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DESIGN AND CHARACTERIZATION OF A NEW SUGAR FREE FORMULATION OF VITAMINS C, E, B6 AND MINERALS Zn^{2+} AND Mn^{2+} FOR CHILDREN IN ORAL VIAL WITH PLUNGER AND TEAR OFF CAP

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ABSTRACT

Objective: Aim of present study was to provide a stable sugar free formulation that contains vitamins C, E, B6 and minerals Zn^{2+} and Mn^{2+} in a unique pharmaceutical dosage form which consists of a plunger containing granules closed with a tear-off cap and a number vial contains solution which is prepared specially to dissolve the last granules. Final form is closed and securely fastened with a plastic cap. This dosage form combines advantages of liquid dosages with those of solid dosages.

Methods: granules were prepared by means of wet granulation method and the granulator was consisted of alcohol and water (80:20). Product in its final form was evaluated for many parameters like, appearance, Loss on drying for granulated part, pH for solution part and assay. The formulation was subjected to stability studies as per ICH guidelines at temperatures and humidity of 40 C^{o} / 75% RH ± 5% RH for six months [1].

Results: results of stability studies for all vitamins and minerals were found within the limits but there was a significant change in assay of vitamins C and B6; in addition, there was failure in meeting the acceptance criteria for color of the granulated part.

Conclusion: it could be concluded that oral vial with plunger and tear off cap can be used as an alternative dosage form for minerals and vitamins, but additional testing at the intermediate storage condition 30°C/65% RH ± 5% RH " If long-term studies were conducted at 25°C /60% RH± 5% RH " should be conducted and evaluated against significant change criteria [1].

Keywords: Vitamins, Minerals, Granulation, Stability, Oral vial.

INTRODUCTION

Vitamins are an essential group of food ingredients which to be supplied in sufficient amounts with diet, vitamins are broad group of organic compounds that are minor, but essential constituents of food required for normal growth, self maintenance and functioning of human and animal bodies [2]. Vitamin C attracts attention of the research community and consumers as a nutrient with a broad biological activity and importance for human health [3]. It is a white, odorless, macro or microcrystalline powder with a strongly acid taste. It dissolves to extent of about 30% in water, is hygroscopic and has a relatively high reduction potential. This is why it undergoes many chemical interactions with other vitamins and has to be regarded as one of the unstable vitamins [4].

Pyridoxine, a water soluble vitamin, is involved principally in amino acid metabolism, but is also involved in carbohydrate and fat metabolism. It is also required for the formulation of hemoglobin [5]. Pyridoxine hydrochloride poses few problems in pharmacy, because it undergoes no chemical interactions with other vitamins and is insensitive to oxygen and reducing agents. The stability of pyridoxine hydrochloride is good in virtually all solid drug forms. It can be granulated without problems. Pyridoxine hydrochloride is also quite stable in liquid drug forms if no oxidizing agents are present and it is not exposed to light [4].

Vitamin E, a fat soluble vitamin, prevents the oxidation of polyunsaturated fatty acids. it reacts with free radical, which are the cause of oxidative damage to cell membranes, without the formation of another free radical in the process. Tocopheryl acetate is insensitive to oxidation, and thus one of the few stable vitamins. Zinc and manganese are essential elements of nutrition. Zinc is a constituent of many enzymes system and is present in all tissues. Water soluble zinc and manganese salts are used as supplements to correct zinc and manganese deficiency [5].

Drugs substances most frequently are administered orally by means of solid dosage forms such as tablets and capsules. Tablets remain popular as a dosage because of the advantages afforded both to the manufacturer (e.g. Simplicity and economy of preparation, stability, and convenience in packaging, shipping, and dispensing) and the patient (e.g. accuracy of dosage, compactness, portability, blandness of taste and ease of administration). Liquid dosage forms are useful for a number of reasons. They can be formulated for different routes of administration: orally, introduction into body cavities or external application the dose can easily be adjusted by dilution, making the oral liquid form ready to be administered to children or people unable to swallow tablets or capsules. Much has been written about the biopharmaceutical properties of solid dosage forms. Many researchers begin their absorption studies of drugs administered in solution to assess the bioavailability relative to tablets and capsules. Absorption occurs when drugs are in a dissolved state, thus it is frequently observed that the bioavailability of oral dosage forms decreases in the following order: aqueous solution> aqueous suspension> tablets or capsule [6].

