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ORIGINAL RESEARCH ARTICLE

Comparative Bioavailability Study of a New Vitamin D3 Orodispersible Film Versus a Marketed Oral Solution in Healthy Volunteers

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Abstract

Background and Objective An orally disintegrating film (ODF) formulation of vitamin D3 that dissolves rapidly in the mouth without drinking or chewing may be a worthwhile alternative to currently available drug products for therapeutic vitamin D supplementation. This study aimed to compare the bioavailability of a single dose of a vitamin D3 25000 I.U. ODF with those of a marketed oral vitamin D3 preparation in healthy subjects.

Methods This Phase 1, randomised, parallel-group, open-label study compared the pharmacokinetics of calcifediol [25(OH) D3], the precursor of bioactive vitamin D3, after a single dose of a new vitamin D3 25,000 I.U. ODF with those of a Reference formulation (vitamin D3 25000 I.U./2.5 mL oral solution) in healthy adult subjects using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. The primary objective was bioavailability under fed conditions, defined as maximum plasma concentration (C_{max}) of 25(OH)D3 and area under the concentration-time curve from time zero to time t, the last quantifiable concentration (AUC_{0-t}). The pharmacokinetics of 25(OH)D3 were also evaluated following the ODF administration under fasting conditions. Subjects were randomised to receive a single dose of the vitamin D3 25000 I.U. ODF or the Reference oral solution under fed conditions or the vitamin D3 ODF under fasting conditions.

Results Forty-eight healthy subjects were randomised and completed the study. Overall, the pharmacokinetic profile was very similar across the three treatment groups, and bioavailability did not significantly differ among treatments. Under fed conditions, mean 25(OH)D3 plasma values for C_{max} were 6.68 ± 2.03 versus 6.61 ± 2.62 ng/mL for the Test versus Reference formulations. Corresponding values for AUC_{0-t} were 2364.80 ± 1336.97 versus 2150.52 ± 1622.76 ng/mL × h. Mean C_{max} was slightly lower (6.68 ± 2.03 vs 7.23 ± 1.48 ng/mL) and the time to reach peak concentration was delayed (144 h [36–312] versus 42 h (2–480]) with the ODF under fed versus fasting conditions ($p = 0.0371$). The point estimates and 90 % CIs of the $Test_{fed}/Reference_{fed}$ ratios of the geometric means showed that the bioavailability of exogenous 25(OH)D3 was, both in rate and extent of absorption, slightly higher with the vitamin D3 ODF than the vitamin D3 oral solution under the administration conditions recommended for the vitamin D3 oral solution. Palatability and ease of use of the ODF were satisfactory.

Conclusion The new ODF 25000 I.U. formulation provided a valuable alternative to the marketed oral solution for therapeutic vitamin D supplementation, with a bioavailability that was slightly higher than that of the vitamin D3 oral solution administered under the same conditions.

Trial Registration The study was retrospectively registered with the ISRCTN Registry (Registry code: ISRCTN13208948) on 27 November 2020.

1 Introduction

Vitamin D has a diverse physiological role beyond its function in skeletal homeostasis, as it is an important regulator of the immune system involved in innate immunity and the adaptive immune system and has an increasingly acknowledged role in the development of several autoimmune diseases, including type 1 diabetes, multiple sclerosis, rheumatoid arthritis, and inflammatory bowel disease [1–4].

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Key Points

A high-potency orally disintegrating film (ODF) formulation that delivers a therapeutic dose of vitamin D3 would be a useful alternative to conventional oil-based oral dosage forms used in vitamin D3 deficiency.

This study compared the bioavailability, palatability, and ease of use of a single-dose vitamin D3 25000 I.U. ODF with that of a marketed oral vitamin D3 preparation in healthy subjects.

The new ODF 25000 I.U. formulation provided a valuable alternative to the marketed oral solution for therapeutic vitamin D supplementation.

Adequate intake of Vitamin D also shows protective properties against acute respiratory tract infections [1, 2, 5], and there is emerging evidence for an association between severe vitamin D deficiency and COVID-19-related mortality [6].

The main source of vitamin D is the endogenous synthesis from 7-dehydrocholesterol of cholecalciferol in the skin following ultraviolet B (UVB) radiation exposure, with subsequent hydrolysis of 25-hydroxyvitamin D [25(OH)D; calcifediol], the primary circulating metabolite of vitamin D, in the liver and kidney to the bioactive form of vitamin D3, 1,25-dihydroxyvitamin D ($1,25(OH)_2D3$; 1,25-dihydroxycholecalciferol; calcitriol) [1, 7]; 25(OH)D is regarded as a reliable marker of vitamin D status [8, 9], and a serum concentration of 25(OH)D between 20 and 50 ng/mL (50–125 nmol/L) is widely considered to be associated with optimal health in the general population, while a concentration < 12 ng/mL (30 nmol/L) represents severe vitamin D deficiency [9, 10].

Sunlight exposure and dietary intake alone are insufficient to maintain optimal vitamin D status in most individuals [8, 11]. To achieve and maintain adequate vitamin D status, supplementation is often required, and there is a need for effective products suitable for routine supplementation that also support good compliance in specific populations at risk, such as the elderly and children. However, international consensus guidelines for the optimal level of vitamin D supplementation needed to maintain human health are currently lacking, with recommendations ranging from 400 to 2000 I.U. (10–50 µg) daily [8, 10, 12–14].

