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Research Article

Development of analytical and stability Testing method for Vitamin-A palmitate formulation

M. S. Charde*, M. M. Shinde, A. S. Welankiwar and Jitendra K.

Government College of Pharmacy, Kathora Naka, Amravati (M.S.) India – 444604

Abstract

The present study deals with the stability testing of formulation of Vitamin A palmitate. The aim of this study is to provide the evidences on how the quality of Vitamin A palmitate varies with the time under the influence of variety of environmental factors like temperature, humidity, light, etc. this study is divided into 2 phases. First phase is the method development for VAP on RP-HPLC. In this phase mobile phase selected was methanol 100% (HPLC grade) with detection wavelength of 326 nm. The method shows the good linearity in the range of 25-125 µg/ml. Once after the method development method validation was carried out as per the ICH Guidelines. Average percentage recoveries were found to be 98.17%, 98.37, and 98.14%. The %RSD for precision studies was found to be 0.393, 0.719. After that phase of stability testing comes which is divided in two parts first is stress testing which was carried out in different medias like Acid, base, oxidative, UV degradation, Degradation in sun light. Then next phase is of accelerated stability testing in which product is stored at different elevated temperatures at interval of 0, 1, 3, 6 months it was sampled and analyzed by the developed method. The Shelf life was then determined by Arrhenius equations and by Q10 method which was found to be 28 months.

Keywords: Accelerated, Stress testing, Activation energy, Arrhenius equations, Q10, VAP, Reaction rate constants.

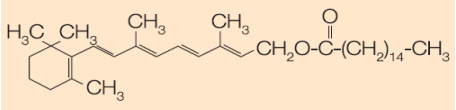
1. Introduction

Stability testing of pharmaceutical products is a complex set of procedures involving considerable cost, time consumption and scientific expertise in order to build in quality, efficacy and safety in a drug formulation. Scientific and commercial success of a pharmaceutical product can only be ensured with the understanding of the drug development process and the various tasks and milestones that are vital to a comprehensive development plan.[1] The most important steps during the developmental stages include pharmaceutical analysis and stability studies that are required to determine and assure the identity, potency and purity of ingredients, as well as those of the formulated products. Stability of a pharmaceutical product may be defined as the capability of a particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, toxicological, protective and informational specifications.[2] In other words, it is the extent to which a product retains, within the specified limits, throughout its period of storage and use, the same properties and characteristics possessed at the time of its packaging. Stability testing thus evaluates the effect of environmental factors on the quality of the a drug substance or a formulated product which is utilized for prediction of its shelf life, determine proper storage conditions and suggest labeling instructions.[3] Moreover, the data generated during the stability testing is an important requirement for regulatory approval of any drug or formulation. Stability testing is a routine procedure performed on drug substances and products and is employed at various stages of the product development. The stability testing is of 4 types[4].

- **Real-Time stability testing** Real time stability testing is normally performed for longer duration of the test period in order to allow significant product degradation under recommended storage conditions.
- **Accelerated stability testing** In accelerated stability testing, a product is stressed at several high (warmer than ambient) temperatures and the amount of heat input required to cause product failure is determined. This is done to subject the product to a condition that accelerates degradation. This information is then projected to predict shelf life or used to compare the relative stability of alternative formulations.
- **Retained sample stability testing** This is a usual practice for every marketed product for which stability data are required. In this study, stability samples, for retained storage for at least one batch a year are selected. If the number of batches marketed exceeds 50, stability samples from two batches are recommended to be taken.
- **Cyclic temperature stress testing** This is not a routine testing method for marketed products. In this method, cyclic temperature stress tests are designed on knowledge of the product so as to mimic likely conditions in market place storage. The period of cycle mostly considered is 24 hours since the diurnal rhythm on earth is 24 hour.

The review of literature[7-15] has suggested that very few works on stability evaluation of the vitamin A palmitate has been done and because of the stability profile of vitamins so this work was undertaken with 2 objectives first is to develop economical, simple, accurate, precise and reproducible stability indicating RP-HPLC analytical method for estimation of vitamin A palmitate in its combined dosage form and to generate a complete accelerate stability profile. Vitamin A palmitate figure 1 chemically is [(2E,4E,6E,8E)-3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexenyl)nona-2,4,6,8-tetraenyl] hexadecanoate. It is Retinyl palmitate, all-trans retinol palmitic acid ester and it is viscous yellow oil at room temperature. It is used in Nutritional Supplement, Improving vision, Enhancing the function of the immune system, Preventing and treating cancer, preventing lung problems in premature infants, treating various skin conditions, Sunburns. It is soluble in methanol, hydrocarbons, ethers, fats, oils but it is insoluble in water and solvent containing peroxides.

Figure 1: Chemical Structure of Vitamin A Palmitate.



* Correspondence Inf0

M. S. Charde
Government College of Pharmacy, Kathora Naka,
Amravati (M.S.) India – 444604
Email: manojudps@rediffmail.com

2. Experimental

Reagents and Chemicals: The standard sample of Vitamin A Palmitate was received as gift sample from Piramal enterprizes Ltd Mahad (Mumbai). The marketed formulation VAP (Piramal enterpizes) was purchased from local market. All reagents and chemicals used were of HPLC grade. The chemical used was Isopropanol**,** Methanol, Water, Glacial Acetic acid.

Instrument: Waters HPLC 2487 Dual λ absorbance, having lichrosphere (100 RP, 18e, 25cm x 4.6mm 5 um) column with particle size 5µm was used for the study. An isocratic elution was performed using Methanol 100% (HPLC grade) as mobile phase with detection wavelength of 326 nm at flow rate of 2 ml/min with injection volume of 20 µl at run time of 30 min.

