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Improving the Photostability of Vitamin K_1 (Phylloquinone) in Organic Solvent

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ABSTRACT

The photostability of vitamin K_1 (phylloquinone) in bulk powder and in its dosage form was studied according to the conditions suggested by ICH Guideline (option 2). The results showed that the rate of photodegradation of vitamin K_1 follows first order kinetics and it is higher in its dosage form than in bulk powder. Attempts have been made to improve the photostability of vitamin K_1 by combination with different photoprotective agents such as p-aminobenzoic acid (PAPA), tartaric acid, boric acid, citric acid, sodium benzoate, titanium dioxide (TiO_2), zinc oxide (ZnO), propyl 4-hydroxybenzoate and methyl 4-hydroxybenzoate). The photodecompositions products were monitored by UV-Vis spectrophotometric method, HPLC. It was found that 0.01% PABA was the best photoprotective agent, which improved the photostability of vitamin K_1 by 46 fold. The method was validated according to ICH guideline. The calibration graph was linear over the concentration range of 1.1×10^{-5}-30.0×10^{-5} mol L^{-1}. The limit of detection and the limit of quantification were 2.42×10^{-6} and 7.35×10^{-6} mol L^{-1}, respectively.

Keywords: Phylloquinone; Photostability; 4-aminobenzoic acid; HPLC; UV-Vis spectrophotometry

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INTRODUCTION

Large number of pharmaceutical compounds undergoes degradation on exposure to light. A wide variety of drugs can undergo changes in color, and nutrient composition when exposed to light. The photostability of a drug substance may be defined as the response of the drug or drug product to the exposure to solar, UV, and visible light in the solid, semisolid, or liquid state that leads to a physical or chemical change [1]. Vitamin K_1 is found in leafy green vegetables, broccoli, and Brussels sprouts. Vitamin K_1 (phylloquinone) is fate soluble vitamin (2-methyl-3-(3, 7, 11, 15-tetramethylhexadec-2-enyl) naphthalene-1, 4-dione (Figure 1). Vitamin K_1 a highly photosensitive compound, Vitamin K exists naturally in two forms; as phylloquinone (PY) and menaquinones (vitamin K_2), they have similar metabolic pathways during reactions. Overall, vitamin K contains a functional naphthoquinone ring and an aliphatic side chain. PY has a phytyl side chain attached to the homolog three synthetic types of vitamin K are known: vitamins K_3 (Menadione), K_4, and K_5,Poor anticoagulant stability in patients using vitamin K antagonists is a risk factor for both bleeding and thrombosis and low dose vitamin K_1 was shown to improve the stability of anticoagulant control [2].

PY is insoluble in water, slightly soluble in alcohol and readily soluble in non-polar organic solvents, for example in n- hexane, ether, and chloroform. The photostability of PY was studied under a combination of a near UV fluorescent lamp and a cool white fluorescent lamp. Moreover, in the present work demonstrate to improve the photostability of PY by addition of some photoprotective agents. Their photodecompositions products were monitored by UV spectrophotometric method, HPLC and LC-MS/MS.

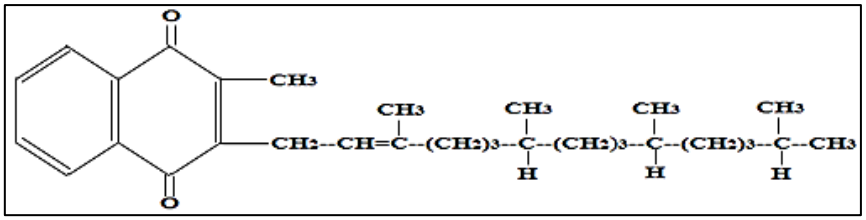


Figure 1: Chemical structures of vitamin K_1 (phylloquinone)

