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RESEARCH ARTICLE

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Formulation Development of Calcium Magnesium Inositol Hexaphosphate, Calcium Gluconate and Low Dosage Ergocalciferol (Vitamin D_2) Combination Granule Using Amixon Mixer Equipment

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Abstract

Ergocalciferol, Calcium Magnesium Inositol Hexaphosphate, Calcium Gluconate combination, prophylaxis and treatment of rickets, osteopathy, spasmophilia, disorders of bone formation and teething, disorders of calcium metabolism, Vitamin D_2 and situations where the demand for calcium is increased, breastfeeding children or children with insufficient intake of fresh air; immunosuppressants, aluminum containing antacids are used to treat patients undergoing treatment with diuretics. The aim of this study is to optimize the mixing time of our Ergocalciferol, Calcium Magnesium Inositol Hexaphosphate, and Calcium Gluconate combination product using Amixon mixer equipment. The suitability of the applied equipment will be evaluated by quantitative analysis and content uniformity analysis.

Keywords: Ergocalciferol (Vitamin D2); Calcium magnesium ınositol hexaphosphate; Calcium gluconate combination granules; Amixon mixer; Bulk homogeneity ın dry mixture; Sandwich method; Product devolopment

Introduction

Ergocalciferol (Vitamin D_2)

Ergocalciferol is an inactivated analog of Vitamin D_2 [1]. It is synthesized by some plants in the presence of UVB light [2]. Ergocalciferol production was accelerated by the identification of dietary deficiency, more specifically Vitamin D_2, as the main causative factor in the development of rickets. Ergocalciferol was first isolated from yeast in 1931 and its structure was elucidated in 1932 [3]. Ergocalciferol is considered the first Vitamin D_2 analogue. FDA records the first approved product containing ergocalciferol was developed by US Pharm Holdings and approved by the FDA in 1941 [4]. Ergocalciferol is a fat-soluble Vitamin that is important for many biochemical processes, including the absorption and metabolism of calcium and phosphorus. Having the right amount of Vitamin D_2, calcium, and phosphorus is important for building and maintaining strong bones. Vitamin D_2 is used to treat and prevent bone disorders (such as rickets, osteomalacia). Vitamin D_2 can be produced by the body when the skin is exposed to sunlight. Ergocalciferol is a form of Vitamin D_2 often found in Vitamin supplements. Ergocalciferol, also known as Vitamin D_2 and non-specific calciferol, is a type of Vitamin D_2 found in foods and used as a nutritional supplement [5]. Additionally, it is used to prevent and treat Vitamin D deficiency [6]. This includes Vitamin D deficiency due to poor absorption by the intestines or liver disease [7]. It can also be used for low blood calcium due to hypoparathyroidism. Used by mouth or by intramuscular injection [6,7].

Ergocalciferol, which is in the form of odorless white crystals, can be dissolved in oil, alcohol and chloroform, but not in water. Ergocalciferol is BCS Class III; it has high resolution and low permeability (Figure 1) [8].

Calcium magnesium inositol hexaphosphate

Calcium Magnesium Inositol Hexaphosphate is a Vitamin-like substance. It is found in animals and many plants, especially cereals, nuts, and legumes [9]. It is a raw material that can also be produced in a laboratory environment. Some people use IP-6 to treat and prevent cancer, including prostate cancer, breast cancer, colon cancer, liver cancer and blood cancers. Researchers have been investigating the role of IP-6 in cancer treatment and prevention since 1988 [10]. However, no studies have been conducted in people with cancer so far. Calcium Magnesium is also used to boost the immune system, treat anemia, and prevent heart disease and kidney stones. It is a white to off-white amorphous powder, practically insoluble in water (Figure 2) [11].

Calcium gluconate

Calcium Gluconate is the gluconate salt of calcium. Calcium, an essential element or mineral for normal nerve, muscle and heart

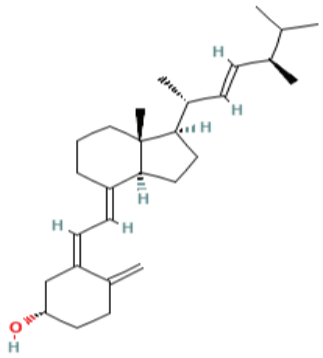

Figure 1: The molecular structure of Ergocalciferol (Vitamin D_2) [8].

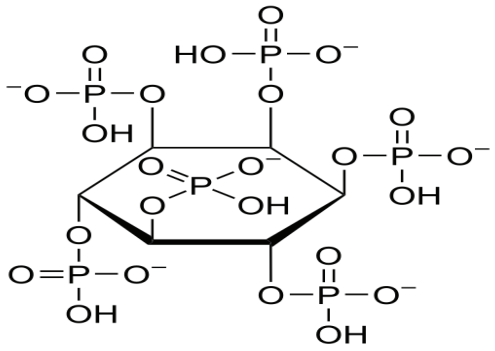

Figure 2: The molecular structure of calcium magnesium inositol hexaphosphate [11].

function, helps to maintain calcium balance and prevent bone loss when taken orally as gluconate salt. This agent can also be chemo-preventive for colon and other cancers. Calcium Gluconate (Figure 3) is the calcium salt of gluconic acid, the oxidation product of glucose, and is about one-third of calcium in the strength of calcium chloride USP [12] contains 9.3% Calcium. Calcium Gluconate is used as a mineral supplement and medicine when there is insufficient calcium in the diet. Supplementation can be done to treat or prevent osteoporosis or rickets, which are consequences of hypocalcemia [13]. It can also be taken orally, but by intramuscular injection is not recommended.

