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(54) Title: IRRADIATION PROCESS OF PRO VITAMIN D

(57) Abstract: The invention discloses an improved process for production of vitamin D3 from 7- dehydrocholesterol (7- DHC) and to a simple process for recovery unreacted 7-DHC for further reuse. The invention further describes a process for isolation and purification of Vitamin D3.

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IRRADIATION PROCESS OF PRO VITAMIN D

Technical filed:

The invention relates to an improved process for production of vitamin D3 from 7- dehydrocholesterol (7- DHC) and to a simple process for recovery unreacted 7-DHC for further reuse. The invention further relates to a process for isolation and purification of Vitamin D3.

Background and prior art:

Conventionally, Vitamin D3 has been produced by an irradiation of 7-dehydrocholesterol with ultraviolet rays in an organic solvent. In such a process, however, there are disadvantages that the solubility of 7-dehydrocholesterol in many organic solvents is less and as a result an effective irradiation of 7-dehydrocholesterol using ultraviolet rays is difficult to achieve and hence pose a challenge to scale up such processes for industrial production of Vitamin D. Accordingly various organic solvents have been studied as a medium for irradiating 7-dehydrocholesterol.

Another drawback is that the starting material (7-DHC), the primary product (pre vitamin D3) as well as by products, absorb with different efficiency in the same UV wavelength range, favours the formation of photochemical by products such as lumisterol and tachysterol which are inactive and in some cases toxic also. Therefore, it is necessary to interrupt the irradiation after relatively low conversion of the 7-DHC to D3. The unconverted 7-DHC is recycled while the primary product (Vitamin D3) is purified.

Moreover vitamin D2 and D3 are also not stable in solutions as they exhibit reversible thermal isomerization to their corresponding pre-vitamins forming equilibrium mixtures. Further, vitamin D2 and D3 are susceptible to decomposition in presence of oxygen and light.

There are few prior art reports available on manufacturing process of vitamin D3, which are as follows.

US3176029A discloses a process for the treatment of the irradiation products of 7-dehydrocholesterol, comprising: treating said irradiation products with ethanol to remove a portion of the unreacted pro-vitamin and provide a residual ethanol gum, dissolving said ethanol gum in methanol, preciptating an adduct of vitamin D3 and pro-vitamin from the methanol solution, and separating said precipitated adduct from said solution.

US3661939 discloses a process for the production of a mixture containing vitamin D3 wherein a solution of 7-dehydrocholesterol organic acid ester in an organic solvent is irradiated with ultraviolet rays to obtain a mixture containing pre-vitamin D3 organic acid ester and then said mixture containing pre-vitamin D3 organic acid ester is subjected to a saponification and heating operation to obtain a mixture containing vitamin D3.

WO2008128783A2 discloses a photochemical process for the preparation of a pre-vitamin D or a derivative thereof from a 7-dehydrosterol or a corresponding derivative thereof which process comprises irradiating the 7-dehydrosterol or the derivative thereof with UV LED(s).

CN1033993A discloses a method of synthesis of vitamin D characterized in that: the use of a YAG laser with a wavelength of 266nm and 355nm laser irradiation alternately in two or pro-vitamins ergosterol (E) or 7-dehydrocholesterol (7-DHC), to synthesize vitamin D2 or vitamin D3.

US6180805B1 discloses a photochemical process for the production of previtamin D3 or a derivative thereof from 7-dehydrocholesterol which comprises:

(a) irradiating 7-dehydrocholesterol in a falling film reactor with a UV radiation source, wherein the UV radiation source comprises an excimer or

exciplex emitter containing XeBr which emits quasi-monochromatically according to the corona discharge mechanism in the UV range; and

(b) recovering the previtamin D3 or derivative thereof.

US20170369436 discloses a method of production of vitamin-D2 using ergosterol or a dihydroxy derivative thereof as a starting material, or production of vitamin-D3 using 7-dehydrocholesterol or a dihydroxy derivative thereof as the starting material, comprising:

(a) irradiating the starting material in a solution comprising an organic or inorganic base with ultraviolet light to obtain a product containing pre-vitamin-D2 or pre-vitamin-D3, and

(b) heating the product to convert the pre-vitamin-D2 or pre-vitamin-D3 to vitamin D2 or vitamin D3.

