

**STABLE ORAL SOLUTION FORMULATION COMPRISING ULTRA-FINE  
AND FINE PARTICLES OF VITAMIN D<sub>3</sub>, A CURCUMINOID AND A NON-  
CURCUMINOID**

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**FIELD OF THE INVENTION**

The present invention generally relates to the field of pharmaceutical preparation.

More particularly present invention relates to stable clear uniformly interspersed particle oral solution formulation comprising ultra-fine and fine particles of vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid for oral administration.

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**BACKGROUND OF THE INVENTION**

Vitamin D<sub>3</sub> chemically referred to as cholecalciferol, has long been recognized as essential to health and used for the treatment of vitamin D deficiency and for the prevention or treatment of various medical conditions associated with vitamin D deficiency. Vitamin D appears to increase the efficiency of the intestine to absorb phosphorus and calcium. By promoting calcium absorption, vitamin D helps to form and maintain strong bones. Vitamin D is also known to modulate the innate and adaptive immune responses. Deficiency in vitamin D is associated with increased autoimmunity as well as an increased susceptibility to infection (*Cynthia Aranow J Invest Med. 2011 Aug*). Naturally, humans acquire vitamin D from sunlight because ultraviolet rays from the sunlight trigger vitamin D synthesis in the skin.

Curcuminoids are the yellow color pigments isolated from turmeric. Curcumin is the major type of Curcuminoids. Apart from their use as natural coloring and flavoring agent, Curcuminoids have been extensively studied for medicinal properties. Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic activities (*Anand P et al, 2007*). Curcumin has also been shown in the last two decades to be a potent immunomodulatory agent that can modulate the activation of T cells, B cells, macrophages, neutrophils, natural killer cells, and dendritic cells (*Ganesh Chandra Jagetia et al, 2007*).

Non-curcuminoids are basically all other biologically active compounds of turmeric excluding curcuminoids. The immunomodulatory and chemo-preventive

activities of  $\alpha$ -turmerone, a non-curcuminoid, was revealed with regards to its potency to be equivalent to curcuminoids (Yue GGL *et al*, 2010). AR-turmerone, a non-curcuminoid, has shown to possess potent anti-inflammatory, anti-oxidative and anti-platelet properties (S Toden *et al*, 2017).

5           The dose of vitamin D is commonly expressed by weight (typically in micrograms) or by international unit (IU), which is a measure of biological activity. One IU unit is equivalent to 0.025 microgram of vitamin D ( $D_3$ ). Vitamin D is very light sensitive and is subject to oxidative degradation, which requires stabilization by means of additives and/or oxygen protective barrier. Despite having various advancements,  
10       there remains a need for a stable vitamin  $D_3$  formulation for maintaining vitamin D levels in people with vitamin D deficiency.

          IN 311468 (1872/CHE/2014) teaches a nano-dispersion with a physical stability upto one year comprising a bioactive compound selected from the group of lipophilic Vitamins, antioxidants and water insoluble drugs; a nano-dispersion stabilizer  
15       modulating interfacial barriers through hydrogen bonding in a concentration range of 1% w/v to 85% w/v, preferably in the range of 25%w/v to 60%w/v, wherein the nano-dispersion stabilizer at concentration ranging from 1% w/v to 85% w/v improves the long term physical stability of the nano-dispersion and facilitates particle size reduction when compared with nano-dispersions without the nano-dispersion stabilizers, wherein  
20       the nano-dispersion stabilizer maintains the physical stability of the nano-dispersion for a period of upto one year at room temperature at concentration ranging from 25%w/v to 60%w/v.

          IN 934/DEL/2015 teaches a liquid dosage form of Vitamin D comprising Vitamin D in the range of 0.03-0.10% w/v, dispersant in the range of 90-99.90% v/v; along with  
25       one or more pharmaceutically acceptable excipients, wherein the vitamin D is dispersed in nano-sized oil droplets in the dispersant.

          IN 201641025758 teaches a stable dispersion comprising an active, primary emulsion, stabilized vehicle base, preservative and aqueous dispersion medium; wherein primary emulsion consists of active, surfactant, emulsifying agent, antioxidant and oil  
30       phase, stabilized vehicle base preservative consists of stevia and glycerol, preservative consist of sodium benzoate and ethylene tetra amine di-acetate and aqueous dispersion

medium, the dispersion medium is stable for a longer period in blow fill tubes made of mixture of polypropylene and polyethylene thereof.

IN 201821039451 teaches an aqueous pharmaceutical composition comprising a therapeutically effective amount of vitamin D<sub>3</sub>. The composition further comprising 1-  
5 25% solvent/vehicle selected from group consisting of castor oil or its derivatives, vegetable glycerin and mixture of thereof and 1% to 15% by weight of a stabilizer selected from group consisting of vegetable glycerine and sorbitol and mixture thereof.

IN 202011006473 teaches a stable pharmaceutical nano-emulsion composition comprising vitamin D<sub>3</sub> compound, lipophilic phase, hydrophilic phase and surfactant,  
10 wherein the amount of the Vitamin D<sub>3</sub> contained in the formulations is not less than 1,20,000 IU of cholecalciferol for dosage form.

*Jean Lucas G da Silva et al., Inflammation.2019 Oct;42(5):1595-1610* teaches effects of a nano-encapsulated association of curcumin and vitamin D<sub>3</sub> on purine metabolism enzymes in neutrophils, lymphocytes, and platelets in a model of adjuvant-  
15 induced arthritis (AIA) in rats.

Vitamin D<sub>3</sub> formulations, particularly the marketed nano-dispersion formulations pose several limitations which include the following:

i. Vitamin D has a fast release and quick absorption in presence of bile. Vitamin D undergoes two hydroxylation in the body for activation. There are several metabolic  
20 products or modified versions of vitamin D. Vitamin D is converted into Calcidiol (25-hydroxyvitamin D<sub>3</sub>) in liver which has a half-life of about 15 days and a T<sub>max</sub> of about 7 days. Calcidiol passes through kidney and converts into Calcitriol (1, 25-dihydroxy vitamin D<sub>3</sub>), the active form of vitamin D, has a half-life of about 5-8 hours. Vitamin D binds to receptors located throughout the body. The vitamin D metabolites are  
25 transported in blood bound primarily to vitamin D binding protein (DBP) (85-88%) and albumin (12-15%). DBP concentrations are normally 4-8μM, well above the concentrations of the vitamin D metabolites, such that DBP is only about 2% saturated. DBP has high affinity for the vitamin D metabolites ( $K_a=5 \times 10^8 M^{-1}$  for 25OHD and 24,25(OH)<sub>2</sub>D,  $4 \times 10^7 M^{-1}$  for 1,25(OH)<sub>2</sub>D and vitamin D), under normal circumstances  
30 approximately 0.03% 25OHD and 24,25(OH)<sub>2</sub>D and 0.4% 1,25(OH)<sub>2</sub>D are free. Conditions such as liver disease and nephrotic syndrome resulting in reduced DBP and

albumin levels will lead to a reduction in total 25OHD and 1,25(OH)<sub>2</sub>D levels. Similarly, DBP levels are reduced during acute illness, potentially obscuring the interpretation of total 25OHD levels. Vitamin D intoxication can increase the degree of saturation sufficiently to increase the free concentrations of 1,25(OH)<sub>2</sub>D and so cause hypercalcemia without necessarily raising the total concentrations.

ii. People with liver and kidney ailments / disorders and organ function impairment have significantly decreased capacity of hydroxylation. This results in decreased conversion of Vitamin D into active metabolite forms which could bind with DBP for active and effective absorption of Vitamin D metabolites.

With this background, the present invention provides a novel formulation having an absorption profile with a desired release pattern which enables release of smaller quantities of Vitamin D<sub>3</sub> for longer periods into blood stream post consumption. Vitamin D<sub>3</sub> formulation of present invention is designed to help in maintaining the rate of permeation through GI track to blood stream in a sustained absorption pattern and ensure a continuous supply of Vitamin D and hence Calcitriol availability, to bind with DBP for maximum therapeutic output. This slow-release pattern also assists in maintaining active levels of Vitamin D metabolites in persons suffering from Kidney and liver diseases which results in decreased capacity of hydroxylation.

Further, the inventors of the present invention have developed a stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid for oral administration which is not taught by the prior-art. The inventors of the present application have developed a stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid with controlled particle size, preferably having a particle size of d<sub>10</sub> not more than 75nm, d<sub>50</sub> not more than 150nm and d<sub>90</sub> not more than 300nm, wherein the uniform interspersing of particles of a curcuminoid and a non-curcuminoid with the particles of Vitamin D<sub>3</sub> results in imparting significantly sustained permeation pattern to Vitamin D<sub>3</sub> particles.

### OBJECTIVE OF THE INVENTION

The main objective of the present invention is to provide a stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid for oral  
5 administration.

Another objective of the present invention is to provide a stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid, a non-curcuminoid and one or more pharmaceutically acceptable excipients.

10 Another objective of the present invention is to provide a method of manufacturing a stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid with controlled particle size, preferably having a particle size of d<sub>10</sub> not more than 75nm, d<sub>50</sub> not more than 150nm and d<sub>90</sub> not more than 300nm, which in turn  
15 imparts significantly sustained permeation pattern which in turn results in a continuous supply of Vitamin D in the blood stream and hence Calcitriol availability to bind with DBP for maximum therapeutic output. The slower rate of permeation also ensures continuous availability of Vitamin D to compensate the decreased capacity of hydroxylation in the patients with liver and kidney disease and/or impairment. The  
20 slower release of Vitamin D<sub>3</sub> into blood stream also mitigates the risk of hypercalcemia due to Vitamin D intoxication.

Another objective of the present invention is to provide a method of reducing particle size and accomplishing a uniformly interspersed particle formulation by using an ultrasonic processor to convert electrical energy into mechanical vibration with a  
25 frequency and transmitting the mechanical energy to the medium (amplitude setting 20% to 100%, energy level between 1000 – 80,000 w/s).

Another objective of the present invention is to provide a stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid, a non-curcuminoid and a carrier system  
30 which comprises non-ionic solubilizer cum stabilizer, which is designed to stabilize vitamin D<sub>3</sub>.

### SUMMARY OF THE INVENTION

The present invention provides a stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid for oral administration.

5       The present invention also provides a stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid, a non-curcuminoid and one or more pharmaceutically acceptable excipients.

10       The present invention also provides a stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid, a non-curcuminoid and one or more pharmaceutically acceptable excipients, wherein a Curcuminoid and a non-curcuminoid in this formulation is manufactured as curcuma nano drops separately by mixing non-ionic solubilizer and emulsifier and a Curcumin removed turmeric oleoresin under heating.

15       The present invention also provides a stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid with controlled particle size, having a particle size of d<sub>10</sub> not more than 75 nm, d<sub>50</sub> not more than 150 nm and d<sub>90</sub> not more than 300 nm.

20       The present invention also provides a stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid, wherein said composition comprising one or more excipients selected from solubilizer and/or emulsifying agent, chelating agents, stabilizers, dispersing agents, sweetening agents, preservatives, buffering agents, 25       flavoring agents and solvents.