Calcium Gluconate is white to whitish in granular structure. Although it dissolves slightly in water, it dissolves freely in boiling water.

Ergocalciferol, Calcium Magnesium Inositol Hexaphosphate, Calcium Gluconate granules combination is A11JB in the class. It is in other Vitamin classes for diseases of the nervous system and metabolism. Since there is no dissolution specification in food supplement products, raw materials particle size were provided in a way to provide D90<250 micron specification, considering the dry mix production method.

Since the Ergocalciferol (Vitamin D_2) raw material in the combination is in low dosage in the formulation, it was decided to use a solvent at the rate of 1/1.000 of the total bulk. Due to the high solubility of Ergocalciferol (Vitamin D_2) in oils, a large amount of olive oil was used in the formulation. In this way, a more homogeneous bulk was obtained while creating a negligible difference in moisture determination analysis and quantification analysis of the final mixture.

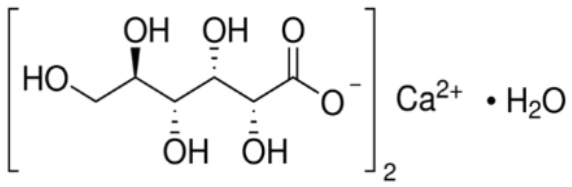

Figure 3: The molecular structure of calcium gluconate [12].

The excipients used in the formulation are: Sucrose (Filler Material), Vanilin (Flavour).

In this study, the history of the formulation of Ergocalciferol (Vitamin D_2), Calcium Magnesium Inositol Hexaphosphate, Calcium Gluconate combination prepared using Amixon mixer equipment is summarized.

Amixon mixer general information

Amixon mixer (Figure 4), which provides ideal mixing quality for dry, moist and viscous materials at variable filling levels, is one of the leading products of pharmaceutical technology [14]. Amixon mixer equipment is conical; it is a device with spiral mixing blade and homogenizer. This spiral blade and homogenizer provides a three dimensional mixing of products by creating a downward flow in the center, while exhibiting a spiral movement up and down the periphery. This equipment guarantees very good mixing results and homogeneity of discharge up to 90%. Together with the Amixon mixer range, it can be used as a powder mixer for dry mixes, wet suspensions as well as liquids, pastes and doughs [14]. Vertical mixer used in R&D, for use as a sterile mixer and reactor that also meets EHEDG requirements FDA and 3A sanitary. It is designed in accordance with the standards [14].

Polyformism is very important especially in pharmaceutical products. The working principle of Amixon mixer is primarily a homogeneous mixture without changing the particle size and structure [14]. The mixing process is more gentle and short-term than the normal container mixer. Conical mixer, including sanitary and pharmaceutical requirements meets all industry standards for surface quality. In addition to all these, jacketed and vacuum types act as dryers or reactors. In the picture below (Figure 5), the structure of the conical bowl Amixon mixer is examined in detail [14].

Amixon mixer has a closed system technology and the prepared bulk mixture is added to it from the feeding area on the top. Single and double shaft device can be supplied according to the studied pharmaceutical form. World Medicine R&D center, there is an image of the mixing mixer with the only shaft that makes a big difference in new product development processes (Figure 6) [14].

Materials and Methods

Materials

The active ingredients in the formulation were supplied; Ergocalciferol (SYNTHESIA-CZECHIA), Calcium Gluconate (GLOBAL CALCIUM - INDIA) Calcium Magnesium Inositol Hexaphosphate (LAIYANG WANJIWEI, CHINA). The excipient which are used as respectively; Sucrose (KONYA SEKER, TURKEY), Vanilin (SOLWAY, SWITZERLAND) supplied. All raw materials used are suitable for European Pharmacopoeia (Table 1).

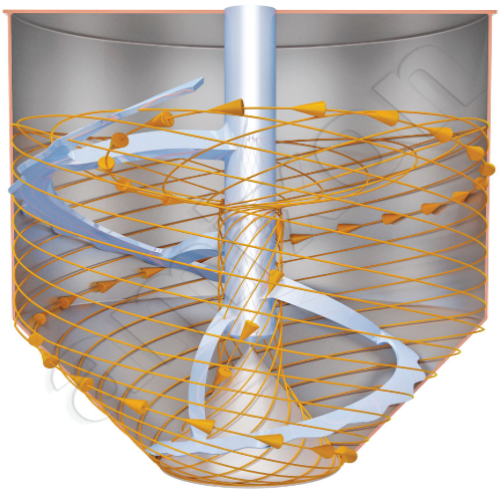

Figure 4: Amixon mixer study principle [14].