The teachings of US'946 is incomplete in view of the percentage conversions and purity of the Vitamin D2 and Vitamin D3. Although demonstrated the irradiation process with very few organic bases, however, fails to provide the applicability of the same with other organic and inorganic bases.

In the light of the above, it is evident that the products of irradiation processes are of widely varying in composition and the quantity of Vitamin D3 depends on the extent of photochemical reaction and the purity of the end product.

Therefore, there remains a need in the art to provide an improved irradiation conditions for production of Vitamin D3 from 7- DHC and for simplified process for recovery unreacted 7-DHC from the reaction mass, which becomes an objective of the present invention, for which protection is sought.

Summary of the invention:

In the light of the above, the present invention provides a photochemical process for the preparation of a vitamin D3 or a derivative thereof which process

comprises irradiating 7-dehydrosterol in combination with a sterol derivative and anti-oxidant in presence of an alkali.

Accordingly, the photochemical process for the preparation of a vitamin D3 which process comprises;

a) Heating the mixture of 7-dehydrocholesterol in combination with a sterol derivative in presence of catalytic amounts of an anti-oxidant and an alkali in an organic solvent at a temperature range of 20-85°C;
b) Irradiating the mixture using a low pressure Mercury lamp at 10-85°C; and
c) Isolating and purifying vitamin D3 from the irradiated mixture.

In another aspect, the invention provides process for isolation and purification of Vitamin D3.

Detailed description of the invention:

The invention will now be described in detail in connection with certain preferred and optional embodiments, so that various aspects thereof may be more fully understood and appreciated.

Accordingly, the present invention provides a photochemical process for the preparation of a vitamin D3 or a derivative thereof which process comprises irradiating the 7-dehydrocholesterol in combination with corresponding derivative thereof in ethanol and in presence of anti-oxidant and an alkali.

The samples of 7-dehydrocholesterol, the starting material used in the present invention is synthesised from Cholesterol isolated either from wool grease which is sourced from New Zealand or Fish oil sourced from Chile or Milk fat sourced from Europe. The Cholesterol used for the synthesis of 7-dehydrocholesterol, the starting material, is isolated from Wool grease, which is sourced from New Zealand.

Accordingly, the photochemical process for the preparation of a vitamin D3 which process comprises;

a) Heating the mixture of 7-dehydrocholesterol in combination with a sterol derivative in presence of catalytic amounts of an anti-oxidant and an alkali in an organic solvent at a temperature range of 20-85°C;
b) Irradiating the mixture using a low pressure Mercury lamp at 10-85°C; and
c) Isolating and purifying vitamin D3 from the irradiated mixture.

The sterol derivative is selected from the group consisting of Cholesterol, Phytosterol, Lanosterol, etc. 7-Dehydrocholesterol is charged with sterol derivative approximately in a ratio of 1:0.1 to 1: 3.

The organic solvent in step a) may be selected from the group consisting of methanol, ethanol, Isopropanol, Tetrahydrofuran, Diethyl ether, methyl-tert-butyl ether, methyl isobutyl ketone & petroleum Ether (40-60°C & 60-80°C).

The use of sterol derivative is to facilitate the solidification of the unreacted 7-DHC from the resinous reaction mass thereby simplifying the recovery and reuse of 7-DHC in the subsequent batch. The use of sterol derivative further makes the isolation of Vitamin D3 less cumbersome.

The anti-oxidant is preferably selected from Butylated hydroxyl toluene, propyl gallate, butylated hydroxyanisole etc. One preferable antioxidant is Butylated hydroxyl toluene.

The alkali is selected from the group consisting of sodium hydroxide, potassium hydroxide, lithium hydroxide or ammonia. In one preferred embodiment, the alkali is preferably 2% to 10% of sodium hydroxide.

The reaction mass is irradiated by a low pressure Mercury lamp at 80-85°C for 30-300 minutes preferably 60 -240 minutes or more preferably 100-200 minutes to accomplish the reaction.