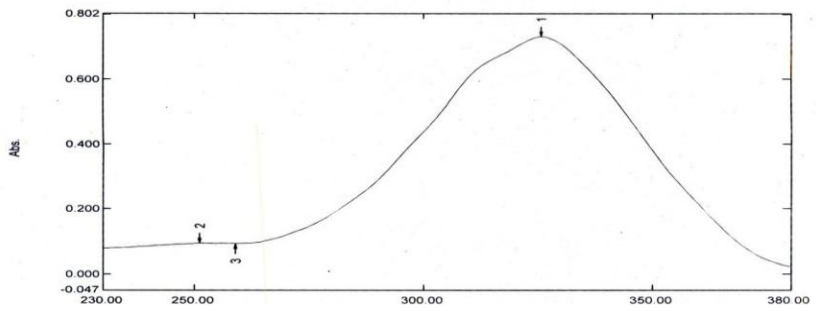
Selection of mobile phase: Initially several mobile phase trials were carried in isocratic elution mode at different flow rate, different solvents (Methanol, Isopropyl alcohol, Glacial acetic acid different pH but most optimized chromatogram was obtained at Methanol 100% (HPLC) grade because at this mobile phase peak shape was good, Retention time 9 min.

VAP standard stock preparation: The stock standard solutions VAP were prepared by dissolving 100.0 mg of drug in 100.0 mL IPA.

VAP working standard solution: The stock standard solution was further diluted with mobile phase i.e 5.0 mL of above solution diluted to 50.0 mL of METH to obtain a concentration of 10 µg/ml of VAP.

Selection of analytical wavelength: From the UV spectrum of the Vitamin A palmitate the wavelength selected was 326 nm.

Figure 2: Determination of λmax of VAP Standard.



Linearity: Aliquot portion of VAP pipette out in 10.0 ml volumetric flasks. The volume was adjusted to the mark with METH to obtain concentrations 80, 90, 100, 110, 120 µg/ml of VAP solution, absorbance were measured at 326 nm. A calibration curve was constructed by plotting absorbance versus concentration. Calibration curve is shown in figure 4.

Method Validation: The developed method was validated as per the ICH guidelines.

1. Accuracy: It was done by recovery study using standard addition method at 80%, 90% and 100% level of VAP and subject to the proposed HPLC method. The percent recovery was then calculated by using following formula

Amount found (mg) =

$$\frac{\text{Sample Area x wt of std (g) x } 1 \text{ x purity x (Y) (Y = 80,100,120)}}{\text{Mean Area of Std x } 100 \text{ x wt of spl(in g) x } 1000}$$

Amount Recovered

% Recovery = Amount found / Amount added x 100

2. Precision: Prepare 6 different sample solutions of the 100 % concentration and measure the absorbance of each solution. Calculate the % assay of each sample and determine the mean, standard deviation and relative standard deviation in the % assay values obtained.

3.Linearity and range: Five different solutions having preparation from of VAP working standard in the range of 80 % ,90%,100 %,110 %, 120 %. Plot a graph of peak response against concentration. Determine the correlation coefficient. Value of correlation coefficient reflects the extent of correlation between the two variables, viz., concentration and the major peaks response.

4. Solution stability: For solution stability of sample to be carried out with precision study by injecting the same sample No. 6 of precision after regular time interval (For e.g. 1,2, hrs.) and calculate cumulative % RSD for assay of VAP with six sample preparations of precision study.

5.Robustness: Robustness of the method was studied by making deliberate variation in parameters such as flow rate (± 0.1mL), % of methanol in the mobile phase composition (± 10%), and change in detection wavelength (±2 nm) and the effect on the results were examined.

6. Specificity: Specificity is defined as the ability to assess the analyte in the presence of components that may be expected to be present such as impurities, degradation products and the matrix components. Prepare solutions as follows,

Placebo Preparation: Weigh accurately about 100.0 mg of vitamin a Pacebo in 100.0 mL volumetric flask.

Dissolve and dilute it with 100.0 mL HPLC grade IPA. Pipette out 5.0 mL of this solution in 50.0mL volumetric flask and dilute with HPLC grade METH.

Spike sample preparation: Weigh accurately about 100.0 mg of VAP working standard and 1.0 mL of placebo in 100 ml volumetric flask Dissolve and dilute it with 100.0 mL HPLC grade IPA. Pipette out 5 ml of this solution in 50ml volumetric flask and dilute with HPLC grade METH.

Sample preparation: Weigh accurately about 100.0 mg of Sample in 100.0 mL volumetric flask, dissolve and dilute it with 100.0 mL HPLC grade IPA. Pipette out 5.0 mL of this solution in 50.0 mL volumetric flask and dilute with HPLC grade METH. Measure the Area of the peak of each of these solutions and see if there is interference between the active ingredient and its excipients.

Stability evaluation of VAP: Mainly 2 types of stability testing are performed on the product.

1. Stress testing.
2. Accelerated stability testing.

Stress testing:

1. Acid degradation: Evaluating the Stability of VAP in acidic conditions using HCL is essential, as it is used for fortification purpose and fortification process includes various acidic conditions to undergoes to form a final product.

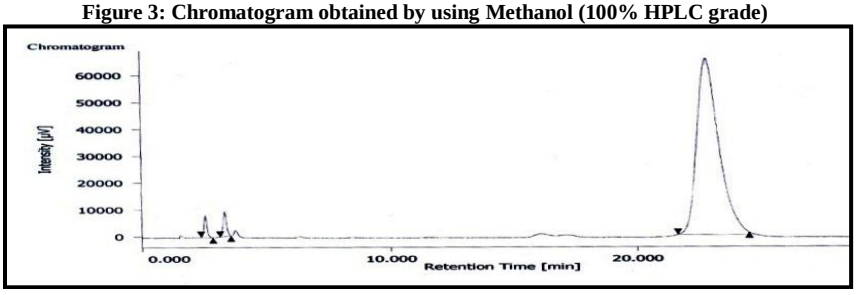
2. Base degradation: The VAP products are degraded with sodium hydroxide having various concentrations and for various times, and results are obtained which shows stability of product in basic conditions.
3. Oxidative degradation: Oxidative degradation study is very important the vitamins are sensitive to oxidation, and stability of VAP is derived mainly by its capability to prevent oxidation of vitamin A, Oxidation studies are performed by taking different concentrations of hydrogen peroxide (H_2O_2).
4. Degradation by different pH solutions: Evaluating stability of VAP at different pH solutions was necessary as VAP in solution form and stability testing at different pH is indicates its behavior in different pH conditions.
5.Degradation study in presence of UV light: Evaluate the stability in presence of UV light is necessary, as it is notified that the vitamin A rapidly degraded in presence of UV light, hence it is necessary to test the stability of VAP in UV light to check its possible degradation. The UV light source used for study is according to ICH guidelines.
6. Degradation in sunlight: The degradation study of VAP in presence of sunlight is necessary, as such products may expose to sunlight during transportation or in any accidental conditions. The degradation study performed by exposing the samples at daylight for different periods and stability of sample evaluated.
Accelerated stability testing: The product is intended to store at refrigerator, so as per guidelines the accelerated stability study performed at 25°C ± 2°C/60% RH ± 5% RH for 6 months. The testing of the samples are performed on the HPLC instrument and concentration of active ingredient calculate by standard assay methods.
The testing intervals for samples are as following:
1) Initial.
2) After 1 month point time.
3) After 3 month point time.
4) After 6 month point time.