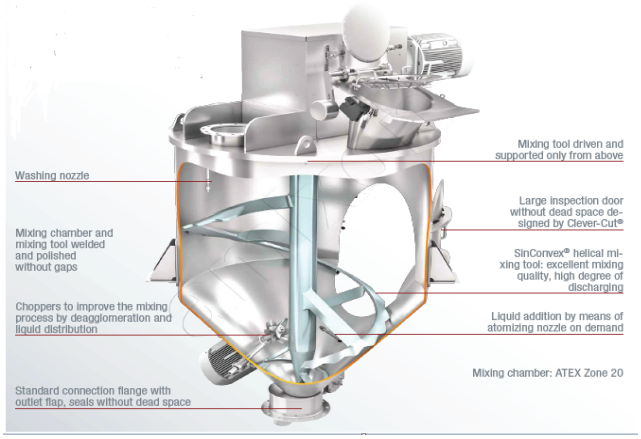

Figure 5: Amixon mixer single shaft device detailed equipment information [14].

Figure 6: Amixon mixer single shaft device [14].

Methods

A. **Stage 1: Trial-1 dry mix production method**

Stage 1.1: Mixing

Ergocalciferol, Calcium Magnesium Inositol Hexaphosphate, Sucrose are mixed with Calcium Gluconate in a cubic mixer.

Stage 1.2: Mixing

Vanillin is added to the powder mixture and the final mixture is provided with the help of a cubic mixer.

Result: Ergocalciferol (Vitamin D_2), which was developed with the dry mix production method, does not provide the specification of content uniformity analysis results. It was decided to make a new trial production with the geometric multiplication method.

B. **Stage 2: Trial-2 dry mix geometric blend production method**

Stage 2.1: Mixing

Ergocalciferol, Calcium Magnesium Inositol Hexaphosphate is mixed with Calcium Gluconate in a cubic mixer.

Stage 2.2: Mixing

It is mixed with sucrose 1/3 pieces 3 times by geometric reproduction method.

Stage 2.3: Mixing

The final mixture is made with the help of a cubic mixer by adding vanillin to it.

Result: Ergocalciferol (Vitamin D_2) content uniformity analysis results, developed with the dry mix geometric reproduction production method, do not provide the specification. It was decided to make a new trial with the wet granulation production method.

C. **Stage 3: Trial-3 wet granulation production method**

Stage 3.1: Pre-mixing

Calcium Magnesium Inositol Hexaphosphate, Sucrose are mixed with Calcium Gluconate in High Shear Mixer granulator.

Stage 3.2: Preparation of granulation solution

Ergocalciferol (Vitamin D_2) is added to 96 % Ethanol under mixing and mixed.

Stage 3.3: Wet granulation

Prepared powder mixture is granulated with granulation solution.

Stage 3.4: Wet sieve

The granules obtained are subjected to wet sieving.

Stage 3.5: Dry

Wet granules are dried in a fluid bed dryer.

Stage 3.6: Dry sieve

Dry granules are sieved through a 630 micron sieve.

Stage 3.7: Final mixture

Vanillin is added to the mixture obtained and the final mixture is made with the help of a cubic mixer.

Table 1: R&D trial formulations.

Ingredients	Function	Trial-1	Trial-2	Trial-3	Trial-4	Trial-5
Calcium Magnesium Inositol Hexaphosphate	Active Substance	125	125	125	125	125
Calcium Gluconate	Active Substance	375	375	375	375	375
Ergocalciferol* (Vitamin D_2)	Active Substance	3000.00 IU	3000.00 IU	3000.00 IU	3000.00 IU	3000.00 IU
Sucrose	Filling Material	-	-	-	-	-
Vanilin	Flavoring Agent	-	-	-	-	-
Olive Oil[1]	Solvent	-	-	-	-	-
Total Amount	-	**5000 mg**	**5000 mg**	**5000 mg**	**5000 mg**	**5000 mg**

[1]It is used in the formulation at the rate of 1/1.000
*3000 IU=0.075 mg

Table 2: Trial-4 long term stability results.

Trial-4 Long Term Stability								
Stability Test Condition: 25°C ± 2°C - 60% RH ± 5								
Tests	**Specifications**	**The beginning of stability**	**3rd Month**	**6th Month**	**9th Month**	**12th Month**	**18th Month**	**24th Month**
Appearance	White-Cream colored vanillin scented granules.	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate
Identification Calcium Magnesium Inositol Hexaphosphate, Calcium Gluconate	Precipitate of yellow color should be observed.	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate
Ergocalciferol	The retention time of the main peak on the chromatogram of the test solution used in the quantification method should correspond to the retention time of the main peak on the chromatogram of the standard solution.							
Average Weight	75 g ± 10.00% (67.50-77.50 g)	75.08 g/bottle	75.02 g/bottle	74.86 g/bottle	73.90 g/bottle	73.52 g/bottle	72.15 g/bottle	70.81 g/bottle
Water Content	Maximum 5.0%	2.05%	2.45%	2.73%	2.88%	3.15%	3.68%	3.98%
Determination of the amount active substance								
Calcium Magnesium Inositol Hexaphosphate	175.00 mg/5 g ± 10% (157.50-192.50 mg)	175.45 mg/5 g	175.56 mg/5 g	173.68 mg/5 g	174.27 mg/5 g	170.81 mg/5 g	168.98 mg/5 g	169.83 mg/5 g
Calcium Gluconate	375.00 mg/5 g ± 10% (337.50-412.50 mg)	373.41 mg/5 g	372.85 mg/5 g	370.93 mg/5 g	372.94 mg/5 g	371.09 mg/5 g	369.02 mg/5 g	365.30 mg/5 g
Ergocalciferol	3000 IU (0.075 mg/5 g ± 10%) (0.068 mg-0.083 mg)	0.076 mg/5 g	0.075 mg/5 g	0.073 mg/5 g	0.070 mg/5 g	0.069 mg/5 g	**0.067 mg/5 g**	**0.063 mg/5 g**
Uniformity of Content	No more than 2 of the single weights can deviate by more than 10% of the average weight, and none can deviate by more than 20%. 5 g ± 10% (4.50-5.50 g)	5.12 g Appropriate	5.08 g Appropriate	5.05 g Appropriate	5.06 g Appropriate	5.02 g Appropriate	5.03 g Appropriate	5.03 g Appropriate