The isolation and purification of vitamin D3 comprises the steps of;

a) Repeatedly cooling the reaction mass to 0-45°C °C to separate the solids consisting of 7-DHC and sterol derivative followed by concentrating the filtrate under vacuum to obtain concentrated mass;
b) Adding an organic solvent to the mass of step a) followed by washing with aq. Ethanol and further distillation under vacuum to obtain residue;
c) Adding an organic solvent to residue followed by cooling up to -5°C-+35°C to separate the solids consisting of Vitamin D3, 7-Dehydrocholesterol and sterol derivative;
d) Evaporating the filtrate under vacuum at 40-45°C to obtain crude Vitamin D3 (resin); and
e) Purifying the crude vitamin D3 by converting into its ester followed by saponification and crystallization from an organic solvent to obtain pure vitamin D3.

The organic solvent used in step b) is selected from the group consisting of methyl tert butyl ether, petroleum ether (40-60°C & 60-80°C), n-Heptane, n-Hexane, ethyl acetate, Diethyl ether, Toluene and Xylene.

The organic solvent used for crystallization in step c) is selected from the group consisting of 2-Butanone, Acetone, Methyl-Isobutyl ketone, petroleum ether (40-6-°C &60-80°C), n-heptane or n-Hexane.

The solids obtained in steps a) and c) according to the process are collected and reused in the subsequent batch.

The esters of vitamin D3 are selected from the group consisting of Acetate, Propionate, Butyrate, Valerate, 2-Nitrobenzoate or 4-Nitrobenzoate, more

preferably Butyrate and can be prepared by treating the crude with the respective acids or anhydrides.

The saponification of the ester is carried out using a base selected from Sodium Hydroxide, Potassium hydroxide, Sodium carbonate, Potassium carbonate, Sodium methoxide, Sodium Ethoxide, Potassium butoxide or Lithium Aluminium Hydride.

The organic solvent used for crystallization of vitamin D3 in step e) is a ketone or an ester selected from the group consisting of acetone, 2-Butanone, Methyl isobutyl ketone, methyl formate, ethyl formate.

In an alternate embodiment, the residue obtained in step b) may be purified directly by column chromatography over silica gel or neutral Alumina or Alumina with 2-10% water, by eluting with Toluene: 2-Butanone in a ratio of 1:99, 2:98, 4:96 & 5: 95 to isolate pure Vitamin D3 /Cholecalciferol crystals. Alternately, pure Vitamin D3 /Cholecalciferol crystals can be obtained by eluting with Dichloromethane: methanol 99:1, 98:2, 95:5, 90:10 or with Ethyl Acetate: methanol 99:1,98:2, 95:5 & 90:10.

In yet another embodiment, the invention provides a composition which comprises:

a) Vitamin D3 in about 98-99.5%; and
b) one or both of lumisterol and tachysterol at a concentration of at least 0.02 % of the vitamin D3 present in the composition;
c) Trans Vitamin D3 at a concentration of at least 0.05%;

Wherein, the vitamin D3 meets potency requirement of 40 MIU.

Accordingly in one preferred embodiment, 7-Dehydrocholesterol is charged with cholesterol approximately in the ratio of 1:0.1 to 1: 3 along with catalytic amounts

of Butylated hydroxyl toluene as an anti-oxidant and 2 to 10% of aq. Sodium hydroxide solution in Ethanol and heated the reaction mass at a temperature range of 0-90°C preferably at 20-85°C or more preferably 75-85°C. The reaction mass is then irradiated by a low pressure Mercury lamp at 80-85°C for 30-300 minutes preferably, 60 -240 minutes or more preferably 100-200 minutes. The reaction mass is further cooled to -10- 50°C, preferably to 15-30°C or most preferably to 0-5°C.

The solids separated out are filtered which contains 7-Dehydrocholesterol and Cholesterol which is the 1st crop.

The filtrate is concentrated to 5-50% of the original volume preferably 10-40% or more preferably 10-20% of the original volume, cooled to 10-15°C and the separated solids are filtered that contains 7-Dehydrocholesterol and Cholesterol, which is the 2nd crop.

The filtrate is again concentrated under vacuum and methyl tert butyl ether is added and washed with 1:1 alcohol and water and further distilled under vacuum to obtain a residue, wherein the alcohol is selected from methanol, ethanol and isopropanol. An organic solvent selected from 2-Butanone (methyl ethyl ketone), Methyl isobutyl ketone, methyl formate or ethyl formate is added to the residue, cooled the mass in the temperature range of -5°C-+35°C and the separated solids are filtered which contains Vitamin D3, 7-Dehydrocholesterol and Cholesterol, which is the 3rd crop. These solids obtained in 1st, 2nd and 3rd crops are reused in subsequent batches for the irradiation to obtain vitamin D3.