Vitamin D deficiency can be treated and prevented through supplements, which may be taken weekly or monthly, given that vitamin D is lipophilic and can be accumulated in fat deposits [15]. The D3 form (cholecalciferol) is preferred over the D2 form (ergocalciferol) for vitamin D supplementation because it appears to be absorbed more efficiently by the small intestine [16–18]. Cholecalciferol is usually administered orally, and available formulations include oral drops, soft capsules, and oily solutions for injection, as the bioavailability of vitamin D3 is generally regarded as being more effective when administered as an oily solution rather than from dry-composition solid dosage forms [19, 20]. Accordingly, a number of different oil-based solution formulations of vitamin D3 (for example, Benferol®, and Colecalciferol Fidia® [soft gelatin capsules] and DIBASE® [oral solution]) are available in Europe, as no large differences in vitamin D3 absorption are to be expected. Although vitamin D supplementation is often expressed as a daily dose, in practice, cumulative doses are more likely to be administered as weekly, biweekly, or monthly doses to enhance compliance, based on the assumption that rapid storage of cholecalciferol in adipose tissue allows for long-term availability after weekly or monthly administration [19].

Orodispersible films (ODFs) are innovative oral dosage systems for delivering active pharmaceutical ingredients and substances for food supplements developed as alternatives to conventional dosage forms. ODFs have an established and growing presence in the pharmaceutical market and can deliver tailored medications to varied patient populations. They require only a small amount of saliva to dissolve in the mouth rapidly and do not need to be administered with water. In addition to their superior dosing accuracy, rapid onset of action, and convenience compared with conventional dosage forms, patient acceptance and compliance is improved, and ODFs are associated with better safety and efficacy, particularly in special patient populations such as children, the elderly, psychiatric patients, and those with difficulty swallowing [21–24]. Moreover, there is a strong patient preference for orodispersible over conventional solid or other oral dosage forms across a wide range of patient groups [21, 23, 24].

A vitamin D3 ODF developed by IBSA, Italy, has been marketed as a food supplement and is available in doses of 1000 and 2000 I.U. [25, 26]. However, due to size and thickness limitations, ODFs can typically be loaded only with limited amounts of active substance per unit volume and surface area [27–29]. The poor water solubility of cholecalciferol represents a challenge in developing aqueous mixture-based ODF formulations with higher drug loading capacities. To address this limitation, the development of a new vitamin D3 ODF with a pharmaceutical dosage comparable to existing high-potency vitamin D3 formulations was undertaken.

The ODF formulation with a 25000 I.U. dosage developed by IBSA, Switzerland has been designed to rapidly dissolve and/or disintegrate when placed in the mouth without drinking or chewing and may provide a valuable alternative

Table 1 Composition and function of excipients used in the pharmaceutical development of Vitamin D3 orally disintegrating film

Ingredients	Function
Vitamin D3	Drug substance
Maltodextrin	Film-forming
Glycerol, mannitol, water	Plasticisers
Glycerol monolinoleate, polysorbate 80, HP βCyclodextrin, purified olive oil, water	Solubilising agents
Copovidone, polyvinyl acetate	Filler
Vitamin C, Vitamin E	Antioxidant agent
Orange, peach and apricot flavours	Flavouring agents
Sunset yellow E131, Titanium dioxide	Colourant agents
Sucralose	Sweetener

to the already marketed drug products for therapeutic vitamin D supplementation.

The objective of this study was to compare the bioavailability of a single dose of the IBSA vitamin D3 25000 I.U. ODF with that of a marketed oral vitamin D3 preparation in healthy subjects.

2 Subjects and Methods

2.1 Materials

The pharmaceutical development of the vitamin D3 ODF was undertaken according to patents describing the preparation process of ODFs for therapeutic and food supplementation use utilising maltodextrin as the only film-forming ingredient [30, 31]. This polymer provides advantages in terms of palatability, physical properties, and stability and was previously used successfully to design a safe and efficacious pharmaceutical oral film formulation containing sildenafil citrate that was shown to be bioequivalent to the conventional branded film-coated tablets [32]. The ingredients used in developing the vitamin D3 ODF are shown in Table 1.

The development of the manufacturing process for the IBSA 25000 I.U. ODF progressed from the initial investigation into critical process parameters that affect the finished product quality at the laboratory scale before scaling up to the pilot scale and finally to the industrial scale using solvent casting as the manufacturing method. Guided by the patents WO2014/049548 and WO2005/039543 [30, 31], the following steps were undertaken: maltodextrin, plasticiser, the active ingredient, and the other excipients are solubilised/dispersed in water; the mixture is coated onto a siliconised PET release liner using the solvent casting method and dried in the oven controlling for temperature, air circulation, and coating speed; the dried mass is cut into reels, and the films are then formed, packaged and sealed in suitable single-dose sachets chosen to protect the film from photodegradation of cholecalciferol and moisture and air exposure.

Manufacturing proceeded according to a standard process developed by the manufacturer and validated for manufacturing other orodispersible films already on the market, such as the pharmaceutical product sildenafil ODF [32]. Using similar technology, ODFs containing 1000 and 2000 I.U. of vitamin D3 were developed and manufactured as food supplements on an industrial scale [25].

In the new pharmaceutical Vitamin D3 ODF the vitamin D3 concentration was increased to 0.5% to obtain a film of 4.5 cm^2 containing 0.625 mg (25000 I.U.) with a weight of 250 g/m^2 of dried mass.