3. Results and Discussion

HPLC method development and Optimization: Initially several mobile phase trials were undertaken at different flow rate and pH values. The mobile phase was selected on the basis of best separation, peak purity index, peak symmetry, theoretical plate etc. After number of trials Methanol 100% (HPLC grade) The selection of analytical wavelength was truly based on the overlain spectra of VAP in IPA as solvent as decipted in Figure 2. So the final optimized chromatographic condition is

Column : Lichrosphere 100 RP, 18e, 25cm x 4.6mm
Partical size packing : 05 µm
Stationary phases : C18
Mobile phase : Methanol (HPLC grade)
Detection wavelength : 326nm
Flow rate : 2 ml/min.
Sample size : 20 µL

Figure 3: Chromatogram obtained by using Methanol (100% HPLC grade)

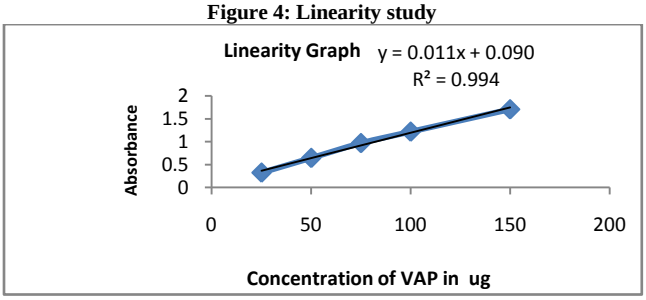


Linearity:

Table No 1: Linearity data of VAP In IPA

Concentration	Absorbance at Wavelengths (326nm)
25	0.323
50	0.645
75	0.97
100	1.22
125	1.71

Figure 4: Linearity study



Method Validation:

1. Accuracy: Determined on the basis of recovery studies at 80, 90, 100 % of levels of VAP and subject to proposed HPLC method.

Table No 2: Accuracy study data for VAP

Sr. No.	% of Conc.	Wt. of drug added in (mg)	Area of peak	Recovered Drug (in mg)	% Recovery	Mean % recovery
1	80	4.05	3228631	3.97	98.04	**98.18**
2	80	4.02	3238971	3.93	98.25	
3	80	4.06	3259647	3.99	98.27	
4	90	4.56	3632470	4.47	98.02	**98.37**
5	90	4.50	3628765	4.41	98.00	
6	90	4.52	3648965	4.48	99.11	
7	100	5.02	4024869	4.92	98.00	**98.14**
8	100	5.08	4023968	4.98	98.03	
9	100	5.01	4326859	4.93	98.40	
				Mean		**98.23**
			RSD (NMT 2.0%)			**0.125**

2. Precision:

Table No 3: Study of Precision.

Samples	R. T.	Area of principal peak	Wt. of sample	% Assay
Sample 1	22.82	3986535	101	98.84454
Sample 2	22.68	3952683	103	98.99514
Sample 3	22.78	3968593	104	100.3875
Sample 4	22.58	3968956	102	99.4027
Sample 5	22.44	3992462	100	101.9912
Sample 6	22.88	3957851	105	97.14208
Mean	22.67	3971180	-----	99.95
SD	0.1646	15621.3927	-----	1.627
% of RSD	0.733	0.393	-----	**1.627**

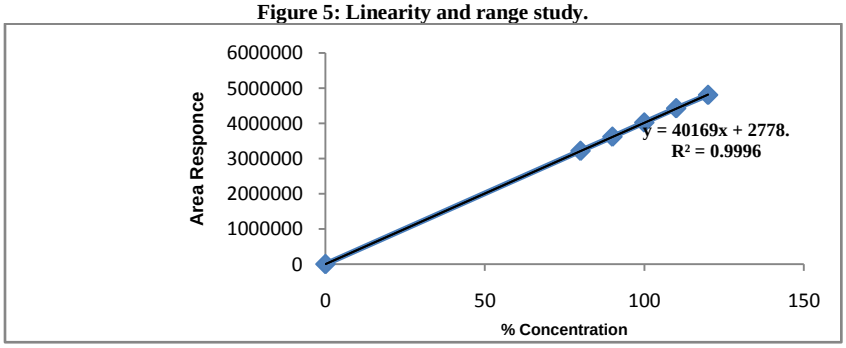
3. Linearity and Range:

This experiment is carried out to demonstrate the linearity of responses of major peaks i .e area of the major peaks of five different solutions having concentrations of VAP in the range 80 % to 120 %.

Table No 4: Linearity and Range study.

Samples	R.T.	Area of principal peak	Theoretical plate
Standard 1	22.46	3998624	3642
Standard 2	22.15	3978963	3574
Standard 3	22.00	3969751	3542
Standard 4	22.98	3924899	3489
Standard 5	22.70	3919714	3411
Mean	22.35	3958390	NA
SD	0.178	34599.77	NA
% of RSD	**0.796**	**0.8740**	NA

Figure 5: Linearity and range study.