The present invention also provides a stable, clear uniformly interspersed particle oral solution formulation comprising;

- a) Vitamin D<sub>3</sub>,
- b) curcuminoid,
- 30       c) non-curcuminoid,
- d) solubilizer and/or emulsifying agent,

- e) dispersing agents,
- f) sweetening agents,
- g) preservatives,
- h) buffering agents,
- 5 i) flavoring agents and
- j) solvents.

The present invention also provides a stable, clear uniformly interspersed particle oral solution formulation comprising;

- a) Vitamin D<sub>3</sub>,
- 10 b) curcuminoid,
- c) non-curcuminoid,
- d) Polyoxyl Hydrogenated castor oil (liquid),
- e) propylene glycol.
- f) maltitol (liquid),
- 15 g) sodium benzoate,
- h) disodium edetate,
- i) sodium citrate,
- j) citric acid monohydrate,
- k) caramel artificial flavour,
- 20 l) ethanol and
- m) purified water.

The present invention also provides a stable, clear uniformly interspersed particle oral solution formulation comprising;

- a) Vitamin D<sub>3</sub>,
- 25 b) curcuminoid,
- c) non-curcuminoid,
- d) polyoxyl Hydrogenated castor oil (liquid),
- e) propylene glycol,
- f) maltitol (liquid),
- 30 g) sodium methylparaben,
- h) sodium propylparaben,

- i) sodium citrate,
- j) citric acid monohydrate,
- k) caramel artificial flavour,
- l) ethanol and
- 5 m) purified water.

The present invention also provides a method of manufacturing a stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid comprising preparing pre-mix 1, preparing pre-mix 2 and preparation of final formulation.

- 10 The present invention also provides a method of manufacturing a stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid with controlled particle size, having a particle size of d<sub>10</sub> not more than 75nm, d<sub>50</sub> not more than 150 nm and d<sub>90</sub> not more than 300nm, wherein a curcuminoid in the said
- 15 formulation enables replacement of synthetic colorant by acting as a natural colorant and the antioxidant property of a curcuminoid and a non-curcuminoid in the said formulation improves the stability as well as supplements the immunomodulatory activity of the oral solution formulation.

- The present invention also provides a method of manufacturing a stable, clear
- 20 uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid comprising steps of:

- (a) weighing and dispensing dispersing agents into a suitable vessel containing dispensed quantity of purified water under stirring for 10 minutes,
- 25 (b) sonicating mixture using sonicator to reduce particle size,
- (c) weighing and dispensing Vitamin D<sub>3</sub> into a suitable vessel containing solvent under stirring until to form clear solution.
- (d) adding water soluble and therapeutically safe curcuma nano drops formulation in solution form under stirring until to form clear yellow color solution,
- 30 (e) sonicating using sonicator to reduce particle size,

- (f) adding required quantity solubilizer/emulsifying agent gradually into a suitable vessel and heat it up to 40°C until to form a clear solution,
- (g) transferring step (f) solution into the vessel containing step (e) solution under stirring for 5 minutes after addition,
- 5 (h) transferring step (b) solution into main manufacturing vessel under stirring for 5 minutes after addition,
- (i) transferring step (g) solution into main manufacturing vessel under stirring for 5 minutes after addition,
- (j) adding preservatives into a suitable vessel containing purified water under stirring  
10 for 5 minutes after addition,
- (k) adding buffering agent into a suitable vessel containing purified water under for 15 minutes after addition and check the clarity of the solution and transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition,
- 15 (l) adding buffering agent solution into main manufacturing vessel under stirring and adjust the pH between 5.00 to 6.00,
- (m) transferring flavour to main manufacturing vessel under stirring for 5 minutes after addition and
- (n) making the volume with purified water under stirring for 5 minutes after addition.
- 20 The present invention also provides a method of manufacturing a stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid comprising steps of:
- (a) weighing and dispensing liquid maltitol, propylene glycol into a suitable vessel  
25 containing dispensed quantity of purified water under stirring for 10 minutes,
- (b) sonicating mixture using sonicator to reduce particle size,
- (c) weighing and dispensing Vitamin D<sub>3</sub> into a suitable vessel containing ethanol under stirring until to form clear solution.
- (d) adding water soluble and therapeutically safe curcuma nano drops formulation in  
30 solution form under stirring until to form clear yellow color solution,
- (e) sonicating using sonicator to reduce particle size,

- (f) adding required quantity of Polyoxyl 40 Hydrogenated castor oil gradually in to a suitable vessel and heat it up to 40°C until to form a clear solution,
- (g) transferring step (f) solution into the vessel containing step (e) solution under stirring for 5 minutes after addition,
- 5 (h) transferring step (b) solution into main manufacturing vessel under stirring for 5 minutes after addition,
- (i) transferring step (g) solution into main manufacturing vessel under stirring for 5 minutes after addition,
- (j) adding preservatives into a suitable vessel containing purified water under stirring  
10 for 5 minutes after addition,
- (k) adding sodium citrate into a suitable vessel containing purified water under for 15 minutes after addition and check the clarity of the solution and transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition,
- (l) adding 10% citric acid solution into main manufacturing vessel under stirring and  
15 adjust the pH between 5.00 to 6.00,
- (m) transferring caramel flavour VV-729-140-3 to main manufacturing vessel under stirring for 5 minutes after addition and
- (n) making the volume with purified water under stirring for 5 minutes after addition.

The present invention further provides a method of reducing particle size along  
20 with uniform interspersing of particle formulation using an ultrasonic processor to convert electrical energy into mechanical vibration with a frequency and transmitting the mechanical energy to the medium (amplitude setting 20% to 100%, energy level between 1000 – 80,000 w/s).

The present invention further provides a carrier system which comprises non-  
25 ionic solubilizer cum stabilizer, which is designed to stabilize vitamin D<sub>3</sub>.

These and other aspects, features and advantages will become apparent to those of ordinary skill in the art from a reading of the following detailed description and the appended claims.

### **BRIEF DESCRIPTION OF DRAWINGS**

**Fig.1:** % permeation comparison at defined time intervals against Reference formulation (existing marketed formulation)

**Fig.2:** Schematic presentation of the In Vitro Permeation Model

5 **Fig.3:** PSD histogram of premix 1 which is used for preparing Vitamin D<sub>3</sub> solution 60,000IU/5ml as detailed in Example No: 4

**Fig.4:** PSD histogram of Vitamin D<sub>3</sub> solution 60,000IU/5ml prepared as detailed in Example No: 4

**Fig.5:** Z-Average of final solution formulation for example No. 4 is: 30.47 (d.nm)

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### **DETAILED DESCRIPTION OF THE INVENTION**

The articles “a” and “an” are used herein refers to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element

15 The term “comprising”, which is synonymous with “including”, “containing”, or “characterized by” here is defined as being inclusive or open-ended, and does not exclude additional, unrecited elements or method steps, unless the context clearly requires otherwise.

20 All percentages are defined as w/v unless stated otherwise. All measurements are defined according to International Standard of Units (SI) unless written otherwise.

The term “water-soluble” is defined as the ability of the curcumin formulation to remain in clear solution form without precipitation or separation of the particles for a minimum period of 24 hours and the ability of curcuma formulation to result into a clear and transparent solution, without precipitation or separation of particles for a minimum  
25 period of 15 minutes when added to water in ratio of 1: 200 and above.

The term Curcumin is defined as the principal biologically active curcuminoid found and derived from Turmeric.

The term Curcuminoid is defined as a linear diarylheptanoid found and derived from Turmeric, comprising curcumin or derivatives of curcumin.

The terms “curcumin”, “curcuminoid” and “curcuminoids” may be used interchangeably unless otherwise stated differently. The native curcumin may comprise curcumin, demethoxycurcumin, bisdemethoxycurcumin and/or mixtures thereof.

5 The term “non-curcuminoid” is defined as a biologically active compound other than a curcuminoid, more particularly a curcumin-free bioactive compound found and derived from Turmeric.

The term curcuma nano drops is used to denote a clear solution formulation comprising “curcuminoids” and “non-curcuminoids” along with one or more pharmaceutically acceptable excipients.

10 The term Turmeric is defined as rhizomatous herbaceous perennial plant of the *Curcuma* species of the ginger family, Zingiberaceae, more particularly *Curcuma Longa*.

Further, the incorporation of a curcuminoid and a non-curcuminoid in the formulation synergistically improves the biological outcomes for the consumer which comprises improved immunity and metabolic wellness.

15 The term “particle” is defined as a minute portion of a substance which when having a size range between 1 and 100 nanometers are called as ultrafine and/or nano particles and when having size range between 100 and 2500 nanometers are termed as fine particles.

20 The term “stable” is defined as the formulation when stored at normal ambient conditions (Temperature 25°C and Relative Humidity 60%) for a period of over 3 months, remains as a clear and transparent solution, without separation or precipitation of the particles.

25 The term “non-ionic solubilizer and /or emulsifying agent” is defined as a compound or substance that acts as a stabilizer for the formulation, wherein the hydrophilic portion of the emulsifying substance does not contain an ionic group.

The term “turmeric oleoresin, TO” is defined as an extract of turmeric in the form of viscous oily fluids, pasty semisolids or hard amorphous solids, and which comprise in varying concentrations, both curcuminoids and essential volatile oil, wherein the volatile oil comprises a mixture of non-curcuminoids in varying concentrations.

30 The term “Turmeric oil, TOIL” is defined as the essential volatile oil comprising a mixture of non-curcuminoids in varying concentrations.

The term “curcumin removed turmeric oleoresin, CRTO” is defined as mother liquor obtained after at least one extraction of curcumin from TO which after removal of residual solvent used in extraction process, comprises the essential volatile oil containing a mixture of non-curcuminoids and may also comprise varying concentrations of curcuminoids.

The terms “pharmaceutically acceptable excipient”, “pharmaceutically acceptable excipients” are defined as substances other than the active pharmaceutical ingredient (API) that have been appropriately evaluated for safety and are intentionally included in a drug delivery system which facilitate processing of the drug formulation during its manufacture; protect, support, and/or enhance stability, bioavailability, or patient acceptability; facilitate in the effectiveness and/or delivery of the drug formulation and assist in maintaining the integrity of the drug formulation during its storage across its shelf life.

The term Carrier is defined as pharmaceutically acceptable excipients which are designed to interact with and enhance the properties of active pharmaceutical ingredient – API.

Disclosed herein are embodiments related to a stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid. The solution formulation has a controlled particle size, preferably comprising a particle size of d<sub>10</sub> not more than 75nm, d<sub>50</sub> not more than 150nm and d<sub>90</sub> not more than 300nm. The controlled particle size along with the uniformly interspersed particle formulation enables significant improvement in the sustained permeation and bioavailability of Vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid.

To achieve stable, clear oral solution, the present invention provides oral solution with a uniformly interspersed particle formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid and a carrier system.