2.2 Subjects

Healthy male and female volunteers aged 40–70 years (inclusive) were eligible for the study if they met the following inclusion criteria: willingness to sign a written informed consent; body mass index (BMI) of 20–29 kg/m^2; vital signs (systolic blood pressure 100–139 mmHg, diastolic blood pressure 50–89 mmHg, heart rate 50–90 bpm, measured after 5 min at rest in the sitting position); the ability to fully comprehend the nature and aims of the study, including possible risks and side effects, and to cooperate with the investigator and comply with the study requirements; the use of at least one reliable method of contraception for females of child-bearing potential and a negative pregnancy test at screening for all female subjects.

The key exclusion criteria were the following: clinically significant abnormalities at a 12-lead electrocardiogram (ECG): abnormal physical findings: clinically significant abnormal laboratory values indicative of physical illness, especially hypercalcaemia and hypercalciuria: hypersensitivity to the active principle and/or ingredients of the formulations: history of medically significant renal, hepatic, gastrointestinal, cardiovascular, respiratory, skin, haematological, endocrine or neurological diseases: prior use of medications or products in general containing calcium, magnesium or vitamin D, for two weeks before the start of the study: abnormal diets (< 1600 or > 3500 kcal/day) or substantial changes in eating habits in the four weeks before this study, or vegetarians, or a high dietary intake of vitamin D and calcium in the four weeks preceding the start of the study; participation in the evaluation of any investigational product or blood donations for three months before this study.

2.3 Study Design

This bioequivalence study was a Phase 1, randomised, parallel-group, open-label study which aimed to compare the bioavailability of calcifediol [25(OH)D3] after a single dose of

the Test formulation (i.e., a new vitamin D3 formulation, the IBSA Vitamin D3 25000 I.U. orodispersible film) versus a Reference formulation (DIBASE®, vitamin D 25000 I.U./2.5 mL oral solution) when administered under fed conditions to healthy subjects. Furthermore, in the study, the bioavailability of 25(OH)D3 was evaluated following single-dose administration of the test ODF formulation both under fasting and fed conditions to investigate the impact of food.

A single dose, parallel design was chosen due to the long half-life of the analyte 25(OH)D3, i.e., 21–30 days. As a cross-over design was likely to cause an elevated drop-out rate, it was not considered.

The study protocol and all relevant documentation were reviewed and approved by an independent Ethics Committee, the Comitato Etico Cantonale, Canton Ticino, Switzerland (Appendix 16.1.3; Project ID: 2019-00932/CE3479, reference number 2019DR1093). The study was conducted in compliance with the tenets of the Declaration of Helsinki and according to the International Conference on Harmonisation Good Clinical Practice guidelines (GCP) and the Swiss "Ordinance on Clinical Trials in Human Research 810.305 (OSRUm)". The study was registered with the ISRCTN Registry (Registry code: ISRCTN13208948) and was designed in agreement with the current European Medicines Agency (EMA) guideline on bioequivalence studies (CPMP/EWP/QWP/1401/98 Rev. 1/Corr**, January 20, 2010). Written informed consent was obtained from all subjects before any study procedures were performed.

The timing of the study was planned for an autumn start, when limited exposure to sunlight was foreseen, and a single dose of 25000 I.U. of vitamin D3 was chosen as sufficient to cover the physiological requirement of vitamin D3 for one month while avoiding problems with treatment compliance and toxicity, as single doses up to 300000 I.U. have been administered safely [33]. Furthermore, the dose chosen is the same as that of a marketed vitamin D3 oral solution for therapeutic vitamin D supplementation.

The planned sample size of 48 subjects (3 groups of 16 subjects) was estimated as sufficient for exploratory descriptive purposes.

2.4 Randomisation, Treatment Assignment, Assessment

Subjects were randomised to receive a single dose of the vitamin D3 25000 I.U. ODF (IBSA, Italy) under fasting conditions; a single dose of the vitamin D3 ODF under fed conditions; or a single dose of the vitamin D3 25000 I.U. oral solution (DIBASE®; Abiogen Pharma S.p.A., Italy) under fed conditions. The allocation of the treatment group was according to a computer-generated randomisation list.

Each study completer underwent nine visits (Fig. 1). The study plan included a screening phase (from day 21 to day

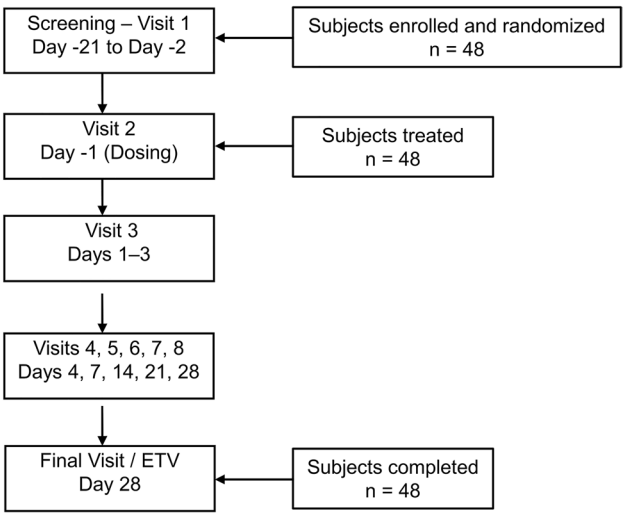


Fig. 1 Flow chart describing study schedule and subject disposition. Each study completer underwent 9 visits. The study plan included a screening phase from day − 21 to day − 2 followed by confinement from the evening before the dosing (day − 1) to ≈ 48 h post-dose. Blood sample collections for pharmacokinetic determinations were performed on day − 1 (Visit 2), days 1–3 (Visit 3), day 4 (Visit 4), day 7 (Visit 5), day 14 (Visit 6), day 21 (Visit 7) and d28 (Visit 8). A Final Visit (Visit 9) was performed on day 28 or at early termination visit (ETV) in case of discontinuation from the study