4. Solution stability:

Table No 5: Solution stability study

Samples	R.T.	Area of principal peak	Wt. of sample	% Assay
Solution stability sample	22.58	3998964	100	99.68057
Precision 1	22.46	3924899	101	96.85603
Precision 2	22.15	3919714	100	97.70513
Precision 3	22.00	3958390	103	95.70912
Precision 4	22.98	3998624	102	97.67865
Precision 5	22.70	3978963	100	99.18201
Precision 6*	22.48	3969751	102	96.97334
Mean	-------	-------	-------	97.68
SD	-------	-------	-------	1.37
% of RSD of Assay	-------	-------	-------	**1.40**

5. Robustness: Change in Flow rate From 2.0 ml to 2.2 ml/min

Table no 5: study of Robustness

Samples	R.T.	Area of principal peak	Wt. of sample	% Assay
Robustness 1	22.75	3998625	103	98.67
Robustness 2	22.68	3986456	102	99.36
Robustness 3	22.58	3996845	103	98.63
Mean	-----	------	-----	98.88
SD	-----	-----	-----	0.4104
% of RSD	-----	------	-----	**0.41**

Table no 6: study of robustness

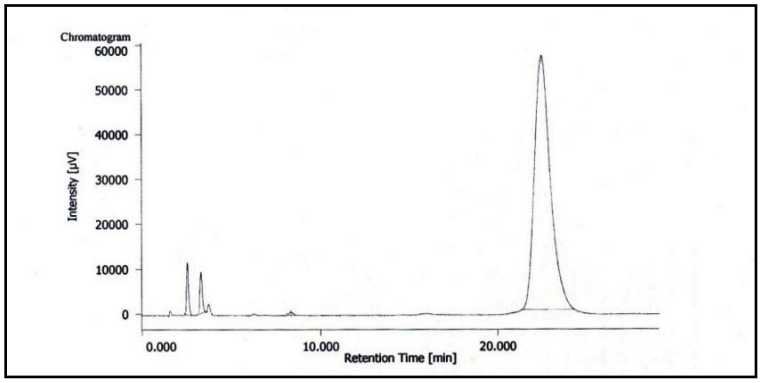
Samples	R. T.	Area of principal peak	Wt. of sample	% Assay
Robustness 1	22.89	3998624	102	99.67
Robustness 2	22.78	3978963	105	96.20
Robustness 3	22.85	3969751	104	96.97
Precision 1	22.46	3924899	101	98.71
Precision 2	22.15	3919714	102	97.70
Precision 3	22.00	3958390	106	94.72
Precision 4	22.98	3998624	102	99.67
Precision 5	22.70	3978963	104	97.19
Precision 6	22.48	3969751	103	97.96
Mean	22.58	3966409	-----	97.64
SD	0.3406	28242.18	-----	1.610
% of RSD	1.50	0.712	-----	**1.6**

6. Specificity:

Table no 6: study of specificity parameters

Samples	R. T.	Area of principal peak	Theoretical plate
Standard 1	22.80	4023005	**3363**
Standard 2	22.83	4025968	3868
Standard 3	22.91	4023896	3465
Standard 4	22.78	4026899	3865
Standard 5	22.69	4021147	**3156**
Mean	22.802	4024183	NA
SD	0.071386	2304.899	NA
% of RSD	**0.31307**	**0.5727**	NA
SNo	Product	Wt taken (in mg)	Retention time at 326 nm
1	a) Blank	------	------
2	b) Placebo	101	1.1, 13.2, 16.1
3	c)Standard	103	22.65
4	e)spike sample		22.66
	a) Std	102	
	b) Placebo	104	1.1,13.2,16.1
5	f) Sample	103	22.80

Figure 6: chromatogram for specificity parameter

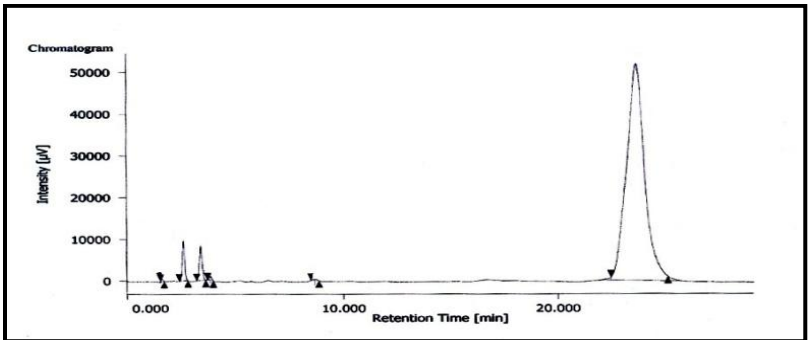


Stability Evaluation of VAP:
Stress testing:
1. Acid degradation:

Table No 7: Results of Acid degradation

Condition	R.T.	Area	% Assay
Standard sample	**22.54**	**3998759**	
Initial sample	**22.68**	**3996578**	**99.99**
0.1 N Hcl acid for 24 hrs	22.57	3268945	81.79
0.1 N Hcl acid for 1 week	22.66	2210107	77.30

Figure 7: Chromatogram of Acid degradation.

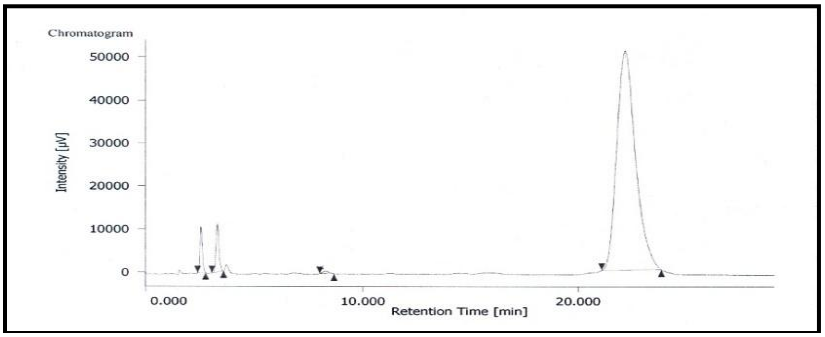


2. Base degradation:

Table No 8: Results of base degradation.

