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FORMULATION AND EVALUATION OF STABILIZED VITAMIN A PALMITATE IN MULTIVITAMIN SYRUP

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ABSTRACT

In liquid oral formulations, the stability of the active and inactive ingredients is of major issue for the formulator. Usually active ingredients are less stable in aqueous formulations than in solid dosage forms. Hence it is important to stabilize and preserve the liquid oral formulations which contains water. This work was aimed to develop and stabilize Vitamin A Palmitate in a multivitamin syrup form. Three formulations (V1, V2 and V3) of Vitamin A Palmitate syrups were developed. All the formulated syrups were evaluated for appearance, colour, taste, pH, wt/ml, viscosity and drug content. Formulation V2 showed good results in terms of general, physical and chemical parameters evaluated compared to formulation V1. Hence, formulation V2 was considered as an optimized formulation. Multivitamins (Vitamin D & E) was incorporated in the optimized formulation (V3) and the stability reports of formulation V3 revealed that Vitamin A Palmitate was stable even in presence of Multivitamins.

KEYWORDS: Multivitamins, Stabilize, Syrup, Vitamin A Palmitate.

INTRODUCTION

Syrup is a concentrated or nearly saturated solution of sucrose in water.[1] Vitamins are essential nutrients found in foods. They perform specific and vital functions in a variety of

body systems and maintaining health. The two different types of vitamins are fat soluble vitamins and water soluble vitamins. Fat soluble vitamins such as A, D, E and K will dissolve in fat before they are absorbed in the bloodstream to carry out their functions.[2] Vitamin A palmitate or Retinyl palmitate is viscous, yellow oil at room temperature that play an essential role in metabolic functioning of the retina, the growth and differentiation of epithelial tissue, the growth of bone, reproduction and the immune response. Healthful sources of vitamin A include dark green leafy vegetables, green stem vegetables, yellow or orange fruits and vegetables.[3] Male adults need 900 µg/d and female adults need 700 µg/d requirement of vitamin A per day.[4] Vitamin A is highly sensitive to oxidation and when oxidized, it loses its valuable therapeutic and prophylactic properties.[5] The principal reason for vitamin A degradation is the oxidation process. The chemical nature of retinoids consisting of polyunsaturated polar lipids, makes them able to interact with oxygen and light to produce reactive oxygen species and free radicals.[6]

Several studies has reported that vitamin A is not stable in multivitamin syrup formulations. In this work, three different trial batches V1, V2 and V3 of Vitamin A palmitate syrups were formulated. Formulation V1 contains Vitamin A palmitate without any antioxidants, stabilizers and buffering agents. Formulation V2 contains Vitamin A palmitate with antioxidants, stabilizers and buffering agents. Formulation V3 is the Multivitamin syrup consisting of Vitamin A palmitate, vitamin D and E.

MATERIALS AND METHODS

Materials

Vitamin A Palmitate was obtained from Piramal Healthcare Ltd., Chennai. Vitamin D3 was procured from Fermenta Biotech Pvt. Ltd., Himachal Pradesh. Vitamin E acetate was purchased from BASF Chemicals Company, Germany. Vitamin C was obtained from HeBei Welcome Pharmaceutical Co., Ltd., China. Sucrose was obtained from E.I.D. Parry, Cuddalore. Disodium edetate was procured from Canton Laboratories Pvt. Ltd., Vadodara. All other chemicals used in the study were of analytical grades.

Methods

PREFORMULATION STUDIES

Description

It is the initial evaluation during preformulation study which assess the colour and taste of the substance. The colour and taste of Vitamin A palmitate was evaluated visually.

Solubility

Solubility was determined as per IP specification. Vitamin A palmitate was dissolved in methanol by using Ultrasonicator.

Drug-excipients Compatibility Studies

Drug-excipients compatibility studies were performed by preparing Drug-excipient blends with different excipients. The blends were kept in the room temperature. The ratio of drug-excipients blend was 1:1. Samples were kept in watch glasses. The samples were evaluated for any changes in the physical characterization with references to its controlled sample kept in room temperature for a period of 30 days.[7]

FORMULATION OF VITAMIN A PALMITATE SYRUPS

Three trial formulations (V1, V2 and V3) containing Vitamin A palmitate syrups were developed with 100% overage. The formulation V1 consists of only Vitamin A palmitate without stabilizers, antioxidants and buffering agents. The formulation V2 consists of Vitamin A palmitate with stabilizers, antioxidants and buffering agents. The formulation V3 consists of Vitamin A palmitate along with vitamin D, E, stabilizers, antioxidants and buffering agents.