Our research presented the product in a dosage form that is widely used in Italy; it combines advantages of liquid dosages with those of solid dosages. it is suitable for patients who have trouble swallowing; it also has fast absorption, accurate dosing and good stability, it is single-dose product, so no need to measure the dose. Use the dosage form as shown in figure1, remove plastic cap, then press the plunger into the vial shaking well then drink the whole solution. Our research presented the product as sugar free, Under the authority of the Nutrition Labeling and Education Act of 1990, FDA issued regulations for the nutrient content claim “sugar free” 58 Federal Register (FR) 2302 at 2415. “Sugar free” is defined in Title 21 of the Code of Federal Regulations 101.60(c) (21 CFR 101.60 (c)) as a claim that may be used on a food that contains less than 0.5 g of sugar [7].

MATERIALS AND METHODS

Tear off caps, molded DED umber glass vial 10 ml, plungers, plastic caps were purchased from P.P.I (Italy), Vitamin C (ascorbic acid) was purchased from Shandong Luwel (China), Vitamin B6 (pyridoxine

HCl) was purchases from jiangxi Senta (China), Vitamin E (alpha tocopheryl acetate) 50% USP was purchased from BASF (Germany), Zinc sulfate monohydrate was purchases from MEDEX (UK), Manganese sulphate monohydrate was purchased from Himedia (India), mannitol was purchased from Cargill (Italy), sodium benzoate was purchased Chem-Base laboratories (China), sucrose was purchased from MITR PHOL (China), sorbitol solution 70% was purchased from Roquette (France), Glycerin was purchased from Acidchem (Malasia), Acesulfame Potassium was purchased from Suzhou hope technology (China), citric acid monohydrate was purchased from Weif and Ensign (China), sodium Citrate dehydrate was purchased from Changzhou(China), Polyethylene Glycol 6000 was purchased from Hopkem (Russia), vanillin flavor was purchased from Jiaxing (China), peppermint oil flavor was purchased from Nectar (India), Strawberry flavor was purchased from Sensiet (Italy) and tween 80 (polysorbate 80) was purchased from Industria Quimica (Mexico).

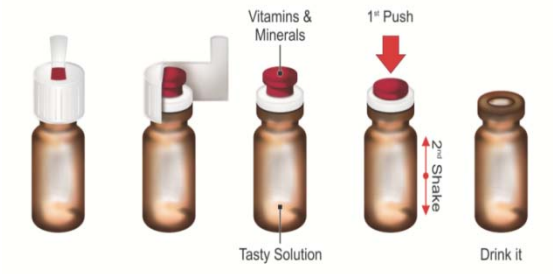

Fig. 1: how to use the dosage form

Formulation of the granulated part

Manganese sulfate, zinc sulfate, vitamin B6, mannitol, sodium benzoate and powdered sucrose were sieved through 20 mesh screen, then they were mixed.

The granulating agent (solution of alcohol and water 80:20) was added to the last mixed materials until quality wet granules mass was obtained. wet granules were dried at 50-55 C° till the Loss on Drying NMT 1%. The resulting dry granules were passed through 20 mesh screen. Vitamin E and vitamin C were sieved through 20 mesh screen then they were blended with last granulated material blend was filled manually into plungers and closed with tear off caps, each cap was contained 200 mg of the blend. The ingredients of granulated part are listed in table1.

Formulation of the solution part

To about 2.8 liter of distilled water its temperature about 50 C°, the following materials were added with continuous mixing. Sorbitol 70 %, glycerin, acesulfame K, sodium benzoate, PEG 6000, tween 80, vanillin powder, peppermint oil, strawberry flavor, sodium citrate and citric acid monohydrate.