Condition	R.T.	Area	% Assay
Standard sample	22.54	3998759	
Initial sample	22.68	3996578	99.99
0.1 N NaOH for 24 hrs	22.72	3618759	90.54
0.1 N NaOH for 1 week	22.48	3168596	79.20

Figure 8: Chromatogram of base degradation

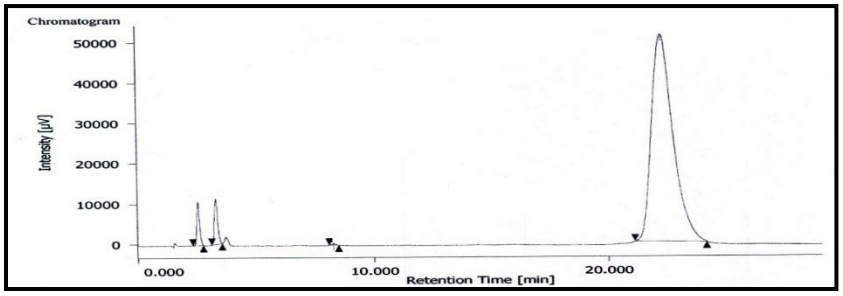


3. Oxidative degradation:

Table No 9: Results of oxidative degradation

Condition	R.T.	Area	% Assay
Standard sample	22.54	3998759	-
Initial sample	22.68	3996578	99.99
3% H_2O_2 for 24 hrs	22.57	3514191	87.93
3% H_2O_2 for 1 week	22.66	3294139	82.42

Figure 9: Chromatogram of oxidative degradation.



4. Degradation by different pH solutions:

Table No 10: Results of pH degradation studies

Condition	R.T.	Area	% Assay
Standard sample	22.54	3998759	
Initial sample	22.68	3996578	99.99
pH 4 for 1 week	22.57	1918357	47.99
pH 7 for 1 week	22.66	3865986	96.73
pH 9 for 1 week	22.48	2835896	70.95

Figure 10: Chromatogram obtained from degradation in pH 4.

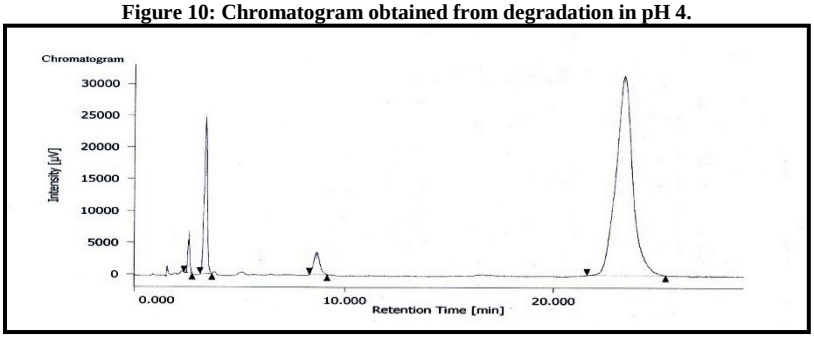


Figure 11: Chromatogram obtained from degradation in pH 7

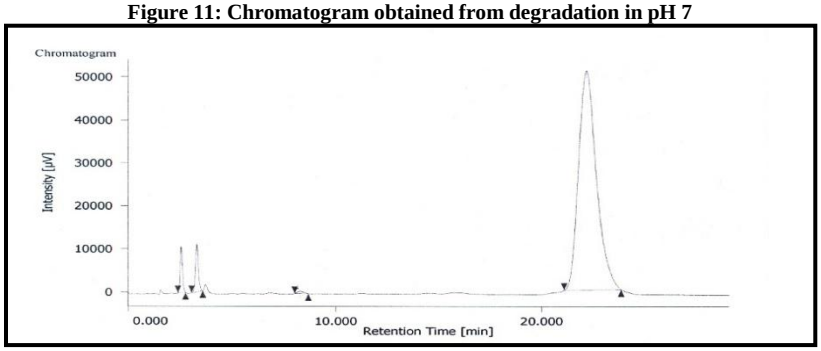
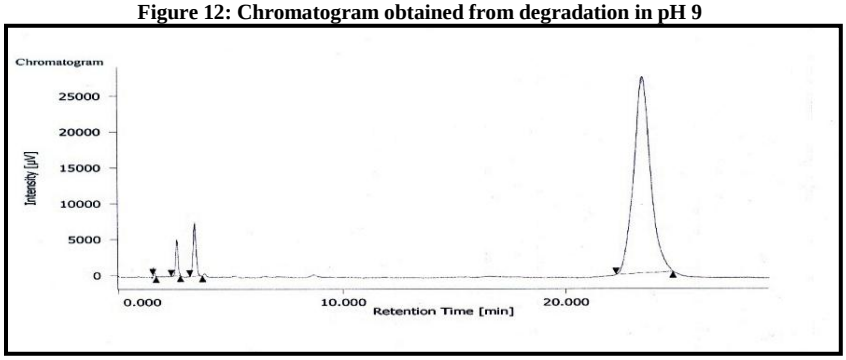


Figure 12: Chromatogram obtained from degradation in pH 9

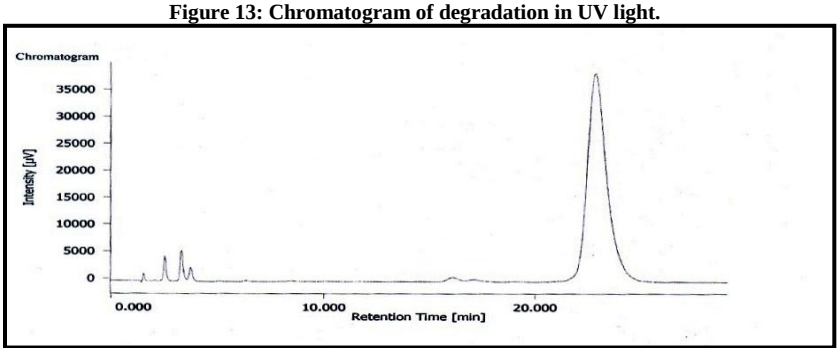


5. Degradation in Presence of UV light:

Table No 11: Results of degradation in UV light.