Table 3: Trial-4 accelerated stability results.

Trial-4 Accelerated Stability				
Stability Test Condition: 40°C ± 2°C - 75% RH ± 5				
Tests	**Specifications**	**The beginning of stability**	**3rd Month**	**6th Month**
Appearance	White-Cream colored vanillin scented granules	Appropriate	Appropriate	Appropriate
Identification Calcium Magnesium Inositol Hexaphosphate, Calcium Gluconate	Precipitate of yellow color should be observed.	Appropriate	Appropriate	Appropriate
Ergocalciferol	The retention time of the main peak on the chromatogram of the test solution used in the quantification method should correspond to the retention time of the main peak on the chromatogram of the standard solution.			
Average Weight	75 g ± 10.00% (67.50-77.50 g)	75.08 g/bottle	73.04 g/bottle	72.18 g/bottle
Water Content	Maximum 5.0%	2.05%	3.10%	4.12%
Determination of the Active Substance				
Calcium Magnesium Inositol Hexaphosphate	175.00 mg/5 g ± 10% (157.50-192.50 mg)	175.45 mg/5 g	170.86 mg/5 g	169.95 mg/5 g
Calcium Gluconate	375.00 mg/5 g ± 10% (337.50-412.50 mg)	373.41 mg/5 g	369.76 mg/5 g	365.29 mg/5 g
Ergocalciferol	3000 IU (0.075 mg/5 g ± 10%) (0.068 mg-0.083 mg)	0.076 mg/5 g	**0.067 mg/5 g**	**0.062 mg/5 g**
Uniformity of Content	No more than 2 of the single weights can deviate by more than 10% of the average weight, and none can deviate by more than 20%. 5 g ± 10% (4.50-5.50 g)	5.12 g Appropriate	5.08 g Appropriate	5.05 g Appropriate

Result: Although the content uniformity results of Trial-3, in which 96% Ethanol wet granulation was performed are appropriate. In our samples produced by wet granulation method using ethanol, there was a significant decrease in Vitamin D quantity determinations in a short stability period. As a result of detailed literature reviews, Vitamin D began to degrade shortly after contact with Ethanol; his knowledge has been reached. For this reason, it was decided to conduct trial production again using oil as a solvent.

D. **Stage 4: Trial-4 wet granulation production method**

Stage 4.1: Pre mixing

Calcium Magnesium Inositol Hexaphosphate, Sucrose is mixed with Calcium Gluconate in a High Shear Mixer granulator.

Stage 4.2: Preparation of granulation solution

Ergocalciferol (Vitamin D_2) is added to olive oil under mixing and mixed.

Stage 4.3: Wet granulation

Prepared powder mixture is granulated with granulation solution.

Stage 4.3: Wet sieve

The granules obtained are subjected to wet sieving.

Stage 4.4: Drying

Wet granules are dried in a fluid bed dryer.

Stage 4.5: Dry sieve

Dry granules are sieved through a 630 micron sieve.

Stage 4.6: Final mixture

Vanillin is added to the mixture obtained and the final mixture is made with the help of a cubic mixer.

Result: Trial-4 provides specifications for the finished product and stability conditions. The results of the stability analysis of our Food Supplement product, whose trial production was carried out, are shared below (Tables 2 and 3). In order to facilitate production, cost and time during the production of our product, it was decided to make a trial production again using Amixon mixer.

Important information: Ergocalciferol, Calcium Magnesium Inositol Hexaphosphate, Calcium Gluconate Combination since the development processes of our product are carried out in the form of food supplements, according to the information received from the European Pharmacopoeia, dissolution and related compounds will not be subjected to analysis.

E. **Stage 5: Trial-5 dry mix production method with Amixon mixer**

Stage 5.1: Loading of raw materials

Calcium Magnesium Inositol Hexaphosphate, Vanilin, Calcium Gluconate and Sucrose are loaded into the Amixon mixer according to the sandwich method.

Stage 5.2: Preparation of solution

Ergocalciferol (Vitamin D_2) is mixed in a very small amount of olive oil until dissolved.

Table 4: Trial-5 long term stability results.