The filtrate is then evaporated under vacuum at 40-45°C and the crude Vitamin D3 (resin) is analysed and the results are shown in table 1.

In an alternate embodiment, after removal of the first crop(unreacted 7-DHC and Cholesterol), the filtrate is concentrated under vacuum and the residue is dissolved

in Methyl tert butyl ether, washed with 10 x100 ml 1:1 ethanol water and distilled under vacuum. The residue is purified by column chromatography over silica gel with Toluene: methyl ethyl ketone 1:99, 2:98, 4:96 & 5: 95 to isolate pure Vitamin D3/Cholecalciferol crystals. The results are shown in table 4.

In another preferred embodiment, 7-Dehydrocholesterol is charged with Phytosterol approximately in the ratio of 1: 0.1 to 1: 3 along with catalytic amounts of Butylated hydroxyl toluene as an anti-oxidant and 2 to 10% of Aq. Sodium hydroxide solution in Ethanol and heated the reaction mass at a temperature range of 0-90°C preferably at 20-85°C. The reaction mass is then irradiated by a low pressure Mercury lamp at 75-85°C for 30-300 minutes preferably 60 -240 minutes or more preferably 100-200 minutes. The reaction mass is cooled to -10 to 50°C preferably to 15-30°C or more preferably to 0-5°C. The solids separated out are filtered which contains 7-Dehydrocholesterol and Phytosterol which is the 1st crop.

The filtrate is concentrated to 5-50% of the original volume preferably 10-40% or more preferably 10-20% of the original volume, cooled to 10-15°C and the separated solids are filtered that contains 7-Dehydrocholesterol and Phytosterol, which is the 2nd crop.

The filtrate is again concentrated under vacuum and methyl tert butyl ether is added and washed with 1:1 ethanol water and distilled under vacuum. 2-Butanone (methyl ethyl ketone) is added to the residue, cooled to (-5) to (-8)°C and the separated solids are filtered which contains Vitamin D3, 7-Dehydrocholesterol and Phytosterol, which is the 3rd crop. These solids obtained in 1st, 2nd and 3rd crops are reused in subsequent batches for the irradiation to obtain Vitamin D3.

The filtrate is then evaporated under vacuum at 40-45°C and the crude Vitamin D3 (resin) is analysed and the results are shown in table 2.

In yet another preferred embodiment, 7-Dehydrocholesterol is charged with Lanosterol approximately in the ratio of 1:0.1 to 1: 3 along with catalytic amounts of Butylated hydroxyl toluene as an anti-oxidant and 2 to 10% of Aq. Sodium hydroxide solution in Ethanol and heated the reaction mass at a temperature range of 0-90°C preferably at 20-85°C or more preferably 75-85°C. The reaction mass is then irradiated by a low pressure Mercury lamp at 80-85°C for 30-300 minutes preferably 60 -240 minutes or more preferably 100-200 minutes. The reaction mass is cooled to 10-15°C.

The solids separated out are filtered which contains 7-Dehydrocholesterol and Lanosterol which is the 1st crop.

The filtrate is concentrated to 5-50% of the original volume preferably 10-40% or more preferably 10-20% of the original volume, cooled to 10-15°C and the separated solids are filtered that contains 7-Dehydrocholesterol and Lanosterol, which is the 2nd crop.

The filtrate is again concentrated under vacuum and methyl tert butyl ether is added and washed with 1:1 ethanol water and distilled under vacuum. 2-Butanone (methyl ethyl ketone) is added to the residue, cooled to (-5) to (-8)°C and the separated solids are filtered which contains Vitamin D3, 7-Dehydrocholesterol and Lanosterol, which is the 3rd crop. These solids obtained in 1st, 2nd and 3rd crops are reused in subsequent batches for the irradiation to obtain Vitamin D3.

The filtrate is then evaporated under vacuum at 40-45°C and the crude Vitamin D3 (resin) is analysed and the results are shown in table 3.