Condition	R.T.	Area	% Assay
Standard sample	22.54	3998759	
Initial sample	22.68	3996578	99.99
UV light for 24 hrs	22.57	2917360	72.99
UV light for 1 week	22.66	2270068	66.83

Figure 13: Chromatogram of degradation in UV light.

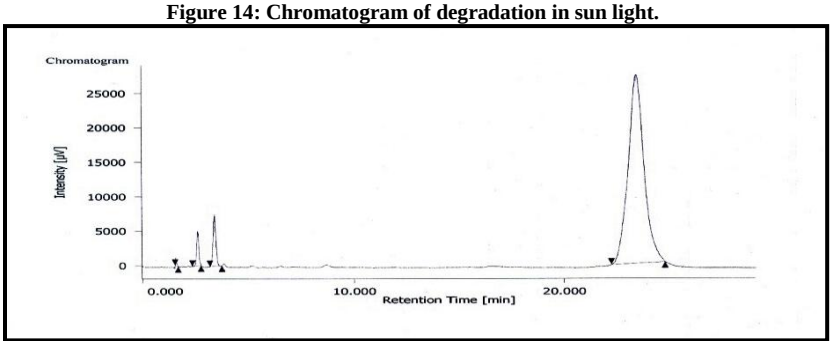


6. Degradation in sun light:

Table No 12: Results of degradation in sun light.

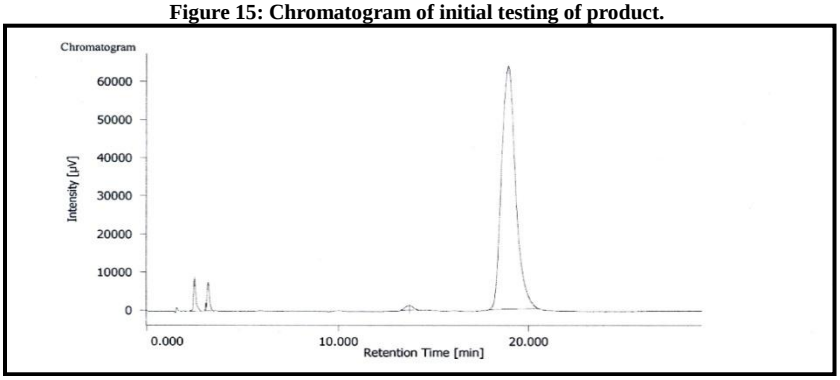
Condition	R.T.	Area	% Assay
Standard sample	22.54	3998759	
Initial sample	22.68	3996578	99.99
Day light for 24 hrs	22.57	3386574	84.73
D light for 1 week	22.66	2210107	77.30

Figure 14: Chromatogram of degradation in sun light.



Accelerated stability testing:

Figure 15: Chromatogram of initial testing of product.



16: Chromatogram obtained from 1 month testing of product.

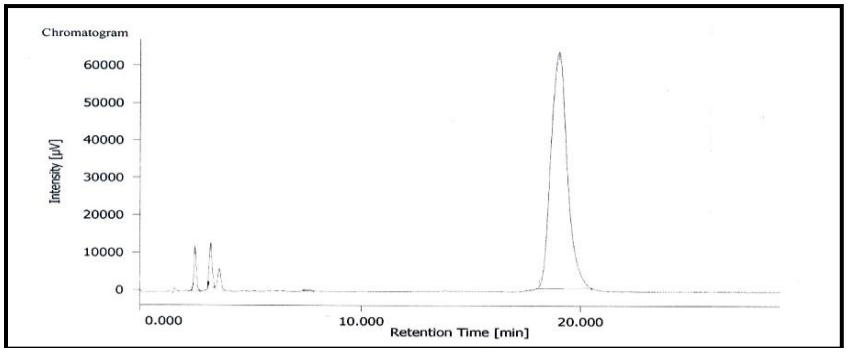


Figure 17: Chromatogram obtained from 3 month testing of product.

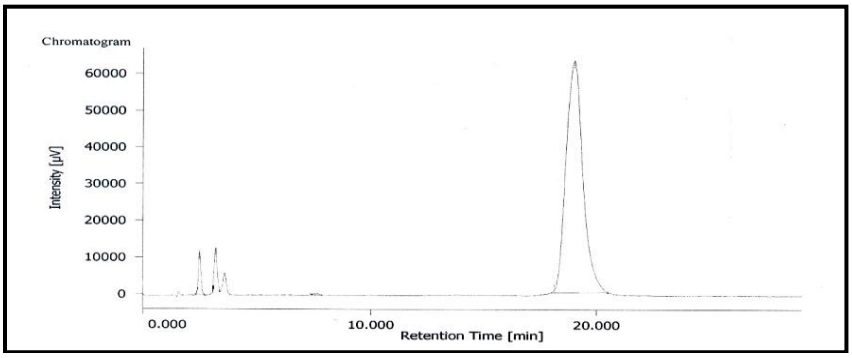


Figure 18: Chromatogram obtained from 6 month testing of product.

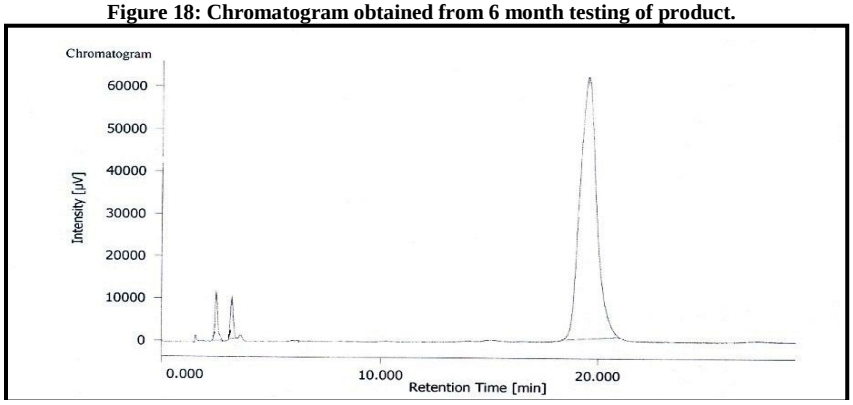


Table No 12: Results of accelerated stability studies.