Formulation of Vitamin A Palmitate Syrup (V2)

Dissolve sucrose in boiling purified water under constant stirring. Dissolve separately sodium benzoate, disodium edetate, citric acid, sodium citrate, ascorbic acid and saccharin sodium in purified water. Disperse xanthan gum in propylene glycol and add to the syrup under constant stirring. Add glycerin and sorbitol to the syrup. Separately warm polysorbate 80 and dissolve butylated hydroxy toluene and vitamin A palmitate. Then add orange flavor to the syrup. Make up the volume using purified water. Filter the syrup using 200 mesh nylon cloth and check the pH of syrup (pH limit: 4.0 - 5.5).

Procedure of formulation V1 is same as formulation V2 except addition of ascorbic acid, disodium edetate, citric acid, sodium citrate and butylated hydroxy toluene. Procedure of formulation V3 is same as formulation V2 along with the addition of Vitamin D3 and Vitamin E acetate.[8] The formulation of Vitamin A palmitate syrups were presented in table no. 1.

Table No. 1: Formulation of Vitamin A Palmitate Syrups

Syrup Formulations For 1000ml				
S. No.	Materials	Formulation Code		
		V1* (%)	V2* (%)	V3* (%)
1	Vitamin A palmitate	0.0176	0.0176	0.0176
2	Vitamin D_3	~	~	0.0002
3	Vitamin E acetate	~	~	0.15
4	Ascorbic acid	~	0.25	0.25
5	Sucrose	25	25	25
6	Sodium benzoate	0.2	0.2	0.2
7	Disodium edetate	~	0.01	0.01
8	Citric acid	~	0.02	0.02
9	Sodium citrate	~	0.1	0.1
10	Glycerin	3	3	3
11	Sorbitol 70% solution	3	3	3
12	Xanthan gum	0.2	0.2	0.2
13	Propylene glycol	5	5	5
14	Polysorbate 80	1	1	1
15	Butylated hydroxy toluene	~	0.001	0.001
16	Saccharin sodium	0.05	0.05	0.05
17	Orange flavor	0.8	0.8	0.8
18	Purified water	QS	QS	QS

***V1-Syrup containing Vitamin A palmitate without stabilizers, antioxidants and buffering agents.**

***V2- Syrup containing Vitamin A palmitate with stabilizers, antioxidants and buffering agents.**

***V3-Syrup containing Vitamin A, with Vitamin D, E, stabilizers, antioxidants and buffering agents.**

EVALUATION OF VITAMIN A PALMITATE SYRUP

The evaluation of syrup such as General parameters (Appearance, colour and taste), Physical parameters (pH, Wt/ml and Viscosity) and Chemical parameter (Assay of Vitamin A palmitate) was carried out. All the three formulations were filled in two separate 100ml amber colored PET bottles and placed in stability chamber maintained at 30±2°C/65±5%RH and at 40±2°C/75±5% RH for 2 months and test were carried out at every 1 month interval.

1. General Parameters

The formulated syrups were stored at 30±2°C/65±5% RH and 40±2°C/75±5% RH for 2 months. The appearance, colour and taste of the syrups were evaluated visually at every 1 month interval.

2. Physical Parameters

All the formulated syrups were stored at 30±2°C/65±5% RH and 40±2°C/75±5% RH for 2 months. The pH, wt/ml and viscosity of the syrups were evaluated at every 1 month interval.

Determination of pH

The determination of pH was carried out by immersing the electrodes in the formulated syrups using Digital pH meter.

Determination of Weight/milliliter

The weight/milliliter of a liquid is the weight, in gram, of 1ml of liquid when weighed in air at 25°C, unless otherwise specified. A pre weighed volumetric flask was taken and the syrup was added up to the mark. The net volume was noted. Then the volumetric flask was weighed and weight/milliliter was calculated accordingly.

Determination of Viscosity

Viscosity is measured using Ostwald-type viscometer. Fill the viscometer, previously washed and completely dried, with the liquid under examination through tube L to slightly above the mark G, using a long pipette. Place the tube vertically in a water-bath maintained at the temperature indicated in the monograph and allow to stand for not less than 30 min to allow the temperature to reach equilibrium. Adjust the volume of the liquid so that the bottom of the meniscus settles at the mark G. Suck or blow the liquid to a point above 5 mm above the mark E. After releasing pressure or suction, measure the time taken for the bottom of the meniscus to fall from the top edge of mark E to the top edge of mark F.[9]

3. Chemical Parameter

This includes assay of Vitamin A palmitate by UV-VIS spectrophotometer at 325 nm.