− 2) followed by confinement from the evening before the dosing (day − 1) to about 48 h post-dose. Both Test and Reference were orally administered on the morning of study day 1, at 08:00 ± 1 h. The subjects allocated to $Test_{fast}$ received the vitamin D3 ODF under prolonged fasting conditions (from 10-h pre-dose to 5-h post-dose), while the subjects allocated to $Test_{fed}$ and $Reference_{fed}$ received their treatment 30 min after having started to eat a light breakfast. Before administration, all the subjects wetted their mouth by drinking 20 mL of still mineral water. Afterward, the contents of one sachet of the vitamin D3 ODF (batch number 9A001V2, expiry January 2020) was placed on the $Test_{fast}$ and $Test_{fed}$ subjects' tongue, allowing it to dissolve without chewing. The contents of the vitamin D3 oral solution (batch number 40419, expiry March 2021) were fully and immediately drunk by $Reference_{fed}$ subjects.

Blood sample collection for pharmacokinetic determinations was performed on day − 1 (Visit 2), days 1–3 (Visit 3), day 4 (Visit 4), day 7 (Visit 5), day 14 (Visit 6), day 21 (Visit 7) and day 28 (Visit 8). A Final Visit (Visit 9) was performed on day 28 or at the early termination visit (ETV) in case of discontinuation from the study (Fig. 1). Blood sampling time-points, selected on the basis of the known pharmacokinetic profile of 25(OH)D3, were scheduled pre-dose (at − 12, − 1 h and 0 h) and post-dose (at 15 and 30 min and 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 144, 312, 480 and 648 h).

The actual sampling times for each subject were recorded in the individual subject case report forms. The actual sampling times were not to exceed the recommended tolerance ranges specified in the study protocol. Collection, handling, and analysis of plasma samples were conducted according to standard procedures defined in the study protocol and detailed in Sect. 2.5.

The clinical phase of the study was performed, in compliance with both international GCP and local legislation in force, at CROSS Research S.A., Switzerland, and was monitored by Clinical Medical Services di Maria Pia Savorelli, Switzerland, who conducted regular onsite monitoring visits and regular inspections of the case report forms. All study documentation and results were reviewed according to the quality assurance standard operating procedures of CROSS Research, Switzerland.

2.5 Sample Collection, Processing, and Bioanalytical Assay Methods

Venous blood samples (12 mL) for 25(OH)D3 pharmacokinetic analysis were collected from a forearm vein at each sampling time point using an indwelling catheter with switch valve. After each sampling, the cannula was rinsed with about 1 mL of sterile saline solution containing 20 I.U./mL Na-heparin. The first 2 mL of blood was discarded at each collection time to avoid contamination of the sample with heparin. The remaining 10 mL were collected from the catheter and transferred into EDTA K2 tubes and were (within 60 min from collection) centrifuged at 4 °C for 10 min at 1900*g* to obtain plasma. Samples were maintained at 4 °C (± 4 °C) during the whole procedure from sample collection until sample freezing. Each plasma sample was immediately divided into three aliquots in pre-labelled polypropylene tubes, with aliquots 1 and 2 each containing at least 1.5 mL of plasma. The aliquots were stored frozen at − 80 °C in different freezers at the clinical unit within 200 min from the end of centrifugation. Aliquot 1, packed in sufficient solid CO_2, was shipped by an authorised courier and under controlled temperature to the bioanalytical laboratory, Anapharm Europe S.L.U., Spain, where the concentration of 25(OH)D3 in plasma was determined, using a fully validated LC-MS/MS method, with a lower quantification limit of 1 ng/mL. Analyses were performed according to the general principles of GLP and GCP. The analytical methodology is provided in detail as Online Resource 1.

2.6 Outcomes

The study's primary objective was to compare the bioavailability of vitamin D3, measured as plasma calcifediol, 25(OH)D3, after a single oral dose of the vitamin D3 25000 I.U. ODF versus the marketed reference oral solution (DIBASE® 25000 I.U.) under fed ($Test_{fed}$) conditions in healthy adult male and female subjects: Extent (area under the concentration-time curve [AUC] from time zero to time t, the last quantifiable concentration [AUC_{0-t}]) and rate (maximum plasma concentration [C_{max}]) of absorption of 25(OH)D3.

Secondary objectives were to (1) evaluate the effect of food on 25(OH)D3 bioavailability after administration of a single dose of the vitamin D3 ODF under fed ($Test_{fed}$) and fasting ($Test_{fast}$) conditions (Extent [AUC_{0-t}] and rate [C_{max}] of absorption of 25(OH)D3); (2) describe the pharmacokinetic profile of 25(OH)D3 after single-dose administration of the vitamin D3 ODF under fed and fasting conditions and the reference oral solution under fed ($Reference_{fed}$) conditions; (3) collect palatability and ease of use data of the vitamin D3 ODF; (4) assess the safety and tolerability of single-dose administrations of the vitamin D3 preparations (treatment-emergent adverse events [TEAEs]; vital signs [blood pressure, heart rate], physical examinations including bodyweight; laboratory tests).