EXPERIMENTAL SECTION

Chemicals and Reagents

Phylloquinone (99.9%) was kindly supplied from European Egypt Pharmaceutical Company, Egypt. Acetonitrile, formic acid and methanol HPLC grade were purchased from E-Merck (Darmstadt, Germany). Tartaric acid was purchased from (ADWIC, Egypt). Methyl 4-hydroxybenzoate and propyl 4-hydroxybenzoate were purchased from (Sigma Company, Germany). 4-aminobenzoic acid was purchased from (Win Lab Company, UK). Sodium benzoate was purchased from (Alexandria Company, Egypt). Boric acid was purchased from (El Gomhouria Company, Egypt). Citric acid, toluene was purchased from (Nasr Company, Egypt). Zinc oxide was purchased from (Merck, Germany). Cyclohexane was purchased from (SdfiNe- Chem Limited Company, India). Titanium dioxide was purchased from (Qualikems, New Delhi, India). Water was obtained from a Milli-Q (ZD60115UV) water purification system (Millipore Company, France). K chewable tablets were kindly supplied by (European Egypt Pharmaceutical IND Company.

Preparation of Standard and Sample Solutions

Standard stock solutions:

The standard stock solutions of concentration 4.43×10^{-3} mol L^{-1} of PY was prepared in cyclohexane. Working standard solution of PY 4.4×10^{-5} mol L^{-1} was freshly prepared by appropriate dilution using cyclohexane. Care was taken to protect the solutions from light at all the time. The solution of PY was prepared freshly for each experiment to avoid any bacterial action or chemical effects.

Preparation of the working solution of vitamin K_1 in combination with different photoprotective agents:

The photostability of PY 4.4×10^{-5} mol L^{-1} solution was also investigated by typical combination of with different photoprotective agents at certain concentration of (0.01, 0.01, 0.01, 0.1, 0.02, 0.01, 2, 0.05and 0.015) tartaric acid, boric acid, citric acid, sodium benzoate, titanium dioxide, zinc oxide, propyl 4-hydroxybenzoate and methyl 4-hydroxybenzoate respectively [3,4].

Preparation of tablet sample solution:

PY market tablet (label K chewable 10 mg phytomenadione per tablet) was extracted purified and prepared in cyclohexane to obtain the same concentration as corresponding standard stock by weight 20 tablets and grained them, weight an amount of the powder equivalent to 4.43×10^{-3} mol L^{-1} of PY was transferred to a 100 ml volumetric flask, complete the volume to the mark with cyclohexane, the solution was filtered and put 0.1 ml of filtered solution into 100 mL volumetric flask complete the volume with cyclohexane to obtain a solution of concentration 4.4×10^{-5} mol L^{-1}.

Method validation for the stabilization of vitamin K_1 in presence of (0.01%) PABA:

The validity of the method was tested regarding to linearity, accuracy, precision, and standard addition technique, limit of detection (LOD) and limit of quantitation (LOQ) according to the International Conference on Harmonization (ICH) guideline. Also, this method was applied to pharmaceutical preparations.

Instrumentation

Irradiation conditions:

CLIMACELL chamber (CLC111, MMM group, Germany) was used as a photostability testing device). It is equipped with a microprocessor-controlled system of humidification and dehumidification with a powerful lighting system that guarantees excellent homogenous parameters for testing (near UV light fluorescent lamp and cool white fluorescent lamp). Working temperature used ranged from 0.0°C up to 99.9°C (without humidity) and from 10°C to 90.0°C (with humidity). It has inner glass door and the inner chamber is made of stainless steel DIN 1.4301 (AISI 304). It contains a shelf for putting samples and a sensor for irradiance measurements. Solution of PY at concentration 4.4×10^{-5} mol L^{-1} was exposed to near UV light fluorescent lamp and cool white fluorescent lamp and the UV spectra of the samples were recorded just after preparation (t=0) and the sample of PY solution was stirred at dark for 30min after the addition of the photoprotective agent, the

samples withdrawn at different time interval (0.5, 1, 3, 5, 7, 10 h) , 5 ml sample was withdrawn and centrifuged immediately at 3000 rpm for 10 min. The supernatant was filtered through 0.45 μm pore size syringe.

UV spectrophotometry:
UV spectrophotometric method was performed on double beam UV-visible spectrophotometer (Shimadzu, model 1700) having two matched quartz cells with 1 cm light path. Connected to an IBM compatible personal computer (PC) and a HP-600 inkjet printer.