The Vitamin D3 (resin) thus obtained is purified by converting into its ester such as Acetate, Propionate, Butyrate, Valerate, 2-Nitrobenzoate, 4-Nitrobenzoate more preferably Butyrate, crystallized and finally saponified using a base selected

from Sodium Hydroxide, Potassium hydroxide, Sodium carbonate, Potassium carbonate, Sodium methoxide, Sodium Ethoxide, Potassium butoxide or Lithium Aluminium Hydride and finally crystallized from acetone.

Alternately, the crude Vitamin D3 thus obtained can be purified by column chromatography using Silica gel or Alumina or Alumina with 2-10% water and using Toluene: 2-Butanone as an eluent.

The present invention is exemplified by the following examples which are provided for illustration only and, should not be construed to limit the scope of the invention.

EXAMPLES:

Example 1

96 gms of 7-Dehydrocholesterol, 90 gms of Cholesterol, 1 gm of Butylated hydroxyl toluene and 25 ml of 2% Aq. Sodium hydroxide solution were dissolved in 1250 ml of **Ethanol** at 75-85°C and the mixture was irradiated by a LOW PRESSURE Mercury lamp at 80-85°C for 180 minutes preferably. The reaction mass was cooled to 25-30°C. The solids separated out were filtered as first crop which contains 7-Dehydrocholesterol (50-60%) + Cholesterol.

The filtrate was concentrated to 20% of the original volume, cooled to 20-25°C and the separated solids were filtered as second crop that contains 7-Dehydrocholesterol (15-20%) + Cholesterol.

The filtrate was again concentrated under vacuum. 2000 ml Methyl *tert* butyl ether was added, washed with 2 *50 ml 1:1 ethanol water and distilled under vacuum. 3000 ml 2-Butanone (methyl ethyl ketone) was added to the residue, cooled to 10°C and the separated solids were filtered as the third crop that contains Vitamin D_3 (5-MIU)+ 7-Dehydrocholesterol 20% + Cholesterol 20%.

All these first, second and third crops are combined and reused in subsequent batches.

The filtrate was then evaporated under vacuum at 40-45°C and the crude Vitamin D_3 (resin) was analysed, as shown in table 1.

Table 1:

S no	Compound	% by HPLC	Potency
1	Vitamin D_3/Cholecalciferol + pre-Vitamin D_3	90-92%	27-29 MIU
2	Cholesterol	0.5-1.3%	NA
3	7-Dehydrocholesterol	0.5-1%	NA
4	Tachysterol	1-2%	NA
5	Lumisterol	1-2%	NA
6	Trans Vitamin D_3	0.5-1%	NA

The crude resin thus obtained can be purified by either of the following methods:

1. Converting the resin to its ester like Acetate or Propionate or Butyrate or Valerate or 2-Nitrobenzoate or 4-Nitrobenzoate more preferably Butyrate, crystallized and finally saponified by a base like Sodium Hydroxide or Potassium hydroxide or Sodium carbonate or Potassium carbonate Sodium methoxide or Sodium Ethoxide or Potassium butoxide or Lithium Aluminium Hydride and finally crystallized from acetone.
2. Purification of the Crude resin by column chromatography using Silica gel or Alumina or Alumina with 2-10% water and using Toluene: 2-Butanone as an eluent.

Example 2

96 gms of 7-Dehydrocholesterol, 50 gms of **Phytosterol**, 1 gm of Butylated hydroxyl toluene and 20 ml of 2% Aq. Sodium hydroxide solution are dissolved

in 1000ml of **Ethanol**, at 75-85 °C and the mixture was irradiated by a low pressure mercury lamp at 80-85°C for 30 minutes. The reaction mass was cooled to 35-40°C.

The solids separated out were filtered as a first crop that contains 7-Dehydrocholesterol (50-60%) + **Phytosterol.**

The filtrate was concentrated to 60% of the original volume, cooled to 25-30°C and the separated solids were filtered as a second crop that contains 7-Dehydrocholesterol (15-20%) + **Phytosterol**.

The filtrate was again concentrated under vacuum. 2000 ml Methyl *tert* butyl ether was added washed with 2*50 ml 1:1 ethanol water and distilled under vacuum. 3000 ml 2-Butanone was added to the residue, cooled to (-5)-(-8)°C and the separated solids were filtered as a third crop that contains Vitamin D_3 (5 MIU)+ 7-Dehydrocholesterol (20%) + **Phytosterol (20%).** All these first, second and third crops are combined and reused in subsequent batches.