Pharmacokinetic analyses were performed in subjects who had completed the study without a major deviation affecting pharmacokinetic results. The plasma pharmacokinetic profiles of 25(OH)D3 after single-dose administration of Test and Reference were calculated from baseline-corrected 25(OH)D3 concentration values using Phoenix WinNonlin® version 6.3 (Pharsight Corporation, St Louis, MO, USA) and included: C_{max}; half-life ($t_{½}$); AUC_{0-t}; AUC extrapolated to infinity ($AUC_{0-\infty}$); time to C_{max} (t_{max}); terminal elimination rate constant (λ_z); percent residual area (C_t/λ_z) extrapolated to infinity in relation to total $AUC_{0-\infty}$ ($\%AUC_{extra}$); and relative bioavailability (F_{rel}).

Adverse events were monitored at each visit throughout the study, and all pre-treatment or TEAEs were coded by System Organ Class and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA) version 23.0. The investigator provided an assessment of relationship to treatment and graded the severity as mild, moderate, or severe.

2.7 Statistical Analysis

The statistical analysis of pharmacokinetic parameters was performed using Phoenix WinNonlin® version 6.3 (Pharsight Corporation, St Louis, MO, USA), and SAS® version 9.3 (TS1M1; SAS Institute, Cary, NC, USA). SAS® version 9.3 was also used to analyse the safety and tolerability data. Data documented in the trial and the clinical parameters measured were analysed using classic descriptive statistics for quantitative variables and frequencies for qualitative variables. C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of 25(OH)D3 were compared between $Test_{fed}$ and $Reference_{fed}$ and, separately, between $Test_{fed}$ and $Test_{fast}$ using analysis of variance (ANOVA) for a

Table 2 Demographic and other baseline characteristics ($n = 48$)

Parameter	Overall $n = 48$	Pharmacokinetic set 1[a] $n = 32$	Pharmacokinetic set 2[b] $n = 32$
Sex			
Female	25 (52.1)	15 (46.9)	17 (53.1)
Male	23 (47.9)	17 (53.1)	15 (46.9)
Age (years)	51.1 ± 6.6	52.0 ± 6.4	51.5 ± 6.8
Body weight (kg)	69.19 ± 9.91	68.96 ± 8.84	70.58 ± 10.44
Height (cm)	168.3 ± 9.4	168.5 ± 8.8	168.8 ± 9.6
BMI (kg/m^2)	24.37 ± 2.25	24.27 ± 2.26	24.69 ± 2.18
Race			
White	47 (97.9)	31 (96.9)	32 (100)
Other	1 (2.1)	1 (3.1)	0

Values are mean ± SD or *n* (%)

BMI body mass index, *SD* standard deviation, *ODF* orally disintegrating film

[a]All subjects randomised to the vitamin D3 ODF ($Test_{fed}$) and the vitamin D3 oral solution fed ($Reference_{fed}$) groups and completed the study

[b]All subjects randomised to the $Test_{fed}$ and the $Test_{fast}$ groups and completed the study

parallel-group design on baseline-corrected log-transformed data, taking treatment into account as a source of variation.

The 90% confidence intervals (CI) were calculated for the point estimates (PE, i.e., the ratio of least square geometric means for $Test_{fed}$/$Reference_{fed}$ or $Test_{fed}$/$Test_{fast}$) of the pharmacokinetic parameters. Values of t_{max} were compared between $Test_{fed}$ and $Reference_{fed}$ and between $Test_{fed}$ and $Test_{fast}$, separately, using the non-parametric Wilcoxon signed-rank test.

3 Results

3.1 Subjects

Forty-eight subjects (25 female and 23 male) with a mean age of 51.1 years were screened, met the eligibility criteria, and were included in the study and randomised as planned. The baseline characteristics of the participants are shown in Table 2. The subjects were all in good physical health, with vital signs (blood pressure, heart rate) within the normal range. All 48 subjects received the study treatment, completed the study as per protocol, and were considered in both the pharmacokinetics and safety analyses. The date of the first enrolment visit was the 24th of September 2019, and the last subject completed the study on the 22nd of November 2019.

The flow of subjects throughout the study is shown in Fig. 1. No major protocol deviations were recorded; minor deviations (mainly deviations from scheduled blood sampling times, difficulty collecting plasma, or consumption of disallowed concomitant medication) were reported in 14.6 % (7 of 48 subjects).

All subjects who completed the study per protocol attended the scheduled study visits up to study end, and all received their planned study dose.

3.2 Pharmacokinetics

Overall, the pharmacokinetic profile was very similar across the three treatment groups, and pharmacokinetics did not significantly differ among treatments. Under fed conditions, the bioavailability of 25(OH)D3 was slightly higher after single-dose administration of the vitamin D3 ODF than after the reference vitamin D3 oral solution, both in terms of rate and extent of absorption (Table 3 and Fig. 2). Under fed conditions, the mean baseline-corrected 25(OH)D3 plasma values for C_{max} were 6.68 ± 2.03 versus 6.61 ± 2.62 ng/mL for the ODF Test formulation versus the Reference oral solution. Corresponding values for AUC_{0-t} were 2364.80 ± 1336.97 versus 2150.52 ± 1622.76 ng/mL × h for the Test versus Reference formulations. $AUC_{0-\infty}$ was 4247.21 ± 3903.59 versus 3582.27 ± 3144.33 ng/mL × h for the Test versus Reference formulations.

When the pharmacokinetics of vitamin D3 ODF under fed and fasting conditions were compared, administration conditions did not substantially affect the extent of absorption of vitamin D; the AUC_{0-t} was similar when under both fed and fasting conditions (Table 3). The rate of absorption was impaired only slightly and to a clinically irrelevant degree when the ODF was taken with food consisting of a standard light breakfast.