Chromatographic conditions and HPLC instrumentation:
High performance liquid chromatographic system (Waters, Milford, USA) equipped with Waters 486 variable wavelength UV detector, Waters 717 auto sampler injector and pump controlled by Waters 610 controller. The output signal was monitored and processed using Empower software. The output signal was monitored operated by Pentium III (450MHz) processor (Lenovo, UK) was used. The ANALYSIS for PY solution before and after illumination was performed by HPLC using Xterre C18; 250×4.6 mm, 5 μm column, mobile phase-A composed of (Acetonitrile and methanol in isocratic elution (5:95 v/v) by isocratic program. Flow rate was set to 2 mL min^{-1} with maintained at ambient temperature, detection wavelength was carried out at 327 nm, injection volume was 20 μL and the retention times of PY was at 3.99 min.

LC-MS/MS instrumentation and its condition:
Separation and detection for photodegradation product was performed on Agilent Triple quadrupole mass spectrometer (USA). Where Agilent Triple quadrupole mass spectrometer with API source coupled with Agilent pump controlled by Agilent 1200 controller and equipped with Agilent1200 auto sampler injector. The analysis was performed in both positive and negative electro spray ionization modes. The capillary voltage was 6000 V. The source temperature was 350°C and the gas flow rate was 13 L min^{-1}. For the data acquisition and integration, Agilent Mass Hunter software operated by Pentium III (450 MHz) processor (HP, USA) .was used Agilent SB-C18 (50×4.6 mm), a 1.8 μm particle size column was used for separation of photodegradation peaks using acetonitrile: 0.1% formic acid (84:16 v/v) as mobile phase pumping at a room temperature using a flow rate of 0.6 $mLmin^{-1}$.

RESULTS AND DISCUSSION

Photodegradition of Vitamin K_1 (PY) in Cyclohexane
Spectrophotometric analysis:
A literature survey reveals no photostability studies for PY was determined according ICH guidelines, thus the goal of this work was to study the photostability of PY in bulk powder and its dosage form. In addition, this work clarifies the effect of photoprotective agents on the photostability of PY in cyclohexane.
Photostability of PY in bulk powder was examined in cyclohexane. Sample was irradiated with two light doses for ten hours, Dose (I) 66 $w.m^{-2}.h^{-1}$ for UV and Dose (II) 80 kilolux for visible. The effect of radiation on drug solution was monitored by recording their absorption spectra at different time intervals.
The maximum absorption of PY was observed at wavelength 327 nm, when solution of PY was irradiated, spectral change was observed, the absorption spectrum of PY was remarkably decreased by increasing irradiation time (Figure 2). Therefore, organic solution of PY in bulk powder is photochemically unstable and its rate of photodegradation obeys first order of kinetic law, the rate constant of photodegradation was 0.185 h^{-1}.

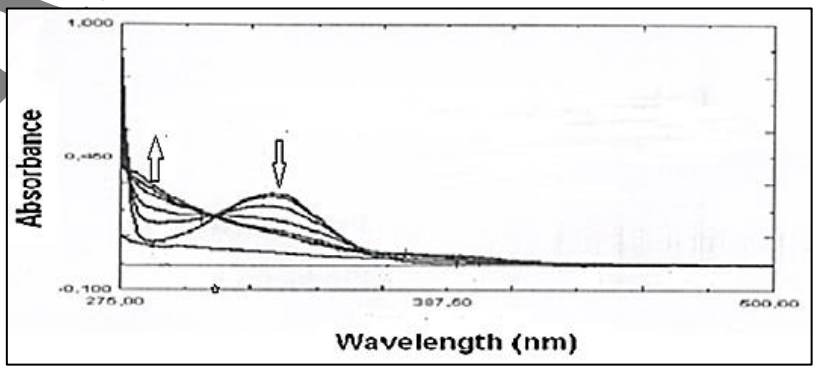


Figure 2: Change in absorption spectra of PY in cyclohexane

HPLC analysis:

On the analysis of non-irradiated and irradiated solutions of PY by HPLC, the chromatogram corresponding to non-irradiated solution showed characteristic peak of the PY at retention time 3.99 min. In the case of irradiated solution the characteristic peak of the PY completely disappeared and no degradation products appeared in the chromatogram **(**Figures 3a and 3b).