The particle size reduction is accomplished by an elaborate process which has been standardized with the use of a particle size reducing processor preferably using the ultrasonic processor “Hielscher Ultrasonics GmbH” Model: UP200St\_\_TD. The

ultrasonic processor is equipped with the ultrasonic transducer UP200St\_TD, the generator UP200St\_G (Operating frequency: 26kHz). The operating mechanism includes the conversion of electrical stimulations into mechanical ultrasonic vibrations that are transmitted directly to the target medium via a sonotrode. The mechanical  
5 amplitude of the processor was standardized and set between 20% and 100% and the energy level was standardized and set between 1000 – 80,000 w/s.

The particle size of the formulation during the entire manufacturing process was measured at various steps of the formulation process using a particle size analyzer, preferably Malvern P analytical Zetasizer ZSP Model No. ZEN5600 which works on  
10 Dynamic Light Scattering technique (DLS technique). The DLS technique sometimes referred to as Photon Correlation Spectroscopy or Quasi-Elastic Light Scattering) is used to measure the size of particles typically in the sub-micron region.

The diameter that is measured in DLS is a value that refers to the average particle diameter expressed in nanometers. The results are reported in Z average values and  $d_{10}$ ,  
15  $d_{50}$ ,  $d_{90}$  values.

The Z-Average size or Z-average mean used in dynamic light scattering is a parameter also known as the cumulants mean. The Z-Average mean is defined in ISO 13321 and more recently ISO22412 which defines this mean as the “harmonic intensity averaged particle diameter”. Detailed dynamic light scattering technique and description  
20 of these values are calculated as known in the art and can be found in, for example ISO 224212.

The term “Dx of the particle size distribution” refers to the xth percentile of the distribution on an equivalent spherical particle volume basis; thus, D90 refers to the 90th percentile, D50 refers to the 50th percentile, and so forth. Taking D90 as an  
25 example, it can be written as D90 or D(90), or D(0.90), or D (0.9) or similar. With respect to median particle size and Dx an uppercase D or lowercase d are interchangeable and have the same meaning. Detailed description of these values is calculated as known in the art and can be found in, for example ISO 9276-2:2014.

The present invention may be understood more readily by reference to the  
30 following detailed description. It is to be understood that this invention is not limited to the specific products, methods, conditions or parameters described and/or shown herein,

and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting of any claimed invention. Similarly, unless otherwise stated, any description as to a possible mechanism or mode of action or reason for improvement is meant to be illustrative only, and the invention herein is not be constrained by the correctness or incorrectness of any such suggested mechanism or more of action or reason for improvement. Throughout this text, it is recognized that the descriptions refer both to the features and methods of making and using the compositions described herein.

In the present disclosure the singular forms “a”, “an” and “the” include the plural reference, and references to a particular numerical value includes at least that particular value, unless the context clearly indicates otherwise. Thus, for example, a reference to “a material” is a reference to at least one of such materials and equivalents thereof known to those skilled in the art, and so forth.

When a value is expressed as an approximation by use of the descriptor “about” it will be understood that the particular value forms another embodiment. In general, use of the term “about” indicates approximations that can vary depending on the desired properties sought to be obtained by the disclosed subject matter and is to be interpreted in the specific context in which it is used, based on its function. The person skilled in the art will be able to interpret this as a matter of routine. In some cases, the number of significant figures used for a particular value may be one non-limiting method of determining the extend of the word “about”. In other cases, the gradations used in a series of values may be used to determine the intended range available to the term “about” for each value. Where present, all ranges are inclusive and combinable. That is, references to values stated in ranges include every value within that range. The term “about” as used herein means  $\pm$  approximately 10% of the indicated value.

It is to be appreciated that certain features of the invention which are, for clarity, described herein in the context of separate embodiments, may also be provided in combination in a single embodiment. That is, unless obviously incompatible or excluded, each individual embodiment is deemed to be combinable with any other embodiment(s) and such a combination is considered to be another embodiment. Conversely, various features of the invention that are, for brevity, described in the

context of a single embodiment, may also be provided separately or in any sub-combination.

The present invention provides a stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>,  
5 a curcuminoid and a non-curcuminoid for oral administration. The stable vitamin D<sub>3</sub> solution comprises an effective dose of vitamin D<sub>3</sub> and a carrier system that stabilizes vitamin D<sub>3</sub>.

The effective dose of vitamin D<sub>3</sub> as used in the present invention may include a dose of vitamin D<sub>3</sub> from 400IU to 60000IU.

10 The carrier system as used in the present invention comprises a non-ionic solubilizer and/or emulsifying agent which also acts as a stabilizer. The non-ionic solubilizer is preferably but not limited to Polyethylene castor oil derivatives like Polyoxyl Hydrogenated castor oil. Other Polyethylene castor oil derivatives include Polyoxyl 35 Hydrogenated castor oil, Polyoxyl 40 Hydrogenated castor oil, Kolliphor  
15 RH 40, Macrogolglycerol hydroxystearate, PEG-40 castor oil, Acrysol. Polyoxyethylene alkyl ethers derivatives, Polyoxyethylene sorbitane derivatives, Sorbitan fatty acid esters and Polyoxyethylene stearate derivatives can also be used as non-ionic solubilizers

The present invention comprises a formulation wherein one of the premix component in solution form comprises a particle comprising a turmeric or a curcumin  
20 removed turmeric oleoresin or a turmeric oil and a particle comprising a curcuminoid in a highly bioavailable, water soluble and therapeutically safe form and to which the required amount of Vitamin D<sub>3</sub> is added to accomplish the manufacture of the premix with a uniformly interspersed particle formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid. The  
25 Curcuminoid majorly comprises Curcumin which as a natural colorant imparts not only color to the formulation but also acts as an antioxidant in combination with the non-curcuminoids thereby enhancing stability of the formulation. The curcuminoids and non-curcuminoids also supplement the immunomodulatory activity of Vitamin D<sub>3</sub>.

The present invention provides a stable, clear uniformly interspersed particle oral  
30 solution formulation comprising Vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid

wherein the inhibition of microbial growth in the formulation is accomplished with use of parabens as preservatives.

The present invention comprising a stable, clear uniformly interspersed particle oral solution formulation comprising Vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid solution wherein the pH of the formulation is maintained between 5–8 to enable the formulation have good palatability. Also, the parabens, particularly Methylparaben and Propylparaben have a broad spectrum of antimicrobial activity and they exhibit their antimicrobial activity at pH 4–8. Antimicrobial activity is further improved by using combinations of parabens.

The present invention provides a novel stable clear uniformly interspersed particle oral solution formulation comprising an ultrafine and a fine particle comprising Vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid in solution form wherein the formulation has low viscosity and is less sticky due to low solid content. The lower viscosity helps in reduction of the dosage loss caused by sticking of the formulation onto the walls of the container during consumption; there by delivering the proper dose on each administration.

The present invention provides a novel stable clear uniformly interspersed particle oral solution formulation comprising an ultrafine and a fine particle comprising Vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid solution which is devoid of any antioxidants wherein the curcuminoid and non-curcuminoid present in the formulation act as antioxidant and stabilize the formulation. The solution formulation is stabilized for packing in convenient to use unit dose packaging formats comprising FFS/BFS packs, mono dose vials or amber/clear bottles. The solution formulation can also be packed in multi dose packaging formats using glass/plastic bottles.

The present invention provides a novel stable clear uniformly interspersed particle oral solution formulation comprising an ultrafine and a fine particle comprising Vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid solution, which has no synthetic colorant and wherein the Curcumin in a curcuminoid added to the formulation acts as a natural colorant cum stabilizing agent.

The present invention provides a novel formulation of curcuma nano drops solution with a uniformly interspersed particle formulation comprising an ultrafine and a

fine particle comprising a curcuminoid and a non-curcuminoid solution which is manufactured by mixing non-ionic solubilizer and emulsifier and a Curcumin removed turmeric oleoresin under heating. Curcumin is added to the mixture under stirring to form a uniform solution. The solution is allowed to cool and then Ethanol is added to the solution. Then the final solution is sonicated to obtain a uniformly interspersed particle formulation of curcuma nano drops solution comprising an ultrafine and a fine particle comprising a curcuminoid and a non-curcuminoid. The obtained curcuma nano drops is a water soluble and therapeutically safe solution which becomes the component to be added to the formulation as specified in the detailed description of invention to obtain the final stable, clear solution formulation.

The formulation of the present invention further comprising one or more excipients of dispersing agents, sweetening agents, preservatives, chelating agent, buffering agents, flavoring agents and solvents.

The dispersing agents used in the formulations of present invention selected from propylene glycol, maltitol (liquid), liquid sucrose, sorbitol, liquid glucose or combinations thereof.

The sweetening agents used in the formulations of present invention selected from sucralose, acesulfame potassium, stevia, glucose or combinations thereof.

The preservatives used in the formulations of present invention selected from sodium benzoate, methylparaben, propylparaben, sodium methylparaben, sodium propylparaben or combinations thereof.

The chelating agent used in the formulations of present invention selected from edetates, disodium edetate or combinations thereof.

The buffering agents used in the formulations of present invention selected from sodium citrate and citric acid or combinations thereof.

The flavoring agents used in the formulations of present invention selected from flavoring agents selected from caramel artificial flavor, peppermint flavor, strawberry flavor, lemon flavor, Pineapple flavor, Mixed fruit flavour, Blueberry flavour, Peach flavour, Raspberry, Lime flavour, Passion Fruit flavour, Mango flavor or any other acceptable flavors. Appropriate other flavours from a wide range of compatible flavours can be used to meet to the consumers taste requirement.

The solvents used in the formulations of present invention selected from ethanol, purified water or combinations thereof.

The following examples are illustrative only, and is not intended to limit the present invention.

- 5 No biological material procured from India is used in the compositions of the present application. All the materials used in the present invention are procured from the commercial sources and the details are given below:

| S. No | Name of Active/Excipient         | Commercial source               | Detailed address of Commercial source                                                                                                                   |
|-------|----------------------------------|---------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1.    | Vitamin D3/Cholecalciferol       | Fermenta Biotech                | Fermenta Biotech Ltd.,<br>Plot No. Z 109 B&C, Dahej SEZ<br>II, Gujarat 392130, India                                                                    |
| 2.    | Curcuma nano Drops               | Stabicon Life Science Pvt. Ltd. | Stabicon Life Science Pvt. Ltd.<br>Plot No. 28, 4th Phase, Jigani hobli<br>Anekal Taluk, Bommasandra<br>Industrial Area, Bengaluru,<br>Karnataka 560099 |
| 3.    | Polyoxyl hydrogenated castor oil | Corel Pharma                    | Corel House,<br>opp. Bhagvat Petrol Pump, SG<br>Highway, Gota, Ahmedabad, Gujarat<br>382481                                                             |
| 4.    | Disodium edetate                 | Finar                           | 184-185-186/P, Village:<br>Chacharwadi-Vasna, Sarkhej Bavla<br>Highway, Ta.: Sanand, Dist.:<br>Ahmedabad - 382110 Gujarat (India)                       |

- 10 **Example 1:** Vitamin D<sub>3</sub> 60,000 IU/5ml oral solution

| Ingredients                                 | gm/100ml |
|---------------------------------------------|----------|
| Cholecalciferol (Vitamin D <sub>3</sub> ) * | 0.0375** |

|                                              |               |
|----------------------------------------------|---------------|
| Polyoxyl 40 Hydrogenated castor oil (liquid) | 1.000         |
| Propylene Glycol                             | 5.000         |
| Maltitol (liquid)                            | 35.000        |
| Disodium Edetate                             | 0.100         |
| Sodium Benzoate                              | 0.100         |
| Ethanol                                      | 0.050         |
| Sodium Citrate                               | 0.052         |
| Citric acid Monohydrate                      | 0.100         |
| Caramel artificial flavour                   | 0.050         |
| Curcuma nano Drops <sup>#</sup>              | 0.005 ml      |
| Purified water                               | q.s. to 100ml |

\* Vitamin D<sub>3</sub> – 40 Mn. IU / gm

\*\* inclusive of 25% overages

<sup>#</sup> Curcuma nano Drops containing

Curcuminoids equivalent to not less than 45 mg of Curcumin.