Trial-5 Long Term Stability								
Stability Test Condition: 25°C ± 2°C - 60% RH ± 5								
Tests	**Specifications**	**The beginning of stability**	**3rd Month**	**6th Month**	**9th Month**	**12th Month**	**18th Month**	**24th Month**
Appearance	White-Cream colored vanillin scented granules	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate
Identification Calcium Magnesium Inositol Hexaphosphate, Calcium Gluconate	Precipitate of yellow color should be observed.	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate
Ergocalciferol	The retention time of the main peak on the chromatogram of the test solution used in the quantification method should correspond to the retention time of the main peak on the chromatogram of the standard solution.							
Average Weight	75 g ± 10.00% (67.50-77.50 g)	75.26 g/bottle	75.03 g/bottle	75.81 g/bottle	74.60 g/bottle	74.32 g/bottle	72.67 g/bottle	71.86 g/bottle
Water Content	Maximum 5.0%	1.48%	1.58%	1.89%	2.34%	2.67%	2.96%	3.35%
Determination of the Amount Substance								
Calcium Magnesium Inositol Hexaphosphate	175.00 mg/5 g ± 10% (157.50-192.50 mg)	178.91 mg/5 g	178.56 mg/5 g	177.05 mg/5 g	176.03 mg/5 g	173.33 mg/5 g	172.61 mg/5 g	170.83 mg/5 g
Calcium Gluconate	375.00 mg/5 g ± 10% (337.50-412.50 mg)	377.88 mg/5 g	376.07 mg/5 g	375.13 mg/5 g	373.30 mg/5 g	372.52 mg/5 g	370.62 mg/5 g	367.49 mg/5 g
Ergocalciferol	3000 IU (0.075 mg/5 g ± 10%) (0.068 mg-0.083 mg)	0.078 mg/5 g	0.075 mg/5 g	0.074 mg/5 g	0.069 mg/5 g	0.069 mg/5 g	0.068 mg/5 g	**0.064 mg/5 g**
Uniformity of Content	No more than 2 of the single weights can deviate by more than 10% of the average weight, and none can deviate by more than 20%. 5 g ±% 10 (4.50-5.50 g)	5.26 g Appropriate	5.18 g Appropriate	5.10 g Appropriate	5.15 g Appropriate	5.09 g Appropriate	5.08 g Appropriate	5.10 g Appropriate

Stage 5.3: Mixing

A small amount of Olive Oil+Ergocalciferol mixture is sprayed into the mixing mixer tank in the mixture state.

Stage 5.4: After mixing

After mixing, it is mixed until the appropriate mixing uniformity is found.

F. **Application of sandwich method in Amixon mixer**

Calcium magnesium inositol hexaphosphate, calcium gluconate, vanilin and powdered sucrose are added to the mix on mixer by the sandwich method as follows, respectively (Figure 7). Ergocalciferol (Vitamin D_2) is dissolved in a small amount of olive oil and sprayed into the powder mixture in the Amixon mixer with a peristaltic pump and atomization pressure. After spraying, it is stirred for 30 minutes.

The results of the analysis of the finished product and stability conditions of Trial-5 are shared (Tables 4 and 5).

Result: It provides the finished product specifications of Trial-5 produced with Amixon mixer equipment. When the stability analysis results of Trial-5 were evaluated, approximately a 20% decrease in Ergocalciferol raw material was observed under stress conditions. In order for our food supplement product to meet the specifications under stability conditions, it was decided to carry out a pilot up study with an excess of 20%.

Although the olive oil and Ergocalciferol mixture was sprayed on the powder mixture in Trial-5, the production method was described as dry mixing. The reason for this is that the amount of solution is very low. Below are two trial productions with wet granulation and dry mixing production methods. The theoretical and post-process moisture levels are given comparatively (Table 6).

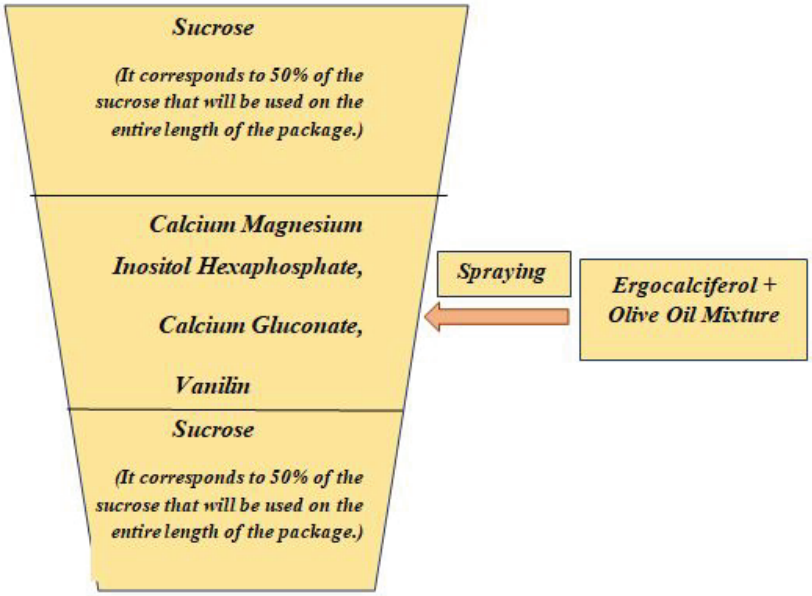

Figure 7: Sandwich method to be applied in the Amixon Mixer manufacturing tank.

Table 5: Trial-5 accelerated stability results.