For the ODF Test formulation under fasting conditions, the mean baseline-corrected 25(OH)D3 plasma values for C_{max} and AUC_{0-t} were 7.23 ± 1.48 ng/mL and 2244.38 ± 1144.26 ng/mL × h (Table 3). $AUC_{0-\infty}$ was 4247.21 ±

Table 3 Main baseline-corrected 25(OH)D3 plasma pharmacokinetic parameters after single-dose administration of Test and Reference preparations of vitamin D3

Parameter	Vitamin D3 ODF		Vitamin D3 oral solution
	Fed ($n = 16$)	Fasting ($n = 16$)	Fed ($n = 16$)
C_{max} (ng/mL)	6.68 ± 2.03	7.23 ± 1.48	6.61 ± 2.62
AUC_{0-t} (ng/mL × h)	2364.80 ± 1336.97	2244.38 ± 1144.26	2150.52 ± 1622.76
$AUC_{0-\infty}$ (ng/mL × h)	Calcifediol.21 ± 3903.59[a]	3328.43 ± 1778.46[b]	3582.27 ± 3144.33[c]
t_{max} (h)	144 (36–312)	42 (2–480)	48 (12–312)
$t_{½}$ (h)	231.75 ± 199.59[a]	236.76 ± 127.62[b]	205.80 ± 142.39[c]
λ_z (1/h)	0.01 ± 0.00[a]	0 ± 0[b]	0.01 ± 0[c]

Values are arithmetic means ± standard deviation (SD), except for t_{max}, which are median (range)

AUC_{0-t} area under the concentration-time curve (AUC) from time zero to last observed concentration-time, $AUC_{0-\infty t}$ AUC extrapolated to infinity, *CI* confidence interval, C_{max} maximum plasma concentration, *ODF* orally disintegrating film, *PE* point estimate, calculated as ratio of geometric means, t_{max} time to maximum plasma concentration, $t_{½}$ half-life, λ_z terminal elimination rate constant

[a]$n = 9$; [b]$n = 11$; [c]$n = 10$

3903.59 versus 3582.27 ± 3144.33 ng/mL × h for the Test versus Reference formulations. The C_{max} was on average slightly lower and the time to reach peak concentration was delayed with the ODF under fed versus fasting conditions ($p = 0.0371$) (Table 3).

The point estimates and 90% CIs of the $Test_{fed}$/$Reference_{fed}$ ratios of the geometric means showed that the bioavailability of exogenous 25(OH)D3 was both in rate (C_{max}) and extent of absorption (AUC_{0-t} and $AUC_{0-\infty}$) slightly higher with the vitamin D3 ODF than the vitamin D3 oral solution when both are taken under the administration conditions recommended for the vitamin D3 oral solution (Table 4).

3.3 Palatability and Ease of Use

The palatability and the ease of use of the vitamin D3 ODF were analogous under both fed and fasting conditions of administration (Online Resource 2). In particular, the ODF mostly had a likable taste of mild intensity, which left an aftertaste. The mouthfeel mainly was judged as pleasant, and the use of the ODF was generally judged as very easy or easy.

3.4 Safety

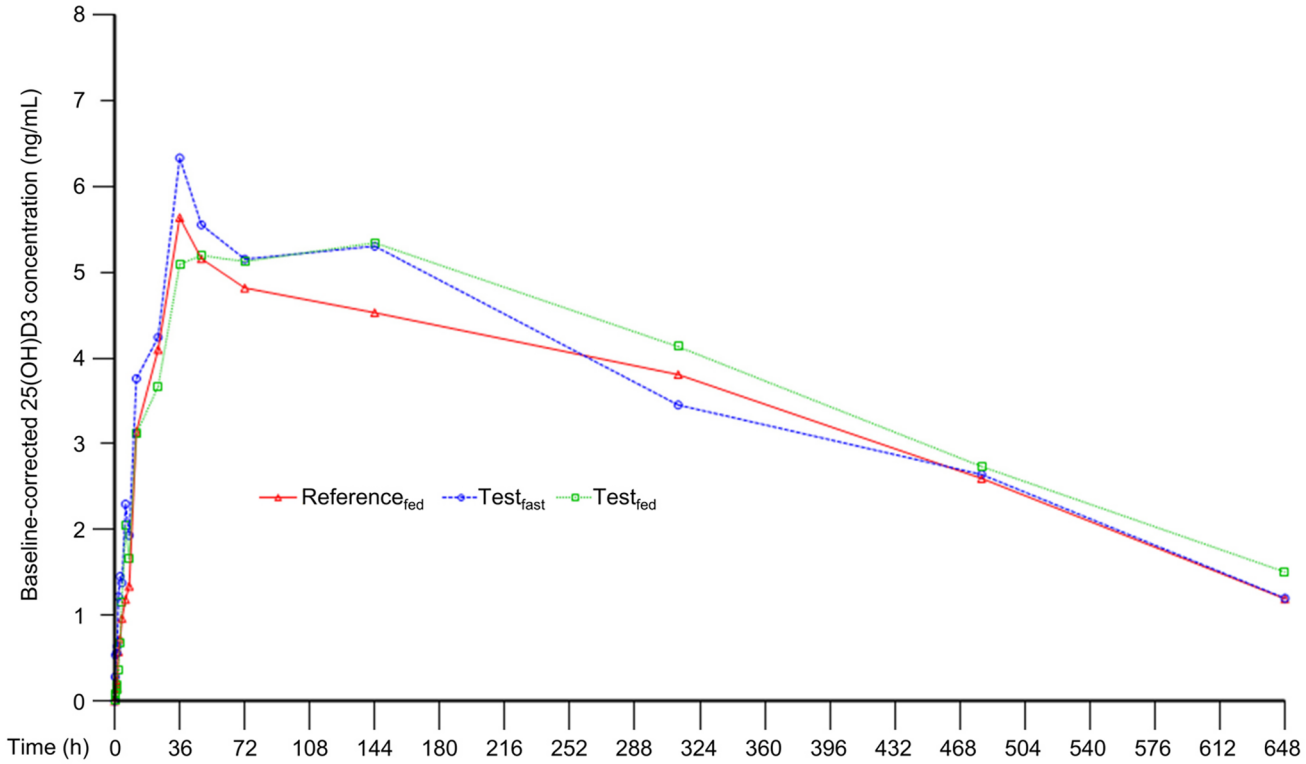
There were no treatment-related TEAEs, and no severe or serious events or discontinuations due to safety reasons (Online Resource 3). Of the16 TEAEs experienced by 12 out of 48 (25.0 %) of subjects, 31.3 % and 18.8 %, respectively, occurred after the vitamin D3 ODF in the fed and fasting condition, respectively, and 25.0 % after the vitamin D3 oral solution. The most common event was headache, which occurred in 12.5 % of subjects in the ODF and oral solution groups in the fed condition, and 6.3 % after the ODF in the fasting condition. Apart from a single case of transient moderate-severity proteinuria judged as unlikely to be related to the study treatment, no significant treatment effects on vital signs, body weight, or other laboratory parameters were observed.