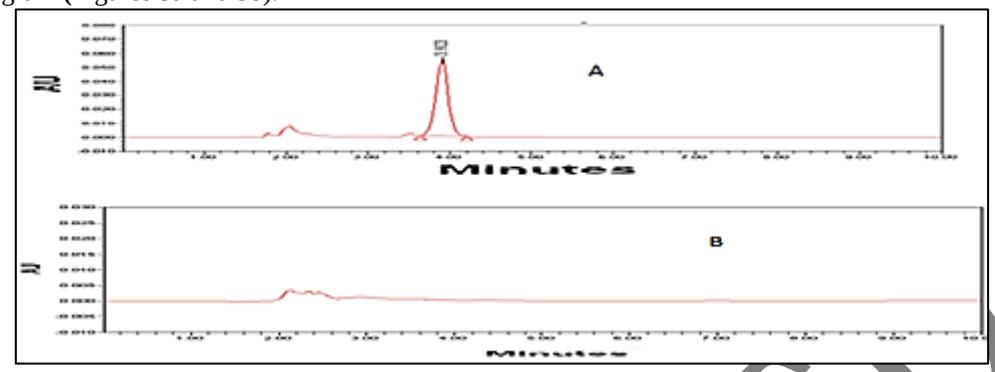


Figure 3: HPLC chromatogram of PY in cyclohexane; (A) pre-exposure and (B) post-exposure to the illumination

LC-MS/MS analysis:

The LC-MS/MS was used to illustrate the photodegradation pathway of PY. The mass spectra for PY solution before and after illumination were recorded. The molecular ion of PY before illumination was detected at m/z 449.6 (M - H) of PY **(**Figure 4a). The mass spectra of PY solution after illumination exhibited a new product was formed in LC/MS chromatogram 171.3 (M- $C_{20}H_{38}$) which indicated that complete degradation for the vitamin $K_1$and formation of new product (Figure 4b). The representative extracted ion chromatograms prove the presence of new product (Figure 5). Photodegradation pathway of PY was proposed and illustrated in Scheme 1.

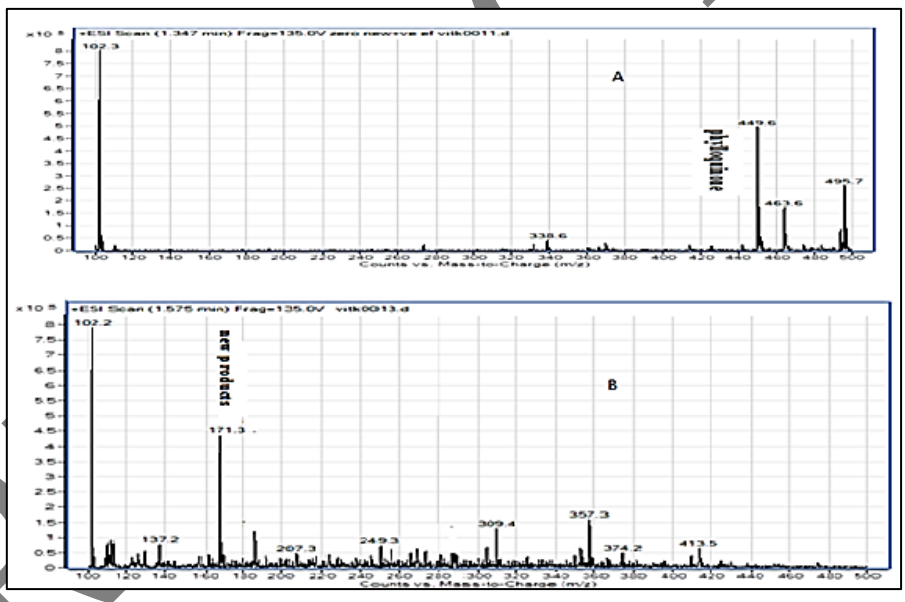
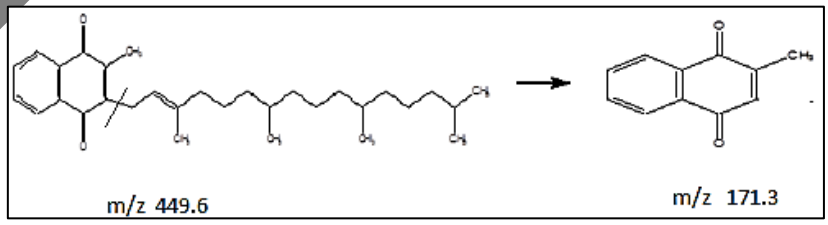


