D as compared to tablet and capsule. Upon assessment of T Vs R1 and T Vs R2 relative bioavailability data, it is found that, in both cases, the 90% confidence intervals of the differences of least squares means calculated for the Ln-transformed pharmacokinetic parameters  $C_{max}$  and AUC for Baseline Corrected 25-hydroxy vitamin D are within the bioequivalence acceptance limits of 80.00 - 125.00%.

To the best of authors' knowledge, this is the first study of single-dose, three treatment, single-period, parallel design oral relative bioavailability study of vitamin D<sub>3</sub> oral solution comparing with tablet and capsule.

## CONCLUSION

The test product vitamin D<sub>3</sub> oral solution formulated with nanotechnology is bioequivalent to conventional vitamin D<sub>3</sub> tablet and capsule. However, oral solution of vitamin D<sub>3</sub> shows higher  $C_{max}$  and AUC when compared to tablet and capsule formulations.

## ACKNOWLEDGEMENTS

The authors are thankful to RHEA Healthcare for conducting the clinical study. This work was sponsored by Akumentis Healthcare Ltd., Mumbai, India.

*Funding: Funding sources from Akumentis Healthcare Ltd.*

*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee*

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**Cite this article as:** Nandgaye KM, Samant RS, Kadam SB, Palkar PJ. Relative oral bioavailability of three formulations of vitamin D3: an open-label, three-treatment study. *Int J Basic Clin Pharmacol* 2019;8:138-42.