4 Discussion

This study aimed to compare the pharmacokinetic profiles of the new vitamin D3 25000 I.U. ODF with those of a reference vitamin D3 25000/2.5 mL oral solution when administered to healthy volunteers. The results support the conclusion that the bioavailability of exogenous 25(OH)D3 following a single 25000 I.U. ODF intake is at least comparable to an equivalent dose of the reference oral solution in terms of both rate (C_{max}) and extent (AUC_{0-t}) of absorption, both of which were slightly higher with the test ODF than the reference vitamin D solution, when administered under the same (fed) conditions. Although the AUC_{0-t} and $AUC_{0-\infty}$ of the exogenous 25(OH)D3 after the Test formulation tended to be slightly higher under fed versus fasting conditions, indicating that the intake of a meal within 30 min of dosing can limitedly enhance the extent of absorption, from a clinical viewpoint, the extent of food effect on the bioavailability of the ODF can be considered negligible or absent.

Adverse events (none of which were considered to be related to the treatments), were reported in the study at a frequency of 25.0%. None were serious or medically significant. No other clinically significant effect of Test or Reference formulation on the safety parameters was observed.

A limitation of the study was the relatively small sample of subjects, which may affect the generalisability of the findings. However, the study design reduced the potential of bias. As the study was conducted in healthy subjects, direct



Time (h)	Test$_{fed}$	Test$_{fast}$	Reference$_{fed}$
0	0.0	0.0	0.0
0.25	0.01 ± 0.04	0.28 ± 0.48	0.14 ± 0.40
0.5	0.07 ± 0.16	0.53 ± 0.55	0.05 ± 0.14
1	0.18 ± 0.40	0.56 ± 0.89	0.24 ± 0.73
1.5	0.13 ± 0.47	0.64 ± 0.77	0.15 ± 0.45
2	0.36 ± 0.62	1.22 ± 1.93	0.58 ± 1.02
3	0.68 ± 0.66	1.45 ± 1.34	0.72 ± 0.77
4	1.16 ± 0.78	1.38 ± 0.87	0.96 ± 0.98
6	2.04 ± 0.95	2.30 ± 1.53	1.18 ± 1.11
8	1.66 ± 1.09	1.93 ± 1.22	1.34 ± 1.34
12	3.12 ± 1.22	3.76 ± 2.11	3.14 ± 2.45
24	3.67 ± 1.01	4.24 ± 1.52	4.10 ± 2.28
36	5.09 ± 1.97	6.33 ± 1.85	5.64 ± 2.83
48	5.20 ± 1.99	5.55 ± 2.21	5.16 ± 2.62
72	5.13 ± 1.75	5.15 ± 1.83	4.82 ± 2.55
144	5.34 ± 2.09	5.30 ± 1.91	4.53 ± 3.24
312	4.13 ± 3.19	3.45 ± 2.49	3.81 ± 3.15
480	2.74 ± 2.59	2.64 ± 2.53	2.60 ± 2.61
648	1.51 ± 2.26	1.20 ± 1.36	1.19 ± 1.95

Data are mean ± standard deviation (SD) baseline-corrected 25(OH)D3 plasma concentrations in ng/mL after a single dose of vitamin D3 ODF under fasting (Test$_{fast}$) or fed (Test$_{fed}$) conditions or the Reference vitamin D3 oral solution (Reference$_{fed}$) under fed conditions

Fig. 2 Mean plasma baseline-corrected 25(OH)D3 concentration (ng/mL) versus time profiles after single-dose administration of Test and Reference vitamin D3 preparations under Fed and Fasting conditions. $n = 16$ in each pharmacokinetic set

extrapolation to patients with vitamin D deficiency cannot be made.