Figure 4: MS/MS spectra of PY (A) pre exposure and (B) postexposureto the illumination



Scheme 1: Photodegradation pathway of PY

Effect of Concentration of Vitamin K_1 on its Rate of Photodegration

Stock solutions of concentration 4.43×10^{-3} mol L^{-1} of PY was prepared, then appropriate dilution in cyclohexane to prepare different concentration ranging from (30.0×10^{-5} - 0.25×10^{-5} mol L^{-1}).

The rate of photodegradation was calculated by plotting ln concentration against time. It was found that the rate of photodegradation increase with increase of concentration of PY, half life time and the percent remaining were also calculated (Table 1).

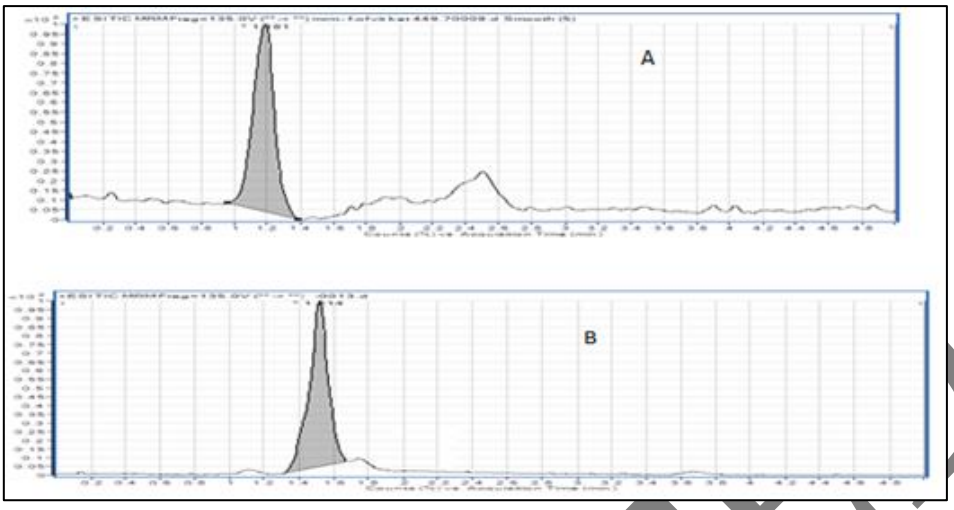


Figure 5: A representative total ion chromatogram in MRM mode of irradiated solution of PY (A) and the extracted ion chromatogram of new products (B)

Effect of the Addition of Photoprotective Agents on the Photostability of Vitamin K_1

UV spectrum and HPLC analysis of vitamin K_1 in the presence of photoprotective agents:

The rates of photodegradation of PY were changed in the presence of PABA, sodium benzoate, methyl 4-hydroxybenzoate, citric acid, boric acid, tartaric acid, propyl 4-hydroxybenzoate, zinc oxide and titanium dioxide respectively (Table 2). It was found that PABA was the best photoprotective agent which improves the photostability of vitamin K_1 by 46 fold (Figure 6). PABA contains a benzene ring in which electrons can resonate, between different locations within the six-sided structure. Electrons can also bounce around in an adjacent carboxyl group, made up of carbon, two oxygens and hydrogen (COOH). The mechanism of action of 4-aminobenzoic acid involves the absorption of UV radiation followed by the excitement of the π HOMO orbital (occupied molecular orbital of higher energy) to the π^* LUMO orbital (unoccupied molecular orbital of lowest energy). These molecules upon returning to their ground states, release excess energy absorbed in the form of heat [5]. The addition of other photoprotective agents slightly improve the photostability of PY about 2-5 fold compared to the photostability of PY without any addition. The half-life and the percent remaining of PY after addition of photoprotective agents (Table 2). The analysis of non-irradiated and irradiated PY solution in the presence of PABA by HPLC was illustrated in Figures 7a and 7b.