The filtrate was then evaporated under vacuum at 40-45°C and the crude Vitamin D_3 (resin) was analysed and the results are as shown in table 2.

Table 2:

S no	Compound	% by HPLC	Potency
1	Vitamin D_3/Cholecalciferol + pre-Vitamin D_3	88-90%	23-24 MIU
2	Phytosterol	2-3%	NA
3	7-Dehydrocholesterol	1-2%	NA
4	Tachysterol	2-2.5%	NA

5	Lumisterol	2-2.5%	NA
6	Trans Vitamin D_3	1-2%	NA

The crude resin was purified by either of the following methods:

1. Converting the resin to its ester like Acetate or Propionate or Butyrate or Valerate or 2-Nitrobenzoate or 4-Nitrobenzoate more preferably Butyrate, crystallized and finally saponified by a base like Sodium Hydroxide or Potassium hydroxide or Sodium carbonate or Potassium carbonate Sodium methoxide or Sodium Ethoxide or Potassium butoxide or Lithium Aluminium Hydride and finally crystallized from acetone.
2. Purification of the Crude resin by column chromatography using Silica gel or Alumina or Alumina with 2-10% water and using Toluene: 2-Butanone as an eluent.

Example 3

96 gms of 7-Dehydrocholesterol, 200 gms of **Lanosterol** 1 gm of Butylated hydroxyl toluene and 1 ml of 2% Aq. Sodium hydroxide solution are dissolved in 6000 ml of **Ethanol**, at 75-85°C and the mixture was then irradiated by a LOW PRESSURE mercury lamp at 80-85°C for 285 minutes. The reaction mass was cooled to 30-35°C.

The solids separated out were filtered as first crop which contains 7-Dehydrocholesterol (50-60%) + **Lanosterol**

The filtrate was concentrated to 50% of the original volume cooled to 25-30°C and the separated solids were filtered as second crop that contains 7-Dehydrocholesterol (15-20%) + **Lanosterol**.

The filtrate was again concentrated under vacuum. 2000 ml Methyl *tert* butyl ether was added washed with 2*50 ml 1:1 ethanol water and distilled under vacuum. 3000 ml 2-Butanone (methyl ethyl ketone) was added to the residue,

cooled to 15°C and the separated solids were filtered to obtain third crop that contains Vitamin D_3 (15MIU)+ 7-Dehydrocholesterol 20%+ Lanosterol 20%. All these first, second and third crops were combined and reused in subsequent batches.

The filtrate was then evaporated under vacuum at 40-45°C and the crude Vitamin D_3 (resin) was analyzed and the results are shown in table 3.

Table 3:

S no	Compound	% by HPLC	Potency
1	Vitamin D_3/Cholecalciferol + pre-Vitamin D_3	85-90%	28-30 MIU
2	Lanosterol	2-3%	NA
3	7-Dehydrocholesterol	1.5-2%	NA
4	Tachysterol	2-4%	NA
5	Lumisterol	1-3%	NA
6	Trans Vitamin D_3	2-3%	NA

The crude resin was purified by either of the following methods:

1. Converting the resin to its ester like Acetate or Propionate or Butyrate or Valerate or 2-Nitrobenzoate or 4-Nitrobenzoate more preferably Butyrate, crystallized and finally saponified by a base like Sodium Hydroxide or Potassium hydroxide or Sodium carbonate or Potassium carbonate Sodium methoxide or Sodium Ethoxide or Potassium butoxide or Lithium Aluminium Hydride and finally crystallized from acetone.
2. Purification of the Crude resin by column chromatography using Silica gel or Alumina or Alumina with 2-10% water and using Toluene: 2-Butanone as an eluent.

Example 4:

96 gms of 7-Dehydrocholesterol, 104 gms of Cholesterol , 1 gm of Butylated hydroxyl toluene and 25 ml of 2% Aq. Sodium hydroxide solution are dissolved in 1250 ml of **Ethanol** at 75-85°C and the mixture was then irradiated by a LOW PRESSURE Mercury lamp at 80-85°C for 180 minutes preferably. The reaction mass was cooled to 25-30°C.