The development of various delivery systems for active pharmaceutical ingredients, vitamins, or food supplements, ranging from thin films, gels, orally disintegrating tablets, and chewing gums to drug-loaded micro-and nanoparticles, has provided several innovative alternatives that address some of the issues particular to conventional dosage forms with the potential of improving convenience and acceptability and enhancing compliance [21, 23, 34]. Among these, ODFs have attracted considerable attention because of their ease of use, readily dissolving in contact with saliva without the need for water intake. Thin and flexible, ODFs can be manufactured in a wide range of shapes and sizes and are easily transported and stored. Various polymers, such as maltodextrin, are basic excipients in thin-film formulations, imparting specific properties that ensure mechanical strength and stability in a film that must disintegrate or dissolve

Table 4 Statistical comparison of baseline-corrected 25(OH)D3 pharmacokinetic parameters

Treatment comparison	Parameter	PE, %	90% CI
$Test_{fed}$ vs $Reference_{fed}$	C_{max}	104.95	83.91–131.25
	AUC_{0-t}	124.60	84.84–183.00
	$AUC_{0-\infty}$	110.14	55.39–218.98[a]
$Test_{fed}$ vs $Test_{fast}$	C_{max}	90.11	77.21–105.17
	AUC_{0-t}	106.34	76.78–147.28
	$AUC_{0-\infty}$	101.60	56.55–182.53[a]

AUC_{0-t} area under the concentration-time curve (AUC) from time zero to last observed concentration-time, $AUC_{0-\infty}$ AUC extrapolated to infinity, *CI* confidence interval, C_{max} maximum plasma concentration, *ODF* orally disintegrating film, *PE* point estimate, calculated as ratio of geometric means, $Test_{fed}$ a single dose of vitamin D3 ODF (Test) under fed conditions, $Test_{fast}$ a single dose of vitamin D3 ODF under fasting conditions, $Reference_{fed}$ a single dose of vitamin D3 oral solution (Reference) under fed conditions

[a] $n = 9$

rapidly when in contact with saliva. Plasticisers, such as glycerol, lower the polymer's glass transition temperature, improving plasticity and elasticity of the film, while taste masking and sweetening agents may be added to cover any unpleasantness associated with the active ingredient. Finally, surfactants act as dispersing, solubilising, and wetting agents [21, 23, 34]. The size and thickness of the film can be modified, and the proportion of excipients adjusted to achieve the desired dose of the active ingredient. ODFs can be manufactured using several methods, including solvent casting, hot-melt extrusion, electrospinning, semisolid casting, or the rolling method [35, 36].

The convenience, superior dosing accuracy, and rapid onset of action of ODFs contribute to a strong preference over conventional solid or oral dosage forms across a wide range of patient groups [21, 23, 32, 37]. However, low drug loading capacity, issues with incorporating bitter medications, and achieving dose uniformity have been seen as limitations of ODFs [27–29]. To overcome such limitations, IBSA undertook the development of a 25000 I.U. formulation of vitamin D3 to recognise the advantages of an orodispersible formulation that dissolved rapidly in the mouth without drinking or chewing while providing an alternative to the currently marketed products for therapeutic vitamin D supplementation.

In achieving an ODF with a dosage level of vitamin D3 necessary for a pharmaceutical dosage form, maltodextrin, a polymer that provides advantages in terms of palatability, physical properties, and stability, was used as the only film-forming ingredient. During the development programme, several formulations using different excipients were prepared in order to study the effect of the different excipients on the properties of the ODF necessary to achieve the desired concentration of vitamin D3 (0.5 %) in a film of 4.5 cm^2 containing 25000 I.U. of drug substance while improving the mechanical properties of the film.

Critical quality attributes of films prepared on an industrial scale were investigated: films were flexible and easily handled, were homogeneous for vitamin D3 content, and vitamin D3 is immediately released. Vitamin D3 ODFs were chemically and microbiologically stable in the tested period, up to 24 months at 25 °C/60% relative humidity.

The development process from laboratory to pilot scale to industrial scale by the solvent casting method showed that an ODF containing 25000 I.U. of vitamin D3 could be manufactured on an industrial scale for commercial purposes. Furthermore, this study has demonstrated that a well-tolerated, palatable, and easy-to-use alternative oral dosage form of vitamin D3, the IBSA 25000 I.U. vitamin D3 ODF, can be regarded as similar to a vitamin D3 oral solution already approved and marketed in the European Union.

5 Conclusion

The new orodispersible formulation developed by IBSA, placed in the mouth of healthy volunteers under fed conditions, provided a valuable alternative to the marketed oral solution, insofar as the bioavailability of the exogenous 25(OH)D3 was both in rate (C_{max}) and extent of absorption (AUC_{0-t} and $AUC_{0-\infty}$) slightly higher than with the vitamin D3 oral solution administered under the same conditions.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40261-021-01113-7.

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Declarations

Funding This study was financially supported by IBSA Institut Biochimique S.A., Pambio-Noranco, Switzerland. IBSA is the owner and manufacturer of the vitamin D3 orally disintegrating film product.

Conflict of interest S.R., C.C, G.M are employees of IBSA Institut Biochimique S.A., Switzerland. I.C, F.M, A.M.G are employees of IBSA Farmaceutici Italia, Italy. M.R is an employee from CROSS Research S.A., Switzerland. The authors report no other conflicts of interest in this work.

Ethics approval The study protocol and all relevant documentation were reviewed and approved by an independent Ethics Committee, the Comitato Etico Cantonale, Canton Ticino, Switzerland; Appendix 16.1.3; Project ID: 2019-00932/CE3479, reference number 2019DR1093). The study was conducted in compliance with the tenets of the Declaration of Helsinki and according to the International Conference on Harmonisation Good Clinical Practice guidelines (GCP) and

the Swiss "Ordinance on Clinical Trials in Human Research 810.305 (OSRUm)".

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication Not applicable.

Availability of data and material De-identified subject data that underlie the results reported in this article are available from the corresponding author on reasonable request.

Code availability Not applicable.

Authors' contributions All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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