Accurately weighed quantity of vitamin A palmitate was suitably diluted in methanol in amber colored standard measuring flask and make up to the volume using methanol. Then filtered through Whatman filter paper (Standard). Accurately weighed quantity of formulated syrup was suitably diluted in methanol in amber colored standard measuring flask and make up the volume to the mark. (Sample). The assay of Vitamin A palmitate was calculated using the formula:

$$\text{Percentage Assay} = \frac{\text{Drug content}}{\text{Label claim}} \times 100$$

RESULTS AND DISCUSSION

1. Preformulation Studies

Organoleptic Properties

The colour of Vitamin A palmitate was found to be yellow. Vitamin A palmitate showed oily taste. Vitamin A palmitate showed similar colour and taste as per the specification.

Solubility Test

The solubility study revealed that Vitamin A palmitate was soluble in methanol (1 in 10 parts).

Drug-Excipients Compatibility Studies

The drug-excipients compatibility study revealed that there was no change or interaction between drug and excipients on storage at room temperature for 30 days. Thus it was concluded that the excipients selected for the formulation were compatible with vitamin A palmitate and suitable for formulation development. The results are shown in table no. 2.

Table No. 2: Drug (Vitamin A palmitate) – Excipients Compatibility Study

S.No.	Composition	Condition: Room Temperature	
		Initial Period	After 30 Days
1	Vitamin A palmitate	Yellow colour viscous liquid	NCC
2	Vitamin A palmitate + Sucrose	Yellow colour oily liquid with white solids	NCC
3	Vitamin A palmitate + Sodium benzoate	Yellow colour oily liquid with white solids	NCC
4	Vitamin A palmitate + Disodium edetate	Yellow colour insoluble Precipitates	NCC
5	Vitamin A palmitate + Citric acid	Yellow colour oily liquid with white solids	NCC
6	Vitamin A palmitate + Sodium citrate	Yellow colour oily liquid with white solids	NCC
7	Vitamin A palmitate + Glycerin	Soluble yellow colour viscous Liquid	NCC
8	Vitamin A palmitate + Ascorbic acid	Yellow colour oily liquid with white solids	NCC
9	Vitamin A palmitate + Sorbitol 70% solution	Soluble yellow colour viscous Liquid	NCC
10	Vitamin A palmitate + Xanthan gum	Yellow colour oily liquid with white solids	NCC
11	Vitamin A palmitate + Propylene glycol	Soluble yellow colour viscous Liquid	NCC
12	Vitamin A palmitate + Polysorbate 80	Soluble yellow colour viscous liquid	NCC
13	Vitamin A palmitate +	Yellow colour oily liquid with	NCC

	Butylated hydroxyl Toluene	white solids	
14	Vitamin A palmitate + Vitamin D3	Soluble yellow colour viscous Liquid	NCC
15	Vitamin A palmitate + Vitamin E acetate	Soluble yellow colour viscous Liquid	NCC
16	Vitamin A palmitate + Saccharin sodium	Yellow colour oily liquid with white solids	NCC
17	Vitamin A palmitate +Orange flavor	Soluble yellow colour viscous Liquid	NCC
18	Vitamin A palmitate + Purified water	Insoluble yellow colour oily drops	NCC

* NCC – No Characteristic Change.

2. Evaluation of Vitamin A Palmitate Syrup

General Parameters

There was no change observed in the appearance, colour and taste in formulation V2 and V3 after 1st and 2nd month even after stored at 30±2°C/65±5% RH and 40±2°C/75±5% RH. But formulation V1 showed change of colour from pale yellow to colorless when stored at 30±2°C/65%±5% RH and 40±2°C/75±5% RH. This may be due to the absence of stabilizers, antioxidants and buffering agents in formulation V1 which affected its stability. The results of general parameters of formulated syrups are shown in table no. 3.

Table No. 3: Stability Report of Syrups Containing Vitamin A Palmitate Stored at 30±2°C/65±5% RH and 40±2°C/75±5% RH (General Parameters)

Formulation Code	Parameters	Initial Period	30±2°C/65±5% RH		40±2°C/75±5% RH	
			1st Month	2nd Month	1st Month	2nd Month
V1	Appearance	Clear viscous liquid	NCC	NCC	NCC	NCC
	Colour	Pale yellow	Colourless	Colourless	Colourless	Colourless
	Taste	Sweet oily taste	NCC	NCC	NCC	NCC
V2	Appearance	Clear viscous liquid	NCC	NCC	NCC	NCC
	Colour	Pale yellow	NCC	NCC	NCC	NCC
	Taste	Sweet oily taste	NCC	NCC	NCC	NCC
V3	Appearance	Clear viscous liquid	NCC	NCC	NCC	NCC
	Colour	Pale yellow	NCC	NCC	NCC	NCC
	Taste	Sweet oily taste	NCC	NCC	NCC	NCC

*NCC – No Characteristic Change.