- 5 Total Non-Curcuminoids determined on the basis of equivalent to not less than 3.5mg of AR Turmerone.

### **Manufacturing Procedure**

#### **Preparing pre-mix 1**

- 10 (a) Weigh and dispense required quantity of Liquid Maltitol and Propylene glycol into a suitable vessel containing dispensed quantity of purified water under stirring for 10 minutes.
- (b) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is
- 15 imparted as per the parameters mentioned below.

|                     |                    |
|---------------------|--------------------|
| Sonotrode           | As per batch size  |
| Energy level        | 50000 w/s          |
| Amplitude           | 30 – 70%           |
| Temperature cut off | Not more than 40°C |

|              |                                                      |
|--------------|------------------------------------------------------|
| On time      | 20-30 seconds                                        |
| Off time     | 20-30 seconds                                        |
| No of cycles | Till the specified Energy is imparted to the mixture |

#### Preparing curcuma nano drops:

- I. Weigh and dispense required quantity of Polyoxyl 35 Hydrogenated castor oil and Curcumin Removed Turmeric Oleoresin (CRTO) into suitable manufacturing vessel and heat it between 80°C to 120°C under stirring.
- II. Add curcumin powder to manufacturing vessel containing Polyoxyl 35 Hydrogenated castor oil and CRTO under stirring at 5000-6000 RPM and maintain at this temperature until dark brown color solution is observed and allow it to cool to room temperature.
- III. Add sucralose to separate vessel containing ethanol under stirring at 2000-3000 RPM until solution is clear.
- IV. Add sucralose solution of step III to step II under continuous stirring at 5000-6000 RPM for 30 to 60 minutes.
- V. Add taste masking flavour to manufacturing vessel under stirring for 5 to 15 minutes after addition.
- VI. Check and record the pH.
- VII. Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) for a duration until the required energy level is imparted as per the parameters mentioned below to obtain soluble and therapeutically safe curcuma nano drop formulation.

|                     |                    |
|---------------------|--------------------|
| Sonotrode           | As per batch size  |
| Energy level        | 50000 w/s          |
| Amplitude           | 30 – 70%           |
| Temperature cut off | Not more than 40°C |
| On time             | 20-30 seconds      |
| Off time            | 20-30 seconds      |

|              |                                                      |
|--------------|------------------------------------------------------|
| No of cycles | Till the specified Energy is imparted to the mixture |
|--------------|------------------------------------------------------|

### Preparing pre-mix 2

- (c) Weigh and dispense required quantity of Vitamin D<sub>3</sub> into a suitable vessel containing Ethanol under stirring until to form clear solution. Add water soluble and therapeutically safe curcuma nano drop formulation in solution form under stirring until a clear yellow color solution is obtained.
- (d) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is imparted as per the parameters mentioned below.

|                     |                                                      |
|---------------------|------------------------------------------------------|
| Sonotrode           | As per batch size                                    |
| Energy level        | 2000 w/s                                             |
| Amplitude           | 30 – 70%                                             |
| Temperature cut off | Not more than 40°C                                   |
| On time             | 10-30 seconds                                        |
| Off time            | 20-30 seconds                                        |
| No of cycles        | Till the specified Energy is imparted to the mixture |

### Remaining Procedure

- (e) Add required quantity of Polyoxyl 40 Hydrogenated castor oil gradually in to a suitable vessel and heat it up to 40°C until to form a clear solution. Transfer this solution into the vessel containing pre-mix 2 solution under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into the vessel containing pre-mix 2 solution and continue stirring for 5-10 minutes after addition.
- (f) Transfer pre-mix 1 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.

- (g) Transfer Pre-mix 2 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.
- 5 (h) Add Sodium benzoate and Disodium Edetate into a suitable vessel containing purified water under stirring for 5 minutes after addition and check the clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.
- 10 (i) Add Sodium citrate into a suitable vessel containing purified water under for 15 minutes after addition and check the clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.
- (j) Add 10% Citric acid solution into main manufacturing vessel under stirring and adjust the pH between 5.00 to 6.00.
- 15 (k) Transfer Caramel flavour VV-729-140-3 to main manufacturing vessel under stirring for 5 minutes after addition.
- (l) Make up the volume with purified water under stirring for 5 minutes after addition.

#### Effect of pH in Vitamin D<sub>3</sub> solution of Example 1

| pH Level | Physical observation                                                                    | Taste Evaluation |
|----------|-----------------------------------------------------------------------------------------|------------------|
| 3.5      | Crystallization was observed after pH adjustment using 10% citric acid solution.        | Not palatable    |
| 4.0      | Slight crystallization was observed after pH adjustment using 10% citric acid solution. | Not palatable    |
| 5.0      | No crystallization was observed.                                                        | Palatable        |
| 5.0-8.0  | No crystallization was observed.                                                        | Palatable        |

#### 20 Example 2: Vitamin D<sub>3</sub> 60,000 IU/5ml oral solution

| Ingredients                                  | gm/100ml |
|----------------------------------------------|----------|
| Cholecalciferol (Vitamin D <sub>3</sub> ) *  | 0.036**  |
| Polyoxyl 40 Hydrogenated castor oil (liquid) | 0.900    |

|                            |               |
|----------------------------|---------------|
| Propylene Glycol           | 5.000         |
| Maltitol (liquid)          | 15.000        |
| Sucralose                  | 0.020         |
| Sodium Methyl Paraben      | 0.090         |
| Sodium Propyl Paraben      | 0.020         |
| Ethanol                    | 0.050         |
| Sodium Citrate             | 0.052         |
| Citric acid Monohydrate    | 0.100         |
| Caramel artificial flavour | 0.050         |
| Curcuma nano Drops#        | 0.040 ml      |
| Purified water             | q.s. to 100ml |

\* Vitamin D<sub>3</sub> – 40 Mn. IU / gm

\*\* inclusive of 20% overages

# Curcuma nano Drops containing

Curcuminoids equivalent to not less than 45mg of Curcumin.

- 5 Total Non-Curcuminoids determined on the basis of equivalent to not less than 3.5mg of AR Turmerone.

### **Manufacturing Procedure**

#### **Preparing pre-mix 1**

- 10 (a) Weigh and dispense required quantity of Liquid Maltitol, Propylene glycol and Sucralose into a suitable vessel containing dispensed quantity of purified water under stirring for 10 minutes.
- (b) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is
- 15 imparted as per the parameters mentioned below.

|                     |                    |
|---------------------|--------------------|
| Sonotrode           | As per batch size  |
| Energy level        | 70000 w/s          |
| Amplitude           | 30 – 70%           |
| Temperature cut off | Not more than 40°C |

|              |                                                      |
|--------------|------------------------------------------------------|
| On time      | 20-30 seconds                                        |
| Off time     | 20-30 seconds                                        |
| No of cycles | Till the specified Energy is imparted to the mixture |

### Preparing pre-mix 2

5 (c) Weigh and dispense required quantity of Vitamin D<sub>3</sub> into a suitable vessel containing Ethanol under stirring until to form clear solution. Add water soluble and therapeutically safe curcuma nano drop formulation (prepared as per detailed process given in Example 1) in solution form under stirring until to form clear yellow color solution.

10 (d) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is imparted as per the parameters mentioned below.

|                     |                                                      |
|---------------------|------------------------------------------------------|
| Sonotrode           | As per batch size                                    |
| Energy level        | 2000 w/s                                             |
| Amplitude           | 30 – 70%                                             |
| Temperature cut off | Not more than 40°C                                   |
| On time             | 10-30 seconds                                        |
| Off time            | 20-30 seconds                                        |
| No of cycles        | Till the specified Energy is imparted to the mixture |

### Remaining procedure

15 (e) Add required quantity of Polyoxyl 40 Hydrogenated castor oil gradually in to a suitable vessel and heat it up to 40°C until to form a clear solution. Transfer this solution into the vessel containing pre-mix 2 solution under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into the vessel containing pre-mix 2 solution and continue stirring for 5-10 minutes after addition.

- (f) Transfer pre-mix 1 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.
- 5 (g) Transfer Pre-mix 2 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.
- 10 (h) Add Sodium methyl paraben and Sodium propyl paraben into a suitable vessel containing purified water under stirring for 5 minutes after addition and check the clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.
- 15 (i) Add Sodium citrate into a suitable vessel containing purified water under for 15 minutes after addition and check the clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.
- (j) Add 10% Citric acid solution into main manufacturing vessel under stirring and adjust the pH between 5.00 to 6.00.
- (k) Transfer Caramel flavour VV-729-140-3 to main manufacturing vessel under stirring for 5 minutes after addition.
- 20 (l) Make up the volume with purified water under stirring for 5 minutes after addition.

**Example 3:** Vitamin D<sub>3</sub> 60,000 IU/5ml oral solution

| Ingredients                                  | gm/100ml |
|----------------------------------------------|----------|
| Cholecalciferol (Vitamin D <sub>3</sub> ) *  | 0.036**  |
| Polyoxyl 40 Hydrogenated castor oil (liquid) | 0.900    |
| Propylene Glycol                             | 5.000    |
| Maltitol (liquid)                            | 15.000   |
| Sucralose                                    | 0.020    |
| Sodium Methyl Paraben                        | 0.090    |

|                            |               |
|----------------------------|---------------|
| Sodium Propyl Paraben      | 0.020         |
| Ethanol                    | 0.050         |
| Sodium Citrate             | 0.052         |
| Citric acid Monohydrate    | 0.100         |
| Caramel artificial flavour | 0.050         |
| Curcuma nano Drops#        | 0.040 ml      |
| Purified water             | q.s. to 100ml |

\* Vitamin D<sub>3</sub> – 40 Mn. IU / gm

\*\* inclusive of 20% overages

# Curcuma nano Drops containing

Curcuminoids equivalent to not less than 45mg of Curcumin.

- 5 Total Non-Curcuminoids determined on the basis of equivalent to not less than 3.5mg of AR Turmerone.