Trial-5 Accelerated Stability				
Stability Test Condition: 40°C ± 2°C - 75% RH ± 5				
Tests	**Specifications**	**The beginning of stability**	**3rd Month**	**6th Month**
Appearance	White-Cream colored vanillin scented granules.	Appropriate	Appropriate	Appropriate
Identification Calcium Magnesium Inositol Hexaphosphate, Calcium Gluconate	Precipitate of yellow color should be observed.	Appropriate	Appropriate	Appropriate
Ergocalciferol	The retention time of the main peak on the chromatogram of the test solution used in the quantification method should correspond to the retention time of the main peak on the chromatogram of the standard solution.			
Average Weight	75 g ± 10.00% (67.50-77.50 g)	75.26 g/bottle	73.04 g/bottle	72.18 g/bottle
Water Content	Maximum 5.0%	1.48%	2.71%	3.52%
Determination of the Amount Active Substance				
Calcium Magnesium Inositol Hexaphosphate	175.00 mg/5 g ± 10% (157.50-192.50 mg)	178.91 mg/5 g	170.86 mg/5 g	169.95 mg/5 g
Calcium Gluconate	375.00 mg/5 g ± 10% (337.50-412.50 mg)	377.88 mg/5 g	369.76 mg/5 g	365.29 mg/5 g
Ergocalciferol	3000 IU (0.075 mg/5 g ± 10%) (0.068 mg-0.083 mg)	0.078 mg/5 g	0.069 mg/5 g	**0.062 mg/5 g**
Uniformity of Content	No more than 2 of the single weights can deviate by more than 10% of the average weight, and none can deviate by more than 20%. 5 g ± 10% (4.50-5.50 g)	5.26 g Appropriate	5.08 g Appropriate	5.05 g Appropriate

The process, time and equipment used in Trial-4 and Trial-5, which provide the finished product analysis specifications, are examined in detail in the table 7.

When the moisture values of the trial productions at the beginning and end of the process are evaluated; while the moisture value of the Trial-4 produced by wet granulation production method increased significantly after the process, the drying process was needed after granulation. There was no significant increase in the dry mix method in which we sprayed the solution, and there was no need for the drying process.

The trial production using Amixon mixer can be completed in a serious production time with a difference of 135 minutes in advance, the use of a total of 3 extra equipments. When we evaluate the cleaning, the cleaning materials to be used in all of these and the ease

Table 6: Comparative moisture value.

Trial Number	Theoretical Moisture	Moisture Value After Wet Granulation	Moisture Value After Dry Mixing
	Specification: It is subjected to drying loss analysis at 105° for 3 minutes		
Trial-4	0.85%	2.05%	-
Trial-5	0.86%	-	1.48%

Table 7: Process equipment comparison.

Used Equipment Names and Time	Trial-4	Trial-5
	Wet Granulation	Dry Mix
Amixon Mixer	X	15 minutes in total
High Shear Mixer	45 minutes in total	X
Fluid Bed Dryer	75 minutes in total	X
Firewitt Hammermill	15 minutes in total	X
Container Mixer	15 minutes in total	X
Total Usage Time	**4 Equipments 15 Minutes**	**1 Equipment 15 Minutes**

Table 8: Pilot study accelerated stability results.

Pilot Study Accelerated Stability				
Stability Test Condition : 40°C ± 2°C - 75% RH ± 5				
Tests	**Specifications**	**The beginning of stability**	**3rd Month**	**6th Month**
Appearance	White-Cream colored vanillin scented granules.	Appropriate	Appropriate	Appropriate
Identification Calcium Magnesium Inositol Hexaphosphate, Calcium Gluconate	Precipitate of yellow color should be observed.	Appropriate	Appropriate	Appropriate
Ergocalciferol	The retention time of the main peak on the chromatogram of the test solution used in the quantification method should correspond to the retention time of the main peak on the chromatogram of the standard solution.			
Average Weight	75 g ± 10.00% (67.50-77.50 g)	75.03 g/bottle	74.95 g/bottle	73.24 g/bottle
Water Content	Maximum 5.0%	1.36%	2.19%	3.27%
Determination of the Amount Active Substance				
Calcium Magnesium Inositol Hexaphosphate	175.00 mg/5 g ± 10% (157.50-192.50 mg)	175.62 mg/5 g	174.67 mg/5 g	172.01 mg/5 g
Calcium Gluconate	375.00 mg/5 g ± 10% (337.50-412.50 mg)	375.03 mg/5 g	373.08 mg/5 g	372.13 mg/5 g
Ergocalciferol	3600 IU (0.090 mg/5 g ± 10%) (0.068 mg-0.108 mg)	0.089 mg/5 g	0.081 mg/5 g	0.076 mg/5 g
Uniformity of Content	No more than 2 of the single weights can deviate by more than 10% of the average weight, and none can deviate by more than 20%. 5 g ± 10% (4.50-5.50 g)	5.05 g Appropriate	5.03 g Appropriate	5.00 g Appropriate

Table 9: Results of the pilot study content uniformity analysis results.