The solids separated out were filtered as first crop which contains 7-Dehydrocholesterol (50-60%) + Cholesterol.

The filtrate was concentrated to 20% of the original volume, cooled to 20-25°C and the separated solids were filtered as second crop that contains 7-Dehydrocholesterol (15-20%) + Cholesterol.

The filtrate was again concentrated under vacuum. 2000 ml Methyl *tert* butyl ether was added washed with 2*50 ml 1:1 ethanol water and distilled under vacuum. 3000 ml 2-Butanone (methyl ethyl ketone) was added to the residue, cooled to 10°C and the separated solids were filtered as the third crop that contains Vitamin D_3 (5-MIU)+ 7-Dehydrocholesterol 20% + Cholesterol 20%. All these first, second and third crops were combined and reused in subsequent batches.

The filtrate was then evaporated under vacuum at 40-45°C.

The residue was purified by column chromatography over silica gel with Toluene: methyl ketone 1:99, 2:98, 4:96 & 5: 95 to isolate pure Vitamin D_3 /Cholecalciferol crystals.

The HPLC analysis of the vitamin D3 crystals are shown in Table 4 as shown below.

Table 4:

S no	Compound	% by HPLC	Potency
1	Vitamin D_3/Cholecalciferol	98-99.5%	40 MIU
2	Cholesterol	ND	NA
3	7-Dehydrocholesterol	ND	NA
4	Tachysterol	0.01%	NA
5	Lumisterol	0.01%	NA
6	Trans Vitamin D_3	0.05%	NA

We claim,

1. A photochemical process for the preparation of a vitamin D3 which process comprises;
   a) Heating the mixture of 7-dehydrosterol in combination with a sterol derivative and catalytic amounts of an anti-oxidant in presence of an alkali in an organic solvent at a temperature range of 20-85°C;
   b) Irradiating the mixture using a low pressure Mercury lamp at 10-85°C; and
   c) Isolating and purifying vitamin D3 from the irradiated mixture.
2. The process as claimed in claim 1, wherein, the sterol derivative is selected from the group consisting of Cholesterol, Phytosterol, Lanosterol, etc.
3. The process as claimed in claim 1, wherein, the organic solvent in step a) is selected from the group consisting of methanol, ethanol, Isopropanol, Tetrahydrofuran, Diethyl ether, methyl-tert-butyl ether, methyl isobutyl ketone & petroleum Ether (40-60°C &60-80°C).
4. The process as claimed in claim 1, wherein, the 7-Dehydrocholesterol is charged with sterol derivative approximately in a ratio of 0.1: 1 to 1: 3.
5. The process as claimed in claim 1, wherein, the anti-oxidant is preferably selected from Butylated hydroxyl toluene, propyl gallate, butylated hydroxyanisole etc.
6. The process as claimed in claim 1, wherein, the anti-oxidant is Butylated hydroxyl toluene.
7. The process as claimed in claim 1, wherein, the alkali is preferably 2% to 10% of sodium hydroxide.
8. The process as claimed in claim 1, wherein, the mixture is irradiated by a low pressure Mercury lamp at 10-85°C for 30-300 minutes preferably 60 - 240 minutes or more preferably 100-200 minutes to accomplish the reaction.

9. The process as claimed in claim 1, wherein, the isolation and purification of vitamin D3 comprises the steps of;
    a) Repeatedly cooling the reaction mass to 0-45°C to separate the solids consisting of unreacted 7-DHC and sterol derivative followed by concentrating the filtrate under vacuum;
    b) Adding an organic solvent followed by washing with aq. alcohol and further distillation under vacuum to obtain residue;
    c) Adding an organic solvent to residue followed by cooling upto -5°C-+35°C to separate the solids consisting of Vitamin D3 (5-10 MIU)+ 7-Dehydrocholesterol + sterol derivative;
    d) Evaporating the filtrate under vacuum at 40-45°C to obtain crude Vitamin D3 (resin); and
    e) Purifying the crude vitamin D3 by converting into its ester followed by saponification and crystallization from an organic solvent to obtain pure vitamin D3.
10. The process as claimed in claim 9, wherein the solids consisting of unreacted 7-DHC and sterol derivative obtained in steps a) and c) are collected to reuse in the subsequent batch.
11. The process as claimed in claim 9, wherein the esters of vitamin D3 are selected from the group consisting of Acetate, Propionate, Butyrate, Valerate, 2-Nitrobenzoate or 4-Nitrobenzoate, more preferably Butyrate.
12. The process as claimed in claim 9, wherein the saponification of the ester is carried out using a base selected from Sodium Hydroxide or Potassium hydroxide or Sodium carbonate or Potassium carbonate Sodium methoxide or Sodium Ethoxide or Potassium butoxide or Lithium Aluminium Hydride.
13. The process as claimed in claim 9, wherein the organic solvent used in step b) is selected from the group consisting of methyl tert butyl ether, petroleum ether (40-60°C & 60-80°C), n-Heptane, n-Hexane, ethyl acetate, Diethyl ether, Toluene and Xylene and the alcohol is selected from methanol, ethanol and isopropanol.