Then the volume was completed to 3 liter with distilled water and solution was adjusted the pH 3-4. Solution was filled manually into 10 ml molded DED umber glass vials. The vials were closed with the last tear off caps and Final forms were closed and securely fastened with a plastic caps. The ingredients of solution part are listed in table 2.

Batch size

3 liter of the solution part and 300 g of the granulated part, each unit contains 200 mg of the granulated part in the plunger and 10 ml of the solution part in the vial.

Stability Studies

The formulation was subjected for stability studies by keeping samples in their final packing in stability chamber (Binder, Germany). The formulations were stored at 40°C ± 2°C/75% RH ± 5% RH for six months as per ICH guidelines. The formulations were subjected to different tests such as appearance, Loss on drying for granulated part, pH for solution part and assay.

Test of loss on drying

loss on drying was measured by taking a 5 g of test specimen. It was dried for 90 minutes at 40 C°. Loss on drying was determined by using LOD tester (Sartorins, Germany).

Table 1: ingredients of the granulated part

Ingredients	Theoretical amounts mg/unit	Actual amounts mg/unit	Actual amounts g/batch
Vitamin C	45.00	63.00 (overage 40%)	94.50
Vitamin E 50%	7.50	22.16 (overage 50 %)	33.24
Vitamin B6	1.50	2.10 (overage 40%)	3.15
Zinc (ZnSO4.H20)	5.00	13.76	20.64
Manganese (MnSO4.H20)	1.50	4.58	6.87
Mannitol	-	85.20	127.80
Sucrose (grind)	-	3.00	4.50
Sodium Benzoate	-	6.20	9.30
total	-	200.00 mg	300.00 g

*Use of an overage of a vitamins to compensate for expected degradation during products shelf life because vitamins decomposes easily.

Table 2: ingredients of the solution part

Ingredients	Quantity per unit / g	Quantity per batch
Sorbitol	2.40000	720.00000
Glycerin	1.00000	300.00000
Acesulfame. K	0.01660	4.98000
Sodium Benzoate	0.01880	0.56300
Citric acid monohydrate	0.04120	12.35100
Sodium citrate	0.00149	0.44700
Vanillin powder	0.00150	0.45000
Peppermint oil	0.00030	0.09000
strawberry	0.05000	15.00000
PEG 6000	0.07000	21.00000
Tween 80	0.11250	33.75000
Purified water	Up to 10.00000 ml	Up to 3000.00000 ml

pH evaluation

For the evaluation of pH, pH meter (Crison, Switzerland) calibrated with buffers, potassium tetraoxalate 0.05m, pH 1.68 and potassium biphthalate 0.05 m, pH 4.01. For each measurement, a 10 ml sample was collected and placed in an amber glass flask. The pH was measured by dipping the electrode directly into the solution, at room temperature.

Assay of Vitamins C & B6 using HPLC

The assay of Vitamins C & B6 were carried out using HPLC (Shimadzo). Buffer solution was prepared by dissolving 1.011 g of heptanesulfonate, sodium salt and 13.600 g of monobasic potassium phosphate in about 800 ml of distilled water, and diluting with water to 1000 ml. Mobile phase was prepared by mixing filtered and degassed buffer solution and acetonitrile (90:10) and adjusting the PH 2.2 with phosphoric acid. sample preparation was prepared by weighing the content of 20 tear off over caps. Average weight was calculated. An accurately weighed one unit of the granules was transferred to a 100 ml volumetric flask, dissolved with water and diluted to volume with the water.

Standard preparation was prepared by transferring an accurately weighed quantity equivalent to 450 mg of vitamin C and 15 mg of vitamin B6 to a 100 ml volumetric flask and dissolving them in water. Pipet 5 ml from the last solution to a 50 ml volumetric flask and dilute to volume with water to obtain a 0.45 mg/ml of vitamin C and 0.015 mg/ml of vitamin B6. Equal volumes (about 20 µl) of sample preparation and standard preparation were injected separately into the chromatograph and chromatograms were recorded. Chromatographic system: The liquid chromatography is equipped with a 275 nm detector and a 4.6 mm × 25 cm column that contains 5 µm packing L1. The column temperature is maintained at ambient temperature, and the flow rate is about 1 ml per minute.