Results of the Pilot Study Content Uniformity				
Locations		Limit and Specifications		
		Ergocalciferol (Vitamin D_2) (90.00%-132.00%)	Calcium Magnesium Inositol Hexaphosphate (90.00%-110.00%)	Calcium Gluconate (90.00%-110.00%)
Pilot Study 1st Minute Results	1st Minute 1st Location	118.66	94.80	93.55
	1st Minute 2nd Location	122.66	96.56	91.81
	1st Minute 3rd Location	120.00	96.00	94.11
	1st Minute 4th Location	121.30	97.12	93.55
	1st Minute 5th Location	120.00	98.80	92.96
	1st Minute 6th Location	121.30	95.44	94.11
	Minimum Value	**118.66**	**94.80**	**91.81**
	Maximum Value	**122.66**	**98.80**	**94.11**
	Standard Deviation Value	**1.39**	**1.41**	**0.87**
	RSD Value	**1.06**	**1.46**	**0.93**
Pilot Study 3rd Minute Results	3rd Minute 1st Location	119.18	94.72	94.11
	3rd Minute 2nd Location	120.26	95.44	94.11
	3rd Minute 3rd Location	120.05	97.68	92.96
	3rd Minute 4th Location	119.85	95.80	93.55
	3rd Minute 5th Location	118.60	96.56	92.40
	3rd Minute 6th Location	121.24	97.71	93.55
	Minimum Value	**118.60**	**95.44**	**92.96**
	Maximum Value	**121.24**	**94.80**	**94.11**
	Standard Deviation Value	**0.91**	**1.27**	**0.67**
	RSD Value	**0.76**	**1.22**	**0.72**
Pilot Study 5th Minute Results	5th Minute 1st Location	120.00	94.80	94.11
	5th Minute 2nd Location	120.18	95.44	93.55
	5th Minute 3rd Location	119.86	95.44	92.40
	5th Minute 4th Location	119.03	96.56	94.11
	5th Minute 5th Location	118.92	94.24	93.55
	5th Minute 6th Location	118.51	97.68	92.96
	Minimum Value	**118.51**	**94.24**	**92.40**
	Maximum Value	**120.18**	**97.68**	**94.11**
	Standard Deviation Value	**0.68**	**1.30**	**0.72**
	RSD Value	**0.57**	**1.24**	**0.62**
Pilot Study 10th Minute Results	10th Minute 1st Location	120.55	98.24	94.11
	10th Minute 2nd Location	120.36	90.80	92.40
	10th Minute 3rd Location	119.80	90.80	92.96
	10th Minute 4th Location	119.63	94.80	93.55
	10th Minute 5th Location	119.07	100.00	93.55
	10th Minute 6th Location	118.01	93.12	94.69
	Minimum Value	**118.01**	**90.80**	**92.40**
	Maximum Value	**120.55**	**100.00**	**94.69**
	Standard Deviation Value	**0.93**	**4.05**	**0.87**
	RSD Value	**0.78**	**3.83**	**0.81**
Pilot Study 15th Minute Results	15th Minute 1st Location	121.08	92.56	92.96
	15th Minute 2nd Location	120.50	98.80	93.55
	15th Minute 3rd Location	120.68	93.68	94.11
	15th Minute 4th Location	118.55	95.44	93.55
	15th Minute 5th Location	118.36	98.24	94.69
	15th Minute 6th Location	119.72	96.56	93.55
	Minimum Value	**118.36**	**92.56**	**92.96**
	Maximum Value	**121.08**	**98.80**	**94.69**
	Standard Deviation Value	**1.14**	**2.47**	**0.59**
	RSD Value	**0.95**	**2.58**	**0.63**
Pilot Study 20th Minute Results	20th Minute 1st Location	121.68	97.68	93.55
	20th Minute 2nd Location	118.80	97.24	94.11
	20th Minute 3rd Location	119.52	97.24	94.69
	20th Minute 4th Location	120.27	94.80	94.11
	20th Minute 5th Location	120.95	96.00	92.96
	20th Minute 6th Location	120.82	99.40	93.92
	Minimum Value	**118.80**	**94.80**	**92.96**
	Maximum Value	**121.68**	**99.40**	**94.69**
	Standard Deviation Value	**1.17**	**1.61**	**0.62**
	RSD Value	**0.97**	**1.56**	**0.59**

Table 9 (Continued)

Pilot Study 25th Minute Results	25th Minute 1st Location	122.56	99.44	94.11
	25th Minute 2nd Location	119.59	98.64	92.96
	25th Minute 3rd Location	120.81	96.56	94.11
	25th Minute 4th Location	121.67	97.12	94.69
	25th Minute 5th Location	118.83	95.44	94.11
	25th Minute 6th Location	119.73	98.80	92.40
	Minimum Value	**118.83**	**95.44**	**92.40**
	Maximum Value	**122.56**	**99.44**	**94.69**
	Standard Deviation Value	**1.17**	**1.58**	**0.86**
	RSD Value	**1.41**	**1.54**	**0.92**
Pilot Study 30th Minute Results	30th Minute 1st Location	122.66	97.68	94.11
	30th Minute 2nd Location	124.00	99.44	94.69
	30th Minute 3rd Location	120.00	93.68	93.55
	30th Minute 4th Location	121.30	100.00	94.11
	30th Minute 5th Location	121.30	96.56	92.96
	30th Minute 6th Location	121.30	98.24	93.55
	Minimum Value	**120.00**	**93.68**	**92.96**
	Maximum Value	**124.00**	**100.00**	**94.69**
	Standard Deviation Value	**1.38**	**2.34**	**0.60**
	RSD Value	**1.14**	**2.38**	**0.64**

of the process, we can see that Amixon mixer is in every sense during commercial production we can easily say that it provides serious benefits.