14. The organic solvent used in step c) is selected from the group consisting of 2-Butanone, Acetone, Methyl-Isobutyl ketone, petroleum ether (40-6-°C &60-80°C), n-heptane or n-Hexane.
15. The organic solvent used in step e) is a ketone or an ester selected from the group consisting of acetone, 2-Butanone, Methyl isobutyl ketone, methyl formate, ethyl formate.
16. The process as claimed in claim 9, wherein the residue obtained in step b) is purified by column chromatography over silica gel/Alumina or 2-10% Hydrated Alumina with Toluene: 2-Butanone in a ratio of 1:99, 2:98, 4:96 & 5: 95 or with Dichloromethane: methanol 99:1, 98.:2,95:5, 90:10 or with Ethyl Acetate: methanol 99:1,98:2, 95:5 & 90:10, to isolate pure Vitamin D3 /Cholecalciferol crystals.
17. A composition comprising :
    a) between about 98-99.5% of D3; and
    b) one or both of lumisterol and tachysterol at a concentration of at least 0.02 % of the vitamin D3 present in the composition;
    c) Trans Vitamin D3 at a concentration of at least 0.05%;

wherein, the vitamin D3 meets potency requirement of 40 MIU.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2020/050426

A. CLASSIFICATION OF SUBJECT MATTER
A61K31/593,C07C401/00,C07J9/00 Version=2020.01

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K, C07C, C07J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

TotalPatent One, IPO Internal Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	IN 201921018751 A (FERMENTA BIOTECH LIMITED) 29 November 2019 (29.11.2019) page 2, line 1- page 18, line 10; abstract; claims.	1-16
X	WO 2008128783 A2 (DSM IP ASSETS BV [NL] ET AL) 30 October 2008 (30.10.2008) page 10, lines 1-30; abstract; claims.	1-16

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03-09-2020	03-09-2020
Name and mailing address of the ISA/ Indian Patent Office Plot No.32, Sector 14,Dwarka,New Delhi-110075 Facsimile No.	Authorized officer Chiranjit Sarkar Telephone No. +91-1125300200

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2020/050426

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

The present application defines the plurality of distinct inventions because the claimed inventions or group of inventions are not linked by a single general inventive concept. The separate inventions or group of inventions are as follows.

Group I: Claims 1-16 relate to a photochemical process for the preparation of vitamin D3.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1-16 (first group of the invention)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IN2020/050426

Citation	Pub.Date	Family	Pub.Date
WO 2008128783 A2	30-10-2018	CN 101668739 A	10-03-2010

International application No.
PCT/IN2020/050426

Continuation of Observations where unity of invention is lacking(Box III)

Group II: Claim 17 relates to a composition comprising vitamin D3.

The above group of inventions is distinct to each other i.e. lack unity within the meaning of Rule 13 PCT since the common technical feature of these group of inventions is "vitamin D3", is already known in the prior art document WO2008128783A2, which discloses a photochemical process for the preparation of a previtamin D and converting the previtamin D or the derivative thereof to the vitamin D or the derivative thereof by thermal rearrangement.
Therefore " vitamin D" cannot serve as a special technical feature over the prior art among these groups of inventions. Further, there is no other special technical feature which correlates these group of inventions in a single inventive concept. Therefore, the present application lacks unity of invention under rule 13(2) of the PCT.