Assay of Vitamins E acetate using HPLC

The assay of Vitamin E acetate using was carried out using (Shimadzo). Mobile phase was prepared by mixing filtered and degassed water and methanol (1:99). sample preparation was prepared by weighing the content of 20 tear off over caps. Average weight was calculated. An accurately weighed one unit of the granules was transferred to a 25 ml volumetric flask, dissolved with methanol and diluted to volume with the same solvent.

Standard preparation was prepared by transferring an accurately weighed quantity equivalent to 30 mg of vitamin E acetate to a 100 ml volumetric flask and dissolving it in methanol. Equal volumes (about 20 µl) of sample preparation and standard preparation were injected separately into the chromatograph and chromatograms were recorded. Chromatographic system: The liquid chromatography is equipped with a 205 nm detector and a 4.6 mm × 25 cm column that contains 5 µm packing L1. The column temperature is maintained at ambient temperature, and the flow rate is about 2 ml per minute.

Assay of Manganese

Dissolve an accurately weighed quantity of Manganse sulfate 1 g, in 200 ml of water. Add 0.5 gr potassium cynide and 20 ml of ammonia-ammonium chloride buffer TS and 0.1 ml of erichrome black TS, and titrate with 0.05 M edetate disodium VS until the solution is deep blue in color. Each 1 ml of 0.05 M edetate disodium is equivalent to 2.75 mg of Manganse. Quantity of Manganse % have calculated as the following equation

$$(\mathrm{Mn})^{2+}\% = \frac{\mathrm{V1} * 2.75}{7.5} * 100$$

Where V1: volume of edentate disodium

Assay of Zinc

Dissolve an accurately weighed quantity of zinc sulfate 1 g, in 200 ml of water. Add 20 ml of ammonia-ammonium chloride buffer TS and 0.1 ml of erichrome black TS, and titrate with 0.05 M edentate disodium VS until the solution is deep blue in color. Each 1 ml of 0.05 M edetate disodium is equivalent to 3.25 mg of Zinc.

The quantity needed to titrate Zn^{2+} = total volume of edetate disodium (V2)- volume needed to titrate Mn^{2+} (V1)

Quantity of Zinc % have calculated as the following equation

$$(\mathrm{Zn})^{2+}\% = \frac{(\mathrm{V2} - \mathrm{V1}) * 3.25}{25} * 100$$

RESULTS AND DISCUSSIONS

Evaluation of the parameters of the granulated part

The ingredients of granulated part, as shown in table 1, include mannitol as a filler, mannitol is ideally suited to this formulation because it is water-soluble, it has a sweet taste, approximately as sweet as glucose and half as sweet as sucrose, and impacts a cooling sensation in the mouth. Sucrose is widely used in oral pharmaceutical formulation. It was used in our formulation as a binding agent for wet granulation. Sodium benzoate was used as antimicrobial preservative [8]. All vitamins and minerals were included in this part to get the maximum stability. Granules were prepared by wet granulation method and the granulator was consisted of alcohol and water (80:20). Our research presented the product as sugar free, it contained only 3 mg per unit. The granules in the plunger were evaluated for various parameters: appearance, loss on drying, and assay. Physical parameters of the granulated part are shown in table 3.

Appearance test results for the granulated part were within the specification i.e. White granules, loss on drying was 0.84 and also was within the specification limit i.e. not more than 3 % at 40 C° for 90 min, we chose this limit because ascorbic acid is unstable even at room temperature, and increased temperature and humidity rapidly increase its degradation [9]. Assay was conducted and results for all vitamins and minerals were found within the limits. Assay of vitamin C was 141.5 %, vitamin B6 was 139.1%, vitamin E was 146.7%, Manganese was 100.3% and Zinc was 99.6%. Results are shown in table 4.