### **Manufacturing procedure**

#### **Preparing pre-mix 1**

- 10 (a) Weigh and dispense required quantity of Liquid Maltitol, Propylene glycol and Sucralose into a suitable vessel containing dispensed quantity of purified water under stirring for 10 minutes.
- (b) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is
- 15 imparted as per the parameters mentioned below.

|                     |                                                      |
|---------------------|------------------------------------------------------|
| Sonotrode           | As per batch size                                    |
| Energy level        | 50000 w/s                                            |
| Amplitude           | 30 – 70%                                             |
| Temperature cut off | Not more than 40°C                                   |
| On time             | 20-30 seconds                                        |
| Off time            | 20-30 seconds                                        |
| No of cycles        | Till the specified Energy is imparted to the mixture |

**Preparing pre-mix 2**

(c) Weigh and dispense required quantity of Vitamin D<sub>3</sub> into a suitable vessel containing Ethanol under stirring until to form clear solution. Add water soluble and therapeutically safe curcuma nano drop formulation (prepared as per detailed process given in Example 1) in solution form under stirring until to form clear yellow color solution.

(d) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is imparted as per the parameters mentioned below.

|                     |                                                      |
|---------------------|------------------------------------------------------|
| Sonotrode           | As per batch size                                    |
| Energy level        | 2000 w/s                                             |
| Amplitude           | 30 – 70%                                             |
| Temperature cut off | Not more than 40°C                                   |
| On time             | 10-30 seconds                                        |
| Off time            | 20-30 seconds                                        |
| No of cycles        | Till the specified Energy is imparted to the mixture |

**Remaining procedure**

(e) Add required quantity of Polyoxyl 40 Hydrogenated castor oil gradually into a suitable vessel and heat it up to 40°C until to form a clear solution. Transfer this solution into the vessel containing pre-mix 2 solution under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into the vessel containing pre-mix 2 solution and continue stirring for 5-10 minutes after addition.

(f) Transfer pre-mix 1 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.

- (g) Transfer Pre-mix 2 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.
- 5 (h) Add Sodium methyl paraben and Sodium propyl paraben into a suitable vessel containing purified water under stirring for 5 minutes after addition and check the clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.
- 10 (i) Add Sodium citrate into a suitable vessel containing purified water under for 15 minutes after addition and check the clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.
- (j) Add 10% Citric acid solution into main manufacturing vessel under stirring and adjust the pH between 5.00 to 6.00.
- 15 (k) Transfer Caramel flavour VV-729-140-3 to main manufacturing vessel under stirring for 5 minutes after addition.
- (l) Make up the volume with purified water under stirring for 5 minutes after addition.

**Example 4:** Vitamin D<sub>3</sub> 60,000 IU/5ml oral solution

| Ingredients                                  | gm/100ml |
|----------------------------------------------|----------|
| Cholecalciferol (Vitamin D <sub>3</sub> ) *  | 0.036**  |
| Polyoxyl 40 Hydrogenated castor oil (liquid) | 0.900    |
| Propylene Glycol                             | 5.000    |
| Maltitol (liquid)                            | 15.000   |
| Sodium Methyl Paraben                        | 0.090    |
| Sodium Propyl Paraben                        | 0.020    |
| Ethanol                                      | 0.050    |
| Sodium Citrate                               | 0.052    |
| Citric acid Monohydrate                      | 0.100    |
| Caramel artificial flavour                   | 0.050    |

|                     |               |
|---------------------|---------------|
| Curcuma nano Drops# | 0.040 ml      |
| Purified water      | q.s. to 100ml |

\* Vitamin D<sub>3</sub> – 40 Mn. IU / gm

\*\* inclusive of 20% overages

# Curcuma nano Drops containing

Curcuminoids equivalent to not less than 45mg of Curcumin.

- 5 Total Non-Curcuminoids determined on the basis of equivalent to not less than 3.5mg of AR Turmerone.

### Manufacturing procedure

#### **Preparing pre-mix 1**

- 10 (a) Weigh and dispense required quantity of Liquid Maltitol and Propylene glycol into a suitable vessel containing dispensed quantity of purified water under stirring for 10 minutes.
- (b) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is
- 15 imparted as per the parameters mentioned below.

|                     |                                                      |
|---------------------|------------------------------------------------------|
| Sonotrode           | As per batch size                                    |
| Energy level        | 50000 w/s                                            |
| Amplitude           | 30 – 70%                                             |
| Temperature cut off | Not more than 40°C                                   |
| On time             | 20-30 seconds                                        |
| Off time            | 20-30 seconds                                        |
| No of cycles        | Till the specified Energy is imparted to the mixture |

#### **Preparing pre-mix 2**

- (c) Weigh and dispense required quantity of Vitamin D<sub>3</sub> into a suitable vessel containing Ethanol under stirring until to form clear solution. Add water soluble
- 20 and therapeutically safe curcuma nano drop formulation (prepared as per detailed

process given in Example 1) in solution form under stirring until to form clear yellow color solution.

- (d) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is imparted as per the parameters mentioned below.

5

|                     |                                                      |
|---------------------|------------------------------------------------------|
| Sonotrode           | As per batch size                                    |
| Energy level        | 2000 w/s                                             |
| Amplitude           | 30 – 70%                                             |
| Temperature cut off | Not more than 40°C                                   |
| On time             | 10-30 seconds                                        |
| Off time            | 20-30 seconds                                        |
| No of cycles        | Till the specified Energy is imparted to the mixture |

#### Remaining procedure

- (e) Add required quantity of Polyoxyl 40 Hydrogenated castor oil gradually in to a suitable vessel and heat it up to 40°C until to form a clear solution. Transfer this solution into the vessel containing pre-mix 2 solution under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into the vessel containing pre-mix 2 solution and continue stirring for 5-10 minutes after addition.
- (f) Transfer pre-mix 1 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.
- (g) Transfer Pre-mix 2 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.
- (h) Add Sodium methyl paraben and Sodium propyl paraben into a suitable vessel containing purified water under stirring for 5 minutes after addition and check the

clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.

- (i) Add Sodium citrate into a suitable vessel containing purified water under for 15 minutes after addition and check the clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.
- (j) Add 10% Citric acid solution into main manufacturing vessel under stirring and adjust the pH between 5.00 to 6.00.
- (k) Transfer Caramel flavour VV-729-140-3 to main manufacturing vessel under stirring for 5 minutes after addition.
- (l) Make up the volume with purified water under stirring for 5 minutes after addition.

**Example 5:** Vitamin D<sub>3</sub> 60,000 IU/5ml oral solution

| Ingredients                                  | gm/100ml      |
|----------------------------------------------|---------------|
| Cholecalciferol (Vitamin D <sub>3</sub> ) *  | 0.036**       |
| Polyoxyl 40 Hydrogenated castor oil (liquid) | 0.900         |
| Maltitol (liquid)                            | 15.000        |
| Sodium Methyl Paraben                        | 0.090         |
| Sodium Propyl Paraben                        | 0.020         |
| Ethanol                                      | 0.050         |
| Sodium Citrate                               | 0.052         |
| Citric acid Monohydrate                      | 0.100         |
| Caramel artificial flavour/Pineapple flavour | 0.050         |
| Curcuma nano Drops#                          | 0.040 ml      |
| Purified water                               | q.s. to 100ml |

\* Vitamin D<sub>3</sub> – 40 Mn. IU / gm

\*\* inclusive of 20% overages

# Curcuma nano Drops containing

Curcuminoids equivalent to not less than 45mg of Curcumin.

Total Non-Curcuminoids determined on the basis of equivalent to not less than 3.5mg of AR Turmerone.

**Manufacturing procedure****Preparing pre-mix 1**

- 5 (a) Weigh and dispense required quantity of Liquid Maltitol into a suitable vessel containing dispensed quantity of purified water under stirring for 10 minutes.
- (b) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is imparted as per the parameters mentioned below.

|                     |                                                      |
|---------------------|------------------------------------------------------|
| Sonotrode           | As per batch size                                    |
| Energy level        | 50000 w/s                                            |
| Amplitude           | 30 – 70%                                             |
| Temperature cut off | Not more than 40°C                                   |
| On time             | 20-30 seconds                                        |
| Off time            | 20-30 seconds                                        |
| No of cycles        | Till the specified Energy is imparted to the mixture |

10 **Preparing pre-mix 2**

- (c) Weigh and dispense required quantity of Vitamin D<sub>3</sub> into a suitable vessel containing Ethanol under stirring until to form clear solution. Add water soluble and therapeutically safe curcuma nano drop formulation (prepared as per detailed process given in Example 1) in solution form under stirring until to form clear yellow color solution.
- 15 (d) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is imparted as per the parameters mentioned below.

|                     |                    |
|---------------------|--------------------|
| Sonotrode           | As per batch size  |
| Energy level        | 2000 w/s           |
| Amplitude           | 30 – 70%           |
| Temperature cut off | Not more than 40°C |

|              |                                                      |
|--------------|------------------------------------------------------|
| On time      | 10-30 seconds                                        |
| Off time     | 20-30 seconds                                        |
| No of cycles | Till the specified Energy is imparted to the mixture |

### Remaining procedure

- 5 (e) Add required quantity of Polyoxyl 40 Hydrogenated castor oil gradually in to a suitable vessel and heat it up to 40°C until to form a clear solution. Transfer this solution into the vessel containing pre-mix 2 solution under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into the vessel containing pre-mix 2 solution and continue stirring for 5-10 minutes after addition.
- 10 (f) Transfer pre-mix 1 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.
- 15 (g) Transfer Pre-mix 2 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.
- 20 (h) Add Sodium methyl paraben and Sodium propyl paraben into a suitable vessel containing purified water under stirring for 5 minutes after addition and check the clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.
- (i) Add Sodium citrate into a suitable vessel containing purified water under for 15 minutes after addition and check the clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.
- 25 (j) Add 10% Citric acid solution into main manufacturing vessel under stirring and adjust the pH between 5.00 to 6.00.
- (k) Transfer Caramel flavour VV-729-140-3 or Pineapple flavour to main manufacturing vessel under stirring for 5 minutes after addition.

- (l) Make up the volume with purified water under stirring for 5 minutes after addition.

**Example 6:** Vitamin D<sub>3</sub> 60,000 IU/5ml oral solution

| Ingredients                                  | gm/100ml      |
|----------------------------------------------|---------------|
| Cholecalciferol (Vitamin D <sub>3</sub> ) *  | 0.036**       |
| Polyoxyl 40 Hydrogenated castor oil (liquid) | 0.900         |
| Sucralose                                    | 0.020         |
| Sodium Methyl Paraben                        | 0.090         |
| Sodium Propyl Paraben                        | 0.020         |
| Ethanol                                      | 0.050         |
| Sodium Citrate                               | 0.052         |
| Citric acid Monohydrate                      | 0.100         |
| Caramel artificial flavour/Pineapple flavour | 0.050         |
| Curcuma nano Drops#                          | 0.040 ml      |
| Purified water                               | q.s. to 100ml |

5 \* Vitamin D<sub>3</sub> – 40 Mn. IU / gm

\*\* inclusive of 20% overages

# Curcuma nano Drops containing

Curcuminoids equivalent to not less than 45mg of Curcumin.