Parameters checked	Initial	After 1 M	After 3 M	After 6 M
Assay %	99.98	99.95	98.86	97.71
Appearance	Light yellow powder	Light yellow powder	Light yellow powder	Light yellow powder
Acid value	1.265	1.452	1.462	1.501
Peroxide value	8.72	8.79	8.92	8.98

Kinetic study of product:

Determination of Arrhenius equation

From the most of literature survey it was found that vitamin A fallows first order of reaction during storage at elevated temp.

A general reaction rate expression for degradation kinetics can be written

$$-d[C]/dt = k[C]^m \quad \ldots\ldots\ldots\ldots (1)$$

Where C is the quantitative value (concentration,mgg−1 or mgml−1) of the component under consideration (Vitamin A palitate in this case),
k is the reaction rate constant, and
m is the order of the reaction.

Since the degradation was found to follow of first order kinetics,

$$m = 1,$$

Integration of eq. (1) over any specified heating period t can be written as

$$\ln([C]0/[C]t) = kt \quad \ldots\ldots\ldots\ldots (2)$$

Where $[C]_0$ is the initial concentration of vitamin A palmitate at time 0 and,
$[C]t$ is the value after storage time t (months).
The relationship of reaction rate to temperature was quantified by the Arrhenius equation,
Where

$$k = A0 \exp(-Ea/RT) \quad \ldots\ldots\ldots\ldots \quad (3)$$

Where, Ea is the activation energy of the degradation reaction (kcalmole−1),
R is the universal gas constant (1.98 kcal mole−1 K−1),
T is absolute temperature (K), and $A0$ is a pre-exponential constant (min−1).

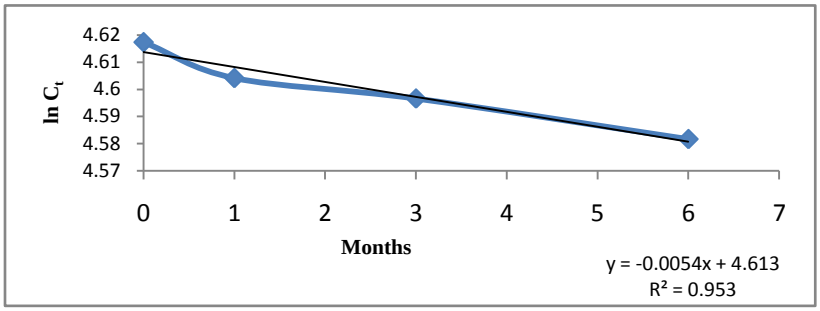
Determination of reaction rate constants:

1. at 25^0c:

Table No 13: Study of reaction rate constants

Time (Months)	% conc. remaining	*ln* of Concentration (C_t)
0	101.23	4.617395
1	99.9	4.60417
3	99.15	4.596634
6	98.68	4.591882

Figure No 19: Study of reaction rate constants at 25^0c



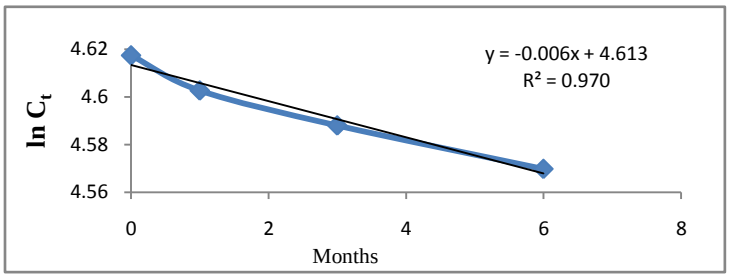
First order of Degradation kinetics K_{25}= **5.4×10^{-3} month**

2. at 30^0c:

Table No 14: study of reaction rate constants at 30^0c

Time (Months)	% conc. remaining	*ln* of Concentration (C_t)
0	101.23	4.617395
1	99.75	4.602667
3	98.3	4.588024
6	96.17	4.566117

Figure 20: study of reaction rate constants at 30^0c

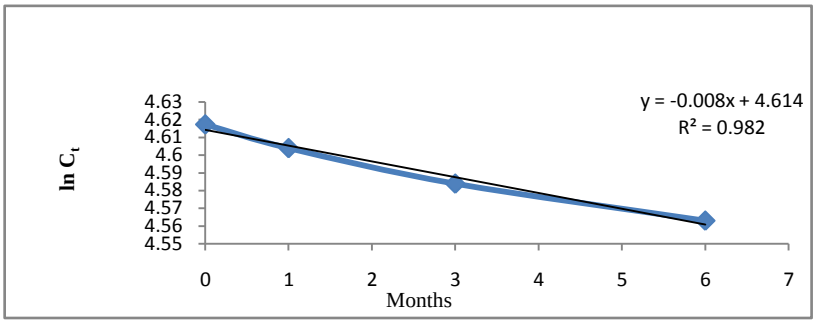


First order of Degradation kinetics K_{30}=**6×10^{-3} month^{-1}**

Table No 15: Study of reaction rate constants at 40^0c

Time (Months)	% concn. remaining	Concentration ln (C_t)
0	101.23	4.617395
1	99.88	4.603969
3	97.89	4.583844
6	95.86	4.562889

Figure 21: Study of reaction rate constants at 40^0c



First order of Degradation kinetics $K_{40}=8\times10^{-3}$ **month**$^{-1}$

Determination of Activation Energy (E_a) of VAP 250CWS SD

Activation energies, *Ea (*kcalM−1*)*, were calculated as a product of

1. gas constant, *R(*1.987 calM−1 K−1*)*,
2. the slope of the graph obtained by plotting *ln k* versus 1/*T*.