Evaluation of the parameters of the solution part

The ingredients of solution part as shown in table 2 include Sorbitol 70% as sweeting agent and vehicle in the formulation, Glycerin as viscosity increasing agent, Acesulfame potassium as sweeting agent, Sodium benzoate as antimicrobial preservative, citric acid monohydrate and sodium citrate as buffering agent, Vanillin powder, peppermint oil and strawberry liquid as flavoring agent, polysorbate 80 is nonionic surfactant it was used as solublizing agent for vitamin E because it is oil soluble vitamin. PEG 6000 was used to enhance the aqueous solubility or dissolution characterization of vitamin E by making solid dispersions [8]. Appearance test results for the solution part was within the specification i.e. Clear colorless solution. PH of solution part was adjusted at 3-4, this value was not represented the pH stability or solubility of vitamins, we chose this value to get the tasty taste. Results are shown in table 5.

Stability Studies

The stability studies, as shown in table 4, revealed that all vitamins and minerals were found within the limits but there was more than 5 percent change in assay of vitamin C and vitamin B6 from their initial value, that mean there was significant change in their assay. in addition to, there was failure in meeting the acceptance criteria for appearance of granulated part because color of granules was changed from white to yellow and this was may be due to the partition oxidation of vitamin C. Loss on drying values were within the limits but they were increased from 0.84 % at initial point to 3.00 % after six months, that mean tear off over cap with plunger and plastic cap is not completely protecting the granules from leaking of moisture at these conditions. For the solution part, as shown in table 5, all parameters, pH and appearance, were found within the limits. Thus our studies conformed that oral vial with plunger and tear off cap can be used as a possible alternative to conventional oral formulation of vitamins and minerals but additional testing at the intermediate storage condition 30°C ± 2°C/65% RH ± 5% RH" If long-term studies were conducted at 25°C ± 2°C/60% RH ± 5% RH" should be conducted and evaluated against

significant change criteria. Testing at the intermediate storage condition should include all tests. The initial application should include a minimum of 6 months' data from a 12-month study at the intermediate storage [1].

Table 3: Physical parameters of the granulated part

Physical parameters	Limits	Reference
Appearance of powder part	White powder	In house specification
Appearance of solution part	Clear colorless solution	In house specification
pH of solution part	3.0-4.0	In house specification
LOD	Not more than 3% at 40 C° for 90 min	In house specification
Assay		
Vitamin C	90-150 %	In house specification
Vitamin B6	90-150 %	In house specification
Vitamin E	90-165 %	In house specification
Zinc	90-105 %	In house specification
Manganese	90-105 %	In house specification

Table 4: stability studies results with initial values for the granulated part

Test	Initial values	1 month	2 month	3 month	6 month
Appearance	White powder	Yellow powder	Yellow powder	Yellow powder	Yellow powder
LOD	0.84 %	1.36 %	2.50 %	2.79 %	3.00 %
Assay:					
Vitamin C	141.5 %	133.9 %	132.4 %	129.1 %	116.3 %
Vitamin B6	139.1 %	126.9%	116.7 %	113.0 %	102.1 %
Vitamin E	146.7 %	144.7 %	148.5 %	139.6 %	146.6 %
Zinc	99.6 %	100.4 %	98.8 %	100.1 %	99.6 %
Manganese	100.3 %	100.7 %	100.7 %	100.4 %	100.7 %

RSD < 2% for all results

Table 5: stability studies results with initial values for the solution part

Test	Initial values	1 month	2 month	3 month	6 month
Appearance	Clear colorless solution	Clear colorless solution	Clear colorless solution	Clear colorless solution	Clear colorless solution
pH	3.28	3.15	3.18	3.26	3.22

CONCLUSION

it could be concluded that oral vial with plunger and tear off cap can be used as an alternative dosage form for minerals and vitamins, but additional testing at the intermediate storage condition 30°C ± 2°C/65% RH ± 5% RH" If long-term studies were conducted at 25°C ± 2°C/60% RH ± 5% RH" should be conducted and evaluated against significant change criteria [1].

CONFLICT OF INTEREST

Declared None

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