Total Non-Curcuminoids determined on the basis of equivalent to not less than 3.5mg of

10 AR Turmerone.

**Manufacturing procedure**

**Preparing pre-mix 1**

- 15 (a) Weigh and dispense required quantity of Vitamin D<sub>3</sub> into a suitable vessel containing Ethanol under stirring until to form clear solution. Add water soluble and therapeutically safe curcuma nano drop formulation (prepared as per detailed process given in Example 1) in solution form under stirring until to form clear yellow color solution.

- (b) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is imparted as per the parameters mentioned below.

|                     |                                                      |
|---------------------|------------------------------------------------------|
| Sonotrode           | As per batch size                                    |
| Energy level        | 2000 w/s                                             |
| Amplitude           | 30 – 70%                                             |
| Temperature cut off | Not more than 40°C                                   |
| On time             | 10-30 seconds                                        |
| Off time            | 20-30 seconds                                        |
| No of cycles        | Till the specified Energy is imparted to the mixture |

## 5 Remaining procedure

- (c) Add required quantity of Polyoxyl 40 Hydrogenated castor oil gradually in to a suitable vessel and heat it up to 40°C until to form a clear solution. Transfer this solution into the vessel containing pre-mix 1 solution under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into the vessel containing pre-mix 1 solution and continue stirring for 5-10 minutes after addition.
- (d) Transfer pre-mix 1 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.
- (e) Add Sucralose into a suitable vessel containing purified water under stirring for 5 minutes after addition and check the clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.
- (f) Add Sodium methyl paraben and Sodium propyl paraben into a suitable vessel containing purified water under stirring for 5 minutes after addition and check the clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.

- (g) Add Sodium citrate into a suitable vessel containing purified water under for 15 minutes after addition and check the clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.
- (h) Add 10% Citric acid solution into main manufacturing vessel under stirring and adjust the pH between 5.00 to 6.00.
- (i) Transfer Caramel flavour VV-729-140-3 or Pineapple flavour to main manufacturing vessel under stirring for 5 minutes after addition.
- (j) Make up the volume with purified water under stirring for 5 minutes after addition.

10

**Example 7:** Vitamin D<sub>3</sub> 400 IU/2ml oral solution

| Ingredients                                  | gm/100ml      |
|----------------------------------------------|---------------|
| Cholecalciferol (Vitamin D <sub>3</sub> ) *  | 0.0006**      |
| Polyoxyl 40 Hydrogenated castor oil (liquid) | 0.900         |
| Propylene Glycol                             | 5.000         |
| Maltitol (Liquid)                            | 15.000        |
| Sucralose                                    | 0.020         |
| Sodium Methyl Paraben                        | 0.090         |
| Sodium Propyl Paraben                        | 0.020         |
| Ethanol                                      | 0.050         |
| Sodium Citrate                               | 0.052         |
| Citric acid Monohydrate                      | 0.100         |
| Caramel artificial flavour/Pineapple flavour | 0.050         |
| Curcuma nano Drops#                          | 0.040 ml      |
| Purified water                               | q.s. to 100ml |

\* Vitamin D<sub>3</sub> – 40 Mn. IU / gm

\*\* inclusive of 20% overages

# Curcuma nano Drops containing

- 15 Curcuminoids equivalent to not less than 45mg of Curcumin.

Total Non-Curcuminoids determined on the basis of equivalent to not less than 3.5mg of AR Turmerone.

**Manufacturing procedure****Preparing pre-mix 1**

- 5 (a) Weigh and dispense required quantity of Liquid Maltitol, Propylene glycol and Sucralose into a suitable vessel containing dispensed quantity of purified water under stirring for 10 minutes.
- (b) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is imparted as per the parameters mentioned below.

|                     |                                                      |
|---------------------|------------------------------------------------------|
| Sonotrode           | As per batch size                                    |
| Energy level        | 50000 w/s                                            |
| Amplitude           | 30 – 70%                                             |
| Temperature cut off | Not more than 40°C                                   |
| On time             | 20-30 seconds                                        |
| Off time            | 20-30 seconds                                        |
| No of cycles        | Till the specified Energy is imparted to the mixture |

10 **Preparing pre-mix 2**

- (c) Weigh and dispense required quantity of Vitamin D<sub>3</sub> into a suitable vessel containing Ethanol under stirring until to form clear solution. Add water soluble and therapeutically safe curcuma nano drop formulation (prepared as per detailed process given in Example 1) in solution form under stirring until to form clear yellow color solution.
- 15 (d) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is imparted as per the parameters mentioned below.

|                     |                    |
|---------------------|--------------------|
| Sonotrode           | As per batch size  |
| Energy level        | 2000 w/s           |
| Amplitude           | 30 – 70%           |
| Temperature cut off | Not more than 40°C |

|              |                                                      |
|--------------|------------------------------------------------------|
| On time      | 10-30 seconds                                        |
| Off time     | 20-30 seconds                                        |
| No of cycles | Till the specified Energy is imparted to the mixture |

### Remaining procedure

- 5 (e) Add required quantity of Polyoxyl 40 Hydrogenated castor oil gradually in to a suitable vessel and heat it up to 40°C until to form a clear solution. Transfer this solution into the vessel containing pre-mix 2 solution under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into the vessel containing pre-mix 2 solution and continue stirring for 5-10 minutes after addition.
- 10 (f) Transfer pre-mix 1 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.
- 15 (g) Transfer Pre-mix 2 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.
- 20 (h) Add Sodium methyl paraben and Sodium propyl paraben into a suitable vessel containing purified water under stirring for 5 minutes after addition and check the clarity of the solution. Transfer the solution into main manufacturing vessel and continue stirring for 5 minutes after addition.
- (i) Add Sodium citrate into a suitable vessel containing purified water under for 15 minutes after addition and check the clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.
- 25 (j) Add 10% Citric acid solution into main manufacturing vessel under stirring and adjust pH between 5.00 to 6.00.
- (k) Transfer Caramel flavour VV-729-140-3 to main manufacturing vessel under stirring for 5 minutes after addition.

- (l) Make up the volume with purified water under stirring for 5 minutes after addition.

**Example 8: Vitamin D<sub>3</sub> 1000 IU/ml oral solution**

| Ingredients                                  | gm/100ml      |
|----------------------------------------------|---------------|
| Cholecalciferol (Vitamin D <sub>3</sub> ) *  | 0.003**       |
| Polyoxyl 40 Hydrogenated castor oil (liquid) | 0.900         |
| Propylene Glycol                             | 5.000         |
| Maltitol (Liquid)                            | 15.000        |
| Sucralose                                    | 0.020         |
| Sodium Methyl Paraben                        | 0.090         |
| Sodium Propyl Paraben                        | 0.020         |
| Ethanol                                      | 0.050         |
| Sodium Citrate                               | 0.052         |
| Citric acid Monohydrate                      | 0.100         |
| Caramel artificial flavour                   | 0.050         |
| Curcuma nano Drops#                          | 0.040 ml      |
| Purified water                               | q.s. to 100ml |

5 \* Vitamin D<sub>3</sub> – 40 Mn. IU / gm

\*\* inclusive of 20% overages

# Curcuma nano Drops containing

Curcuminoids equivalent to not less than 45mg of Curcumin.

Total Non-Curcuminoids determined on the basis of equivalent to not less than 3.5mg of

10 AR Turmerone.

**Manufacturing procedure**

**Preparing pre-mix 1**

- (a) Weigh and dispense required quantity of Liquid Maltitol, Propylene glycol and  
 15 Sucralose into a suitable vessel containing dispensed quantity of purified water  
 under stirring for 10 minutes.

- (b) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is imparted as per the parameters mentioned below.

|                     |                                                      |
|---------------------|------------------------------------------------------|
| Sonotrode           | As per batch size                                    |
| Energy level        | 50000 w/s                                            |
| Amplitude           | 30 – 70%                                             |
| Temperature cut off | Not more than 40°C                                   |
| On time             | 20-30 seconds                                        |
| Off time            | 20-30 seconds                                        |
| No of cycles        | Till the specified Energy is imparted to the mixture |

## 5 Preparing pre-mix 2

- (c) Weigh and dispense required quantity of Vitamin D<sub>3</sub> into a suitable vessel containing Ethanol under stirring until to form clear solution. Add water soluble and therapeutically safe curcuma nano drop formulation (prepared as per detailed process given in Example 1) in solution form under stirring until to form clear yellow color solution.

10

- (d) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is imparted as per the parameters mentioned below.

|                     |                                                      |
|---------------------|------------------------------------------------------|
| Sonotrode           | As per batch size                                    |
| Energy level        | 2000 w/s                                             |
| Amplitude           | 30 – 70%                                             |
| Temperature cut off | Not more than 40°C                                   |
| On time             | 10-30 seconds                                        |
| Off time            | 20-30 seconds                                        |
| No of cycles        | Till the specified Energy is imparted to the mixture |

15

**Remaining procedure**

- 5 (e) Add required quantity of Polyoxyl 40 Hydrogenated castor oil gradually in to a suitable vessel and heat it up to 40°C until to form a clear solution. Transfer this solution into the vessel containing pre-mix 2 solution under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into the vessel containing pre-mix 2 solution and continue stirring for 5-10 minutes after addition.
- 10 (f) Transfer pre-mix 1 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.
- 15 (g) Transfer Pre-mix 2 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.
- 20 (h) Add Sodium methyl paraben and Sodium propyl paraben into a suitable vessel containing purified water under stirring for 5 minutes after addition and check the clarity of the solution. Transfer the solution into main manufacturing vessel and continue stirring for 5 minutes after addition
- (i) Add Sodium citrate into a suitable vessel containing purified water under for 15 minutes after addition and check the clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.
- 25 (j) Add 10% Citric acid solution into main manufacturing vessel under stirring and adjust pH between 5.00 to 6.00.
- (k) Transfer Caramel flavour VV-729-140-3 to main manufacturing vessel under stirring for 5 minutes after addition.
- (l) Make up the volume with purified water under stirring for 5 minutes after addition.

30

**Example 9:** Vitamin D<sub>3</sub> 1000 IU/2ml oral solution

| Ingredients                                  | gm/100ml      |
|----------------------------------------------|---------------|
| Cholecalciferol (Vitamin D <sub>3</sub> ) *  | 0.0015**      |
| Polyoxyl 40 Hydrogenated castor oil (liquid) | 0.900         |
| Propylene Glycol                             | 5.000         |
| Maltitol (Liquid)                            | 15.000        |
| Sucralose                                    | 0.020         |
| Sodium Methyl Paraben                        | 0.090         |
| Sodium Propyl Paraben                        | 0.020         |
| Ethanol                                      | 0.050         |
| Sodium Citrate                               | 0.052         |
| Citric acid Monohydrate                      | 0.100         |
| Caramel artificial flavour                   | 0.050         |
| Curcuma nano Drops#                          | 0.040 ml      |
| Purified water                               | q.s. to 100ml |

\* Vitamin D<sub>3</sub> – 40 Mn. IU / gm

\*\* inclusive of 20% overages

# Curcuma nano Drops containing

5 Curcuminoids equivalent to not less than 45mg of Curcumin.

Total Non-Curcuminoids determined on the basis of equivalent to not less than 3.5mg of AR Turmerone.