Sampling plan

The detailed production sampling plan of Ergocalciferol, Calcium Magnesium Inositol Hexaphosphate, Calcium Gluconate granule combination are as follows.

During the mixing after spraying, samples are provided from the designated locations within 1, 3, 5, 10, 15, 20, 25, 30 minutes and the final formula and production method are obtained by evaluating the result of the combination produced with Amixon mixer equipment at which time the appropriate AW homogeinty specifications will be met.

Sampling locations

Samples from 6 different locations in the mixing mixer at different times in order to accurately find the time when the appropriate homogeneity is achieved in the mixing process after receipt, the content will be subjected to uniformity analysis. Below is the location information of the samples to be taken during production (Figure 8).

Location descriptions

1st Location: Top Left
2nd Location: Top Right
3rd Location: Left Centre
4th Location: Top Right
5th Location: Bottom Left
6th Location: Bottom Right

After the determined mixing times, 6 different samples are taken for each minute from the designated locations inside the Amixon mixer by means of the sampling stick (Figure 9).

After all the product development studies, the pilot study of 150.00 kg was carried out by increasing the Trial-5 formula 100 times the serial length. During the pilot study, Ergocalciferol raw material was added more than 20%. When evaluated in the results of accelerated stability analysis under the worst condition, it was observed that the final product developed complies with the finished product and shelf life specifications.

The results of the stability analysis of the combination (Accelerated Period) are given below (Table 8). Final production process flow chart

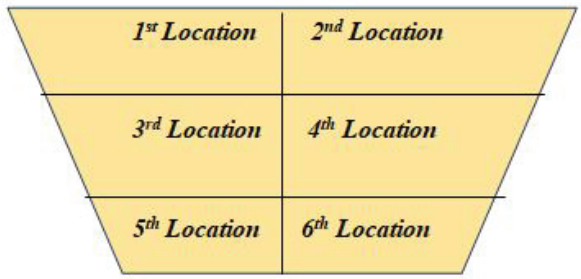

Figure 8: Amixon mixer sampling location.

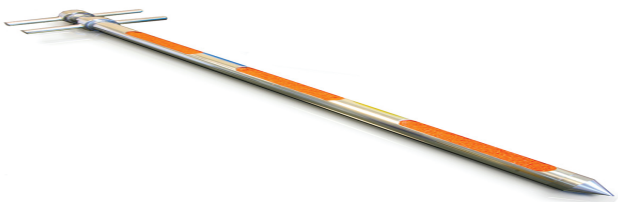

Figure 9: Sampling stick.

is shown in figure 10. Pilot Study Batch Size: 150.000 kg/ 20.000 Bottle (1 bottle contains 75 g of product).

Results

When all content uniformity results are examined (Table 9), while Ergocalciferol, Calcium Magnesium Inositol Hexaphosphate, Calcium Gluconate granule combination was developed, when Amixon mixer equipment was used in product development studies; It is clear that only the 1st minute content uniformity analysis results meet the specifications of commercial productions.

Discussion and Conclusion

After the completed R&D studies, trial production and 1 pilot

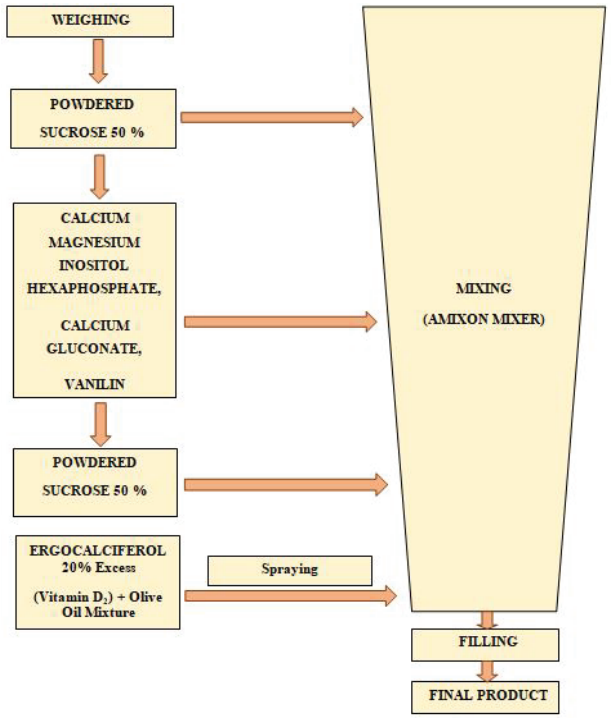

Figure 10: Final production process flow chart.

production were made with 5 different production methods and formulations. According to the results of the finished product analysis, while the Trial-4 and Trial-5 results provided the appropriate finished product results, it was decided to continue with Trial-5 due to the convenience in the production method, cheap cost and big time difference.

When the results of the Trial-4 and Trial-5, which are suitable for the finished product results, are compared in detail, in terms of production technologies and cost, while more than one process is applied in the wet granulation production method, when we use Amixon mixer; we can clearly say that we provide convenience from many factors such as equipment, time, equipment, cost and ease of operation.

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