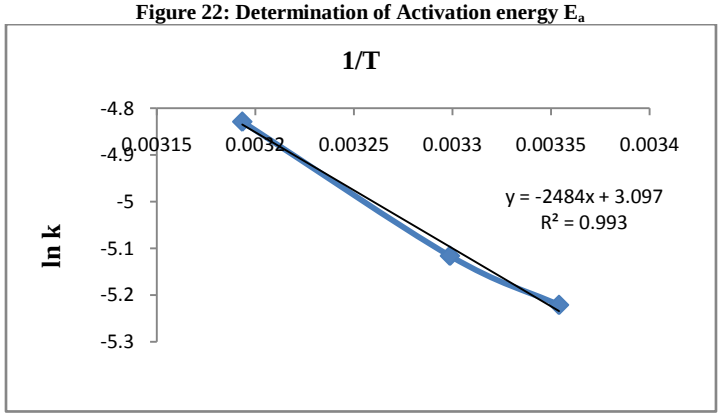
Following Figure 22 shows the Arrhenius plot for the degradation of Vitamin A palmitate Rate constants reported for Vitamin A palmitate ranged from 0.005 to 0.008 min−1 for a Temperature range of 25–40 ◦C

Activation energy E_a

Table No 16: Determination of activation energy E_a

1/ T	ln *K*
0.003354	-5.22136
0.003299	-5.116
0.003193	-4.82831

Figure 22: Determination of Activation energy E_a



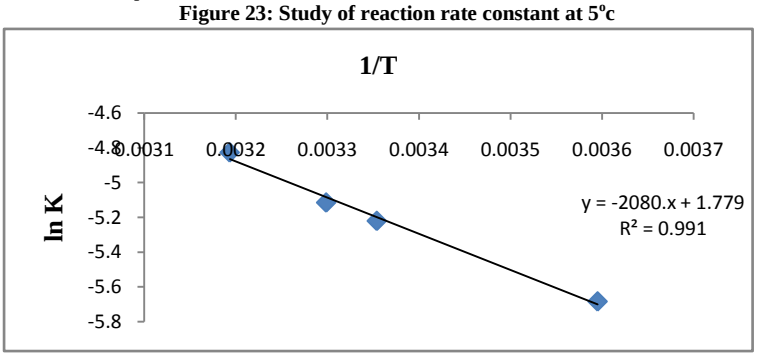
Activation energy = 1.987*2484 / 100 = **4.93 kcalM−1**

Determination of shelf life:

The rate of drug decomposition is accelerated by storage under drastic conditions as elevated temperature. Arrhenius equation is then applied by plotting *ln* K versus 1/T , getting the degradation rate constant by extrapolation to desired temperature. the shelf-life can be calculated from the equation :

t90% = 0.105 / K temp.

Figure 23: Study of reaction rate constant at 5^oc



K at 5^0 C = 2.9×10^{-3} month

So, Shelf life at 5^0C = 0.105 /0.0029 = **28 months.**

Q_{10} Method of Shelf life estimation

The term Q10 is the factor by which the rate constant increases for a 10 °C rise in temperature2.

$$Q10 = k (T+10)/kT \quad .]\ldots\ldots. (1)$$

The Q10 factor can also be calculated from the exponential form of Arrhenious equation derivatives:

$$k = A. \exp (-Ea/RT) \quad \ldots\ldots\ldots (2)$$

Where k is the reaction rate constant of any order, A and Ea are the constants and T is the absolute temperature. R is the gas constant (1.987cal/mol/°). In simplified form it can be written as:

$$\text{Log } k = \log A - Ea/2.303\ RT \quad \ldots\ldots\ldots (3)$$

To evaluate the effect of temperature on shelf life we can correlate the QΔ T value with the shelf life.

Most of the degradation reactions usually follow either zero order or first order or pseudo first order.

$$t90 = 0.105/k1$$

Where the reaction is a first order process, and 0.1, D0, 0.105 all are respective constants.

So the shelf lives can be written in a general correlating order of reactions,

$$t90 = a / kT$$

Table No 17: Determination of Q10 Value

T	R
30	6.00
40	8.00

$$\mathbf{Q_{10} = (R_2/R_1)^{(10/T_1-T_2)}}$$

$$Q_{10} = (8/6)^{(10/40-30)}$$

Q_{10} = 1.33

R_1 = Rate Constant at higher temp

R_2 = Rate Constant at lower temp

$T_{90}(T_2)$ = Shelf life to be Estimated.

$T_{90}(T_1)$ = Given shelf life at given temp.

dT = Difference in temp.

Vitamin Apalmitate 1.7 mIU have shelf life of 28 month at 5^0C (Refrigerator)

Determination of shelf life at 25,30, 40^0C

At 25^0C

$$T_{90}(T_2) = T_{90}(T_1) / Q_{10}^{(dT/10)}$$

$$= 28 / 1.33^{(25-5/10)}$$ **At 25^0C= 15.8 Months**

At 30^0C

$$T_{90}(T_2) = T_{90}(T_1) / Q_{10}^{(dT/10)}$$

$$= 28/ 1.33^{(30-5/10)}$$ **At 30^0C = 13.7 Months**

At 40^0C $T_{90}(T_2) = T_{90}(T_1) / Q_{10}^{(dT/10)}$

$$= 28/ 1.33^{(40-5/10)}$$ **At 40^0C = 12.90 Months**

4. Conclusion

Stability testing is now the key procedural component in the pharmaceutical development program for a new drug as well as new formulation. Stability tests are carried out so that recommended storage conditions and shelf life can be included on the label to ensure that the medicine is safe and effective throughout its shelf life. Over a period of time and with increasing experience and attention, the regulatory requirements have been made increasingly stringent to achieve the above goal in all possible conditions to which the product might be subjected during its shelf life. The RP HPLC method validated for parameters like accuracy, precision, linearity, specificity, robustness, etc. and used for determining the assay of drugs. And method found to be accurate, precise, linear, specific and robust. The accelerated stability studies carried out on marketed formulation of VAP. These formulations kept for six month in different stability condition as per protocol and then evaluated for different stability parameter, with the help of validated RP HPLC method. From the stress tastings it can be concluded that aluminum type of container closure is required for accelerated stability tastings as well as for marketing of products. And also stress study suggested that products are degraded in presence of acid, base, oxidative condition, UV light, day light, etc. Shelf life of products was determined by using Arrhenius equation and shelf life of products was found to be 28 months for VAP 250 CWS SD.

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