**Manufacturing procedure****10 Preparing pre-mix 1**

(a) Weigh and dispense required quantity of Liquid Maltitol, Propylene glycol and Sucralose into a suitable vessel containing dispensed quantity of purified water under stirring for 10 minutes.

15 (b) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is imparted as per the parameters mentioned below.

|                     |                                                      |
|---------------------|------------------------------------------------------|
| Sonotrode           | As per batch size                                    |
| Energy level        | 50000 w/s                                            |
| Amplitude           | 30 – 70%                                             |
| Temperature cut off | Not more than 40°C                                   |
| On time             | 20-30 seconds                                        |
| Off time            | 20-30 seconds                                        |
| No of cycles        | Till the specified Energy is imparted to the mixture |

### Preparing pre-mix 2

5 (c) Weigh and dispense required quantity of Vitamin D<sub>3</sub> into a suitable vessel containing Ethanol under stirring until to form clear solution. Add water soluble and therapeutically safe curcuma nano drop formulation (prepared as per detailed process given in Example 1) in solution form under stirring until to form clear yellow color solution.

10 (d) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is imparted as per the parameters mentioned below.

|                     |                                                      |
|---------------------|------------------------------------------------------|
| Sonotrode           | As per batch size                                    |
| Energy level        | 2000 w/s                                             |
| Amplitude           | 30 – 70%                                             |
| Temperature cut off | Not more than 40°C                                   |
| On time             | 10-30 seconds                                        |
| Off time            | 20-30 seconds                                        |
| No of cycles        | Till the specified Energy is imparted to the mixture |

### Remaining procedure

15 (e) Add required quantity of Polyoxyl 40 Hydrogenated castor oil gradually in to a suitable vessel and heat it up to 40°C until to form a clear solution. Transfer this solution into the vessel containing pre-mix 2 solution under stirring for 5 minutes

after addition. Rinse the vessel with purified water and transfer rinsed solution into the vessel containing pre-mix 2 solution and continue stirring for 5-10 minutes after addition.

5 (f) Transfer pre-mix 1 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.

10 (g) Transfer Pre-mix 2 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.

15 (h) Add Sodium methyl paraben and Sodium propyl paraben into a suitable vessel containing purified water under stirring for 5 minutes after addition and check the clarity of the solution. Transfer the solution into main manufacturing vessel and continue stirring for 5 minutes after addition

(i) Add Sodium citrate into a suitable vessel containing purified water under for 15 minutes after addition and check the clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.

20 (j) Add 10% Citric acid solution into main manufacturing vessel under stirring and adjust pH between 5.00 to 6.00.

(k) Transfer Caramel flavour VV-729-140-3 to main manufacturing vessel under stirring for 5 minutes after addition.

25 (l) Make up the volume with purified water under stirring for 5 minutes after addition.

**Example 10:** Vitamin D<sub>3</sub> 2000 IU/ml oral solution

| Ingredients                                  | gm/100ml |
|----------------------------------------------|----------|
| Cholecalciferol (Vitamin D <sub>3</sub> ) *  | 0.006**  |
| Polyoxyl 40 Hydrogenated castor oil (liquid) | 0.900    |
| Propylene Glycol                             | 5.000    |

|                            |               |
|----------------------------|---------------|
| Maltitol (Liquid)          | 15.000        |
| Sucralose                  | 0.020         |
| Sodium Methyl Paraben      | 0.090         |
| Sodium Propyl Paraben      | 0.020         |
| Ethanol                    | 0.050         |
| Sodium Citrate             | 0.052         |
| Citric acid Monohydrate    | 0.100         |
| Caramel artificial flavour | 0.050         |
| Curcuma nano Drops#        | 0.040 ml      |
| Purified water             | q.s. to 100ml |

\* Vitamin D<sub>3</sub> – 40 Mn. IU / gm

\*\* inclusive of 20% overages

# Curcuma nano Drops containing

Curcuminoids equivalent to not less than 45mg of Curcumin.

- 5 Total Non-Curcuminoids determined on the basis of equivalent to not less than 3.5mg of AR Turmerone.

### **Manufacturing procedure**

#### **Preparing pre-mix 1**

- 10 (a) Weigh and dispense required quantity of Liquid Maltitol, Propylene glycol and Sucralose into a suitable vessel containing dispensed quantity of purified water under stirring for 10 minutes.
- (b) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is
- 15 imparted as per the parameters mentioned below.

|                     |                    |
|---------------------|--------------------|
| Sonotrode           | As per batch size  |
| Energy level        | 50000 w/s          |
| Amplitude           | 30 – 70%           |
| Temperature cut off | Not more than 40°C |
| On time             | 20-30 seconds      |

|              |                                                      |
|--------------|------------------------------------------------------|
| Off time     | 20-30 seconds                                        |
| No of cycles | Till the specified Energy is imparted to the mixture |

### Preparing pre-mix 2

(c) Weigh and dispense required quantity of Vitamin D<sub>3</sub> into a suitable vessel containing Ethanol under stirring until to form clear solution. Add water soluble and therapeutically safe curcuma nano drop formulation (prepared as per detailed process given in Example 1) in solution form under stirring until to form clear yellow color solution.

(d) Carry out the sonication using Sonicator (Ultrasonic processor, Hielscher, Germany) to reduce particle size for a duration until the required energy level is imparted as per the parameters mentioned below.

|                     |                                                      |
|---------------------|------------------------------------------------------|
| Sonotrode           | As per batch size                                    |
| Energy level        | 2000 w/s                                             |
| Amplitude           | 30 – 70%                                             |
| Temperature cut off | Not more than 40°C                                   |
| On time             | 10-30 seconds                                        |
| Off time            | 20-30 seconds                                        |
| No of cycles        | Till the specified Energy is imparted to the mixture |

### Remaining Procedure

(e) Add required quantity of Polyoxyl 40 Hydrogenated castor oil gradually in to a suitable vessel and heat it up to 40°C until to form a clear solution. Transfer this solution into the vessel containing pre-mix 2 solution under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into the vessel containing pre-mix 2 solution and continue stirring for 5-10 minutes after addition.

(f) Transfer pre-mix 1 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed

solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.

5 (g) Transfer Pre-mix 2 solution into main manufacturing vessel under stirring for 5 minutes after addition. Rinse the vessel with purified water and transfer rinsed solution into main manufacturing vessel and continue stirring for 5-10 minutes after addition.

10 (h) Add Sodium methyl paraben and Sodium propyl paraben into a suitable vessel containing purified water under stirring for 5 minutes after addition and check the clarity of the solution. Transfer the solution into main manufacturing vessel and continue stirring for 5 minutes after addition.

(i) Add Sodium citrate into a suitable vessel containing purified water under for 15 minutes after addition and check the clarity of the solution. Transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition.

15 (j) Add 10% Citric acid solution into main manufacturing vessel under stirring and adjust pH between 5.00 to 6.00.

(k) Transfer Caramel flavour VV-729-140-3 to main manufacturing vessel under stirring for 5 minutes after addition.

(l) Make up the volume with purified water under stirring for 5 minutes after addition.

20

#### **An *in vitro* drug permeation evaluation model of the Vitamin D<sub>3</sub> solution formulation**

25 Vitamin D when taken orally absorbs quickly in presence of bile. Vitamin D undergoes two hydroxylation in the body for activation. There are several metabolic products or modified versions of vitamin D. Vitamin D is converted into Calcidiol (25-hydroxyvitamin D<sub>3</sub>) in liver which has a half-life of about 15 days and a Tmax of about 7 days. Calcidiol passes through kidney and converts into Calcitriol (1,25-dihydroxyvitamin D<sub>3</sub>), the active form of vitamin D, has a half-life of about 5-8 hours. Vitamin D binds to receptors located throughout the body.

30 The vitamin D metabolites are transported in blood bound primarily to vitamin D binding protein (DBP) (85-88%) and albumin (12-15%). DBP concentrations are

normally 4-8 $\mu$ M, well above the concentrations of the vitamin D metabolites, such that DBP is only about 2% saturated. DBP has high affinity for the vitamin D metabolites ( $K_a=5 \times 10^8 M^{-1}$  for 25OHD and 24,25(OH) $_2$ D,  $4 \times 10^7 M^{-1}$  for 1,25(OH) $_2$ D and vitamin D), under normal circumstances approximately 0.03% 25OHD and 24,25(OH) $_2$ D and 0.4% 1,25(OH) $_2$ D are free. Conditions such as liver disease and nephrotic syndrome resulting in reduced DBP and albumin levels will lead to a reduction in total 25OHD and 1,25(OH) $_2$ D levels. Similarly, DBP levels are reduced during acute illness, potentially obscuring the interpretation of total 25OHD levels. Vitamin D intoxication can increase the degree of saturation sufficiently to increase the free concentrations of 1,25(OH) $_2$ D and so cause hypercalcemia without necessarily raising the total concentrations.

The intent of Vitamin D $_3$  with a curcuminoid and a non-curcuminoid formulation developed as detailed in this patent application is to provide an absorption profile with a desired release pattern enabling smaller quantities of release of Vitamin D $_3$  for slightly longer period into the blood stream after consumption of the dose. Vitamin D $_3$  formulation developed should help in maintaining the rate of permeation through GI track to blood stream in a sustained absorption pattern and ensure a continuous supply of Vitamin D and hence Calcitriol availability, to bind with DBP for maximum therapeutic output. Yet another intent was to maintain active levels even in the patients with liver and kidney disease and with impairment of organ function resulting in decreased capacity of hydroxylation. The above intent were to be accomplished based on the hypothesis that the Vitamin D $_3$  in the formulation would get appropriate coating of the constituents of curcuma nano drops formulation during the preparation of said premix containing Vitamin D $_3$  and the Curcuminoids and Non-Curcuminoids in curcuma nano drops formulation.