Physical Parameters

In Physical parameters (pH, wt/ml and viscosity) evaluation the pH of formulation V1 changed after 1st and 2nd month when stored at 30±2°C/65±5% RH and 40±2°C/75±5% RH as this syrup does not contain buffering agent. In formulation V2 and V3 all the parameters

were within the limit and both the formulation were stable at 30±2°C/65±5% RH and 40±2°C/75±5% RH. The results of physical parameters of syrup are shown in table no. 4.

Table No. 4: Stability Report of Syrups Containing Vitamin A Palmitate Stored at 30±2°C/65±5% RH and 40±2°C/75±5% RH (Physical Parameters)

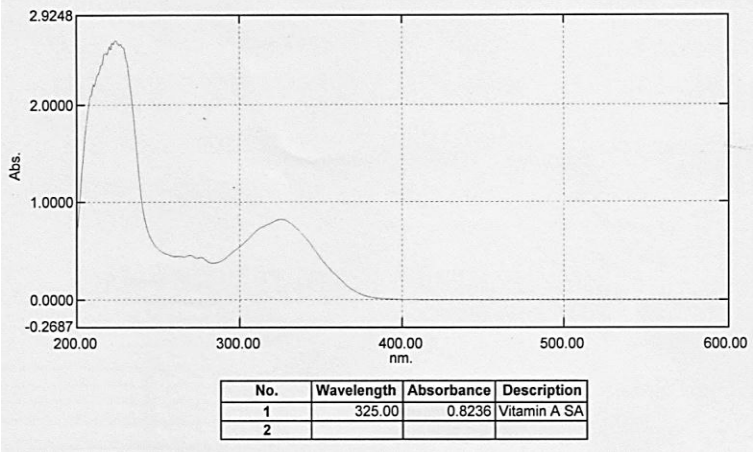
Formulation Code	Parameters	Limit	Initial Period	30±2°C/65±5%RH		40±2°C/75±5%RH	
				1st Month	2nd Month	1st Month	2nd Month
V1	pH	4.0 - 5.5	6.47	5.95	5.89	6.04	6.01
	Wt/ml	1.0 - 1.25 g/ml	1.107 g/ml	1.105 g/ml	1.102 g/ml	1.099 g/ml	1.097 g/ml
	Viscosity	18 - 30 mm^2s^{-1}	19.66 mm^2s^{-1}	19.54 mm^2s^{-1}	19.50 mm^2s^{-1}	19.59 mm^2s^{-1}	19.56 mm^2s^{-1}
V2	pH	4.0 - 5.5	4.51	4.51	4.50	4.51	4.50
	Wt/ml	1.0 - 1.25 2.0 g/ml	1.110 g/ml	1.108 g/ml	1.107 g/ml	1.110 g/ml	1.109 g/ml
	Viscosity	18 - 30 mm^2s^{-1}	27.67 mm^2s^{-1}	27.62 mm^2s^{-1}	27.56 mm^2s^{-1}	27.33 mm^2s^{-1}	27.33 mm^2s^{-1}
V3	pH	4.0 - 5.5	4.51	4.51	4.50	4.51	4.50
	Wt/ml	1.0 - 1.25 g/ml	1.104 g/ml	1.102 g/ml	1.101 g/ml	1.097 g/ml	1.086 g/ml
	Viscosity	18 - 30 mm^2s^{-1}	18.66 mm^2s^{-1}	18.61 mm^2s^{-1}	18.59 mm^2s^{-1}	18.33 mm^2s^{-1}	18.31 mm^2s^{-1}

Chemical Parameter

In Chemical parameter evaluation it was observed that the drug content of formulation V1 decreased when stored at both 30±2°C/65±5% RH and 40±2°C/75±5% RH for 2 months. The reason for decline in drug content in formulation V1 may be that the syrup was formulated without antioxidants, stabilizers and buffering agents. The drug content of formulation V2 and V3 were found to be stable at 30±2°C/65±5% RH and 40±2°C/75±5% RH. The results were presented in table no. 5 and the UV spectrum of Vitamin A palmitate syrups are shown in fig. 1, 2 and 3.