In order to demonstrate the above desired absorption pattern for the Vitamin D $_3$  formulation, an *in vitro* permeation model was designed developed which essentially targeted towards mimicking of the biological membrane of GI tract. The biological membrane of mucosa comprises a hydrophobic part in the GI track and the sandwiched hydrophilic middle part which is near to blood capillaries. As most of the drugs are known to be absorbed through passive diffusion, the permeation model was appropriate

for simulating the passive diffusion of drug from one compartment to another. The invitro permeation model so designed comprised the following:

1. A donor compartment and an acceptor compartment separated by a lipid infused artificial membrane installed in specific way between the donor and acceptor compartments wherein the membrane had a layer of 1%Lecithin in dodecane as the hydrophobic layer on the donor side and a hydrophilic layer of 0.22  $\mu$  PVDF on the acceptor side. The hydrophobic layer faced the product to be evaluated and hydrophilic part faced towards receiving media.  
PBS buffer with 1% PEG400 and having 7.4pH was used as the Donor media
2. PBS buffer having 7.4 pH was used as receptor media.
3. The product dilution was taken in 1:1 ratio.
4. Temperature was maintained at 37°C
5. Sampling timepoints: at interval of 0.5, 1, 2, 3, 4, 5 and 6 hours
6. Detection of Vitamin D<sub>3</sub> was done on HPLC with UV detector @ 269nm

The study was conducted on the developed formulation and compared with an existing marketed formulation by taking samples at the defined intervals and analysing the Vitamin D<sub>3</sub> content in the receptor compartment using HPLC system and computing the % permeation at each interval. The results obtained were as follows: (Reference 1 and 2 are 2 batches of existing marketed product. Test 1 and 2 are 2 batches of the developed formulation (Example 1 and 4).

| % Drug permeation |             |             |           |              |              |           |
|-------------------|-------------|-------------|-----------|--------------|--------------|-----------|
| Time              | Reference-1 | Reference-2 | Avg       | Example No.1 | Example No.4 | Avg       |
| <b>30 minutes</b> | 15          | 13          | <b>14</b> | 4            | 4            | <b>4</b>  |
| <b>1 Hr</b>       | 28          | 31          | <b>30</b> | 16           | 13           | <b>15</b> |
| <b>2 Hr</b>       | 49          | 49          | <b>49</b> | 31           | 23           | <b>27</b> |
| <b>3 Hr</b>       | 59          | 63          | <b>61</b> | 42           | 33           | <b>38</b> |
| <b>4 Hr</b>       | 70          | 70          | <b>70</b> | 52           | 42           | <b>47</b> |
| <b>5 Hr</b>       | 83          | 82          | <b>83</b> | 62           | 51           | <b>57</b> |
| <b>6 Hr</b>       | 91          | 87          | <b>89</b> | 70           | 55           | <b>63</b> |

Based on the study results, it can be deduced that the Vitamin D<sub>3</sub> formulation developed exhibits a slower and more sustained rate of permeation through the artificial membrane simulating the diffusion characteristics from GI track to blood stream in comparison to the existing marketed product. The results indicate a significantly sustained permeation pattern which would result in a continuous supply of Vitamin D in the blood stream and hence Calcitriol available to bind with DBP for maximum therapeutic output. The slower rate of permeation also ensures continuous availability of Vitamin D to compensate the decreased capacity of hydroxylation in the patients with liver and kidney disease and/or impairment. The slower release of Vitamin D<sub>3</sub> into blood stream will also enable mitigation of the risk of hypercalcemia in cases of Vitamin D intoxication.

Stability of Vitamin D<sub>3</sub> solution 60,000IU/5ml prepared as detailed in Example No: 1

| S. No | Parameters             | Initial | 1M<br>(40/75) | 2M<br>(40/75) | 3M<br>(40/75) | 6M<br>(40/75) |
|-------|------------------------|---------|---------------|---------------|---------------|---------------|
| 1     | Assay                  |         |               |               |               |               |
|       | Vitamin D <sub>3</sub> | 113.20  | 108.41        | 109.62        | 109.17        | 111.40        |
|       | Sodium benzoate        | 94.1    | 99.3          | 96.90         | 101.70        | 97.9          |
| 2     | pH                     | 5.87    | 5.87          | 5.96          | 5.92          | 5.90          |
| 3     | Weight /mL             | 1.1012  | 1.0990        | 1.0990        | 1.0995        | 1.1008        |

| S. No | Parameters             | Initial       | 3M (25/60)    | 6M (25/60)    | 20M (25/60) |
|-------|------------------------|---------------|---------------|---------------|-------------|
| 1     | Assay                  |               |               |               |             |
|       | Vitamin D <sub>3</sub> | 113.20        | 111.77        | 111.99        | 107.33      |
|       | Sodium benzoate        | 94.10         | 101.18        | 97.40         | 98.70       |
|       | Total Curcuminoids     | Not performed | Not performed | Not performed | 10 mcg      |
|       | Non                    | Not           | Not           | Not           | 9.6 mcg     |

|   |                                         |           |           |           |        |
|---|-----------------------------------------|-----------|-----------|-----------|--------|
|   | Curcuminoids<br>eq. to AR<br>Turmerones | performed | performed | performed |        |
| 2 | pH                                      | 5.87      | 5.94      | 5.88      | 5.60   |
| 3 | Weight /mL                              | 1.1012    | 1.0985    | 1.1007    | 1.0972 |

Physical and Chemical evaluation results of Vitamin D<sub>3</sub> solution prepared as detailed in Example No. 4

#### Evaluation of Vitamin D<sub>3</sub> solution

| Test Parameters                                      | Results                                            |
|------------------------------------------------------|----------------------------------------------------|
| Description                                          | A pale yellow coloured clear solution with flavour |
| pH                                                   | 5.64                                               |
| Density (g/ml)                                       | 1.0431                                             |
| Viscosity (cps)                                      | 2.00                                               |
| Buffer Capacity                                      |                                                    |
| Quantity of 0.1N HCl consumed (to change 1 unit pH)  | 1.2ml                                              |
| Quantity of 0.1N NaOH consumed (to change 1 unit pH) | 0.6ml                                              |
| Acid value                                           | 1.10                                               |
| Assay                                                |                                                    |
| Vitamin D <sub>3</sub>                               | 69540 IU (115.9%)                                  |
| Total Curcuminoids                                   | 80mcg of Curcumin                                  |
| Non-Curcuminoids eq. to AR Turmerones                | 12.00 mcg of AR Turmerones                         |
| Sodium methyl paraben                                | 4.35mg (96.70%)                                    |
| Sodium propyl paraben                                | 0.946mg (94.60%)                                   |
| PSD                                                  |                                                    |
| D10                                                  | 19.7 nm                                            |
| D50                                                  | 31.2 nm                                            |
| D90                                                  | 54.9 nm                                            |

PSD histogram of water soluble and therapeutically safe curcuma nano drop formulation in solution which is used for preparing Vitamin D<sub>3</sub> solution 60,000IU/5ml as detailed in Example No: 4 is given in figure 3

| PSD |         |
|-----|---------|
| D10 | 17.4 nm |
| D50 | 30.7 nm |
| D90 | 58.4 nm |

- 5 PSD histogram of premix 1 which is used for preparing Vitamin D<sub>3</sub> solution 60,000IU/5ml as detailed in Example No: 4 is given in figure 4

| PSD |         |
|-----|---------|
| D10 | 1.06 nm |
| D50 | 266 nm  |
| D90 | 366 nm  |

PSD histogram of Vitamin D<sub>3</sub> solution 60,000IU/5ml prepared as detailed in Example No: 5 is given in figure 5

| PSD |         |
|-----|---------|
| D10 | 19.7 nm |
| D50 | 31.2 nm |
| D90 | 54.9 nm |

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**WE CLAIM:**

1. A stable, clear uniformly interspersed particle oral solution formulation comprising an ultra-fine and a fine particle comprising vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid, wherein the formulation further comprises pharmaceutically acceptable  
5 excipients.
2. The stable oral solution formulation as claimed in claim 1, wherein the particles in the formulation having a particle size of d<sub>10</sub> not more than 75 nm, d<sub>50</sub> not more than 150 nm and d<sub>90</sub> not more than 300 nm.
3. The stable oral solution formulation as claimed in claim 1, wherein the size of the  
10 particles in the formulation are reduced using an ultrasonic processor.
4. The stable oral solution formulation as claimed in claim 1, wherein the formulation further comprises a carrier system which is a non-ionic solubilizer and a stabilizer such as polyoxy hydrogenated castor oil
5. The stable solution formulation as claimed in claim 1, wherein the dose of vitamin D<sub>3</sub>  
15 particles is in the range of 400IU to 60000IU.
6. The stable solution formulation as claimed in claim 1, wherein the curcumin in a curcuminoid becomes the natural colorant in the formulation.
7. The stable solution as claimed in claim 1, wherein the excipients are selected from chelating agents, dispersing agents, sweetening agents, preservatives, buffering agents,  
20 flavoring agents and solvents.
8. The stable solution formulation as claimed in claim 7, wherein preservative is selected from sodium benzoate, methylparaben, propylparaben, sodium methylparaben, sodium propylparaben and combination thereof, said chelating agents are selected from disodium edetate.
- 25 9. The stable solution formulation as claimed in claim 7, wherein said dispersing agents are selected from propylene glycol, maltitol (liquid), liquid sucrose, sorbitol, liquid glucose or combinations thereof.
10. The stable solution formulation as claimed in claim 7, wherein said sweetening  
30 agents are selected from sucralose, acesulfame potassium, stevia, glucose or combinations thereof.

11. The stable solution formulation as claimed in claim 7, wherein said flavoring agents are selected from caramel artificial flavor, peppermint flavor, strawberry flavor, lemon flavor, Pineapple flavor, Mixed fruit flavour, Blueberry flavour, Peach flavour, Raspberry, Lime flavour, Passion Fruit flavour, Mango flavor or any other acceptable  
5 flavors.
12. The stable solution formulation as claimed in claim 7, wherein said buffering agents are selected from sodium citrate and citric acid or combinations thereof, and said solvents are selected from ethanol, purified water or combinations thereof.
13. The stable solution formulation as claimed in claim 1, wherein the solution is having  
10 pH in the range of 5 to 8 and a viscosity in the range of 15 to 40 cp.
14. The stable solution formulation as claimed in claim 1, wherein said formulation comprising uniformly interspersed ultra-fine and fine particles of vitamin D<sub>3</sub>, a curcuminoid and a non-curcuminoid enables slow and steady permeation of Vitamin D<sub>3</sub> resulting from uniform interspersed of Vitamin D<sub>3</sub> particles with the particles of a  
15 curcuminoid and a non-curcuminoid.
15. The process for the preparation of stable solution formulation as claimed in claim 1, comprising steps of:
- (a) weighing and dispensing dispersing agents into a suitable vessel containing dispensed quantity of purified water under stirring for 10 minutes,
  - 20 (b) sonicating mixture using sonicator to reduce particle size,
  - (c) weighing and dispensing Vitamin D<sub>3</sub> into a suitable vessel containing solvent under stirring until to form clear solution.
  - (d) adding water soluble and therapeutically safe curcuma nano drops formulation in solution form under stirring until to form clear yellow color solution,
  - 25 (e) sonicating using sonicator to reduce particle size,
  - (f) adding required quantity solubilizer/emulsifying agent gradually in to a suitable vessel and heat it up to 40°C until to form a clear solution,
  - (g) transferring step (f) solution into the vessel containing step (e) solution under stirring for 5 minutes after addition,
  - 30 (h) transferring step (b) solution into main manufacturing vessel under stirring for 5 minutes after addition,

- (i) transferring step (g) solution into main manufacturing vessel under stirring for 5 minutes after addition,
- (j) adding preservatives into a suitable vessel containing purified water under stirring for 5 minutes after addition,
- 5 (k) adding buffering agent into a suitable vessel containing purified water under for 15 minutes after addition and check the clarity of the solution and transfer solution into main manufacturing vessel and continue stirring for 5 minutes after addition,
- (l) adding buffering agent solution into main manufacturing vessel under stirring and  
10 adjust the pH between 5.00 to 6.00,
- (m) transferring flavour to main manufacturing vessel under stirring for 5 minutes after addition and
- (n) making the volume with purified water under stirring for 5 minutes after addition.

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