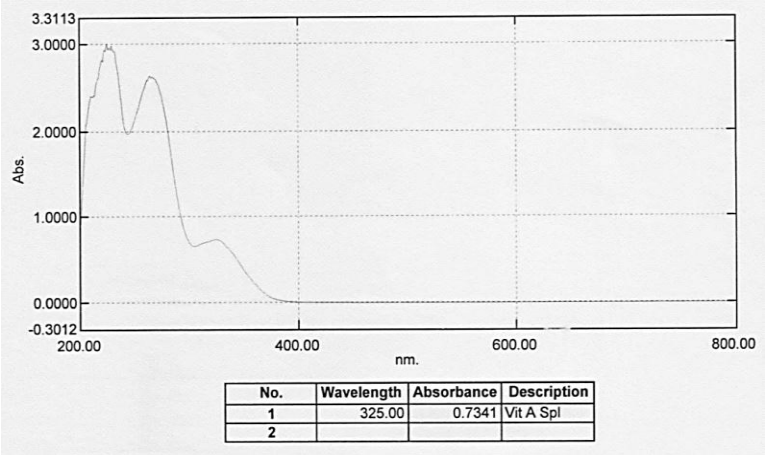
Table No. 5: Stability Report of Syrups Containing Vitamin A Palmitate Stored at 30±2°C/65±5% RH and 40±2°C/75±5% RH (Chemical Parameter)

Formulation Code	Parameter	Initial Period	30±2°C/65±5%RH		40±2°C/75±5%RH	
			1st Month	2nd Month	1st Month	2nd Month
V1	Drug content (%)	197.93	177.84	147.45	160.67	136.69
V2		197.90	195.88	195.16	190.56	189.56
V3		198.94	196.76	195.64	194.87	191.91



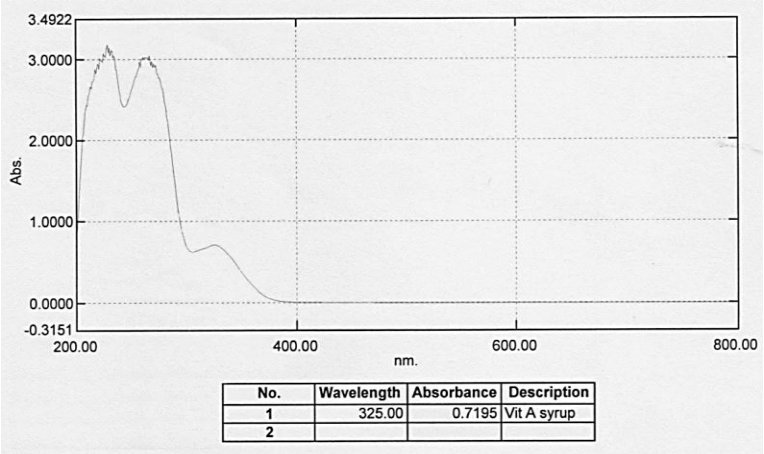
No.	Wavelength	Absorbance	Description
1	325.00	0.8236	Vitamin A SA
2			

Fig. 1: UV Spectrum of Vitamin A Palmitate Syrup (Formulation V1)



No.	Wavelength	Absorbance	Description
1	325.00	0.7341	Vit A Spl
2			

Fig. 2: UV Spectrum of Vitamin A Palmitate Syrup (Formulation V2)



No.	Wavelength	Absorbance	Description
1	325.00	0.7195	Vit A syrup
2			

Fig. 3: UV Spectrum of Vitamin A Palmitate Syrup (Formulation V3)

CONCLUSION

From the results, it can be concluded that the stability report of the formulation V1 was not good. The absence of stabilizers, antioxidants and buffering agents in the formulation V1

could be a reason behind the decline in drug content of Vitamin A palmitate. Whereas, the formulation V2 showed good stability of Vitamin A palmitate as it contains stabilizers, antioxidants and buffering agents. Formulation V2 also showed good stability in terms of General, Physical and Chemical parameters and was selected as optimized formulation. Multivitamins (Vitamin D and E) were incorporated in the optimized formulation and the formulation was termed as V3. The stability of formulation V3 was evaluated in terms of General, Physical and Chemical parameters. The stability report of formulation V3 revealed that Vitamin A palmitate was stable even in presence of Vitamin E and Vitamin D (Multivitamins).

The study concludes that stable formulation of multivitamin syrup with Vitamin A palmitate (V3) could be successfully developed and the stability results showed that the developed formulations were found to be stable in terms of General, Physical and Chemical parameters evaluated at 30±2°C/65±5% RH and 40±2°C/75±5% RH for 2 months.

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