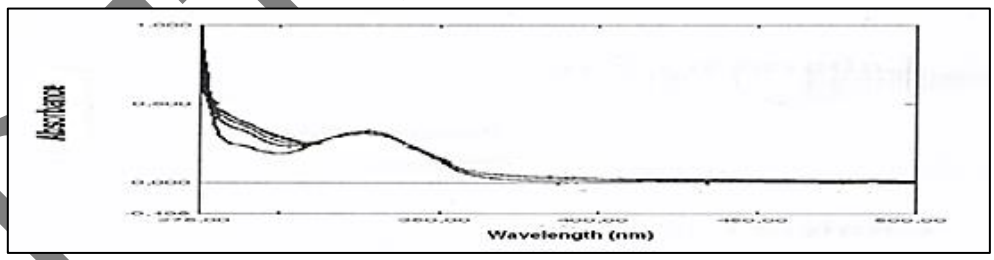


Figure 6: Absorption spectra of PY in presence of PABA

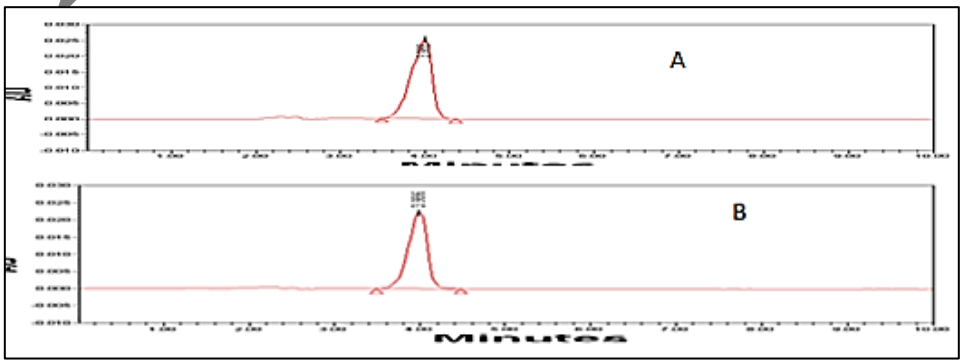


Figure 7: HPLC chromatograms of PY with in presence of PABA pre-exposure(A) and post-exposure (B)

Effect of Concentration of PABA and Propyl 4-Hydroxybenzoate on the Rate of Photodegration of Vitamin K_1

Different concentrations of PABA (0.4, 0.25, 0.1, 0.05, 0.01% (w/v)) and also different concentrations of propyl 4-hydroxybenzoate (0.4, 0.25, 0.15, 0.1, 0.05% (w/v)) were added to the working solution of PY 4.4×10^{-5} mol L^{-1}. The rate of photodegradation was plotted against the concentration (Figure 8). It is observed that the rate of photdegradation increases as concentration of PABA and propyl 4-hydroxybenzoate increase.

Table 1: Rate of photodegradation of PY solution at different concentration of VitaminK_1

Concentration of PY $\times10^{-5}$mol L^{-1}	Rate of photodegradation (×10-3h-1)	Half-life times $t_{1/2}$(h)	Percentage remaining (%)
30	250.74	3.98	8.72
25	243.57	4.1	9.03
20	243.87	4.1	9.38
15	229.28	4.36	10.14
12	228.71	4.37	10.81
10	224.83	4.44	10.87
8.8	216.47	4.61	11.44
4.4	187.93	5.32	16.27
2.2	136.45	7.32	24.7
1.1	90.01	11.1	41.37
0.5	20.67	48.36	80.48
0.25	18.48	54.08	84.61

Table 2: Rates of photodegradation and Half-life times of PY in cyclohexane with photoprotective agents

PY with photoprotective agents	Rate of photodegradation PY with Photoprotective agents ($\times10^{-3}$)h^{-1}	Half-life times $t_{1/2}$(h)	Percentage remaining (%)
PY without any additives	185.84	5.39	14.64
PABA	4.01	249.37	95.91
Sodium benzoate	61.07	16.37	54.43
Methyl -4-hydroxybenzoate	57.46	17.4	56.25
Citric acid	74.9	13.35	47.5
Boric acid	58.43	17.11	55.77
Tartaric acid	65.69	15.22	52.12
Propyl 4-hydroxybenzoate	49.46	20.21	60.8
ZnO	72.11	13.86	48.48
TiO_2	80.59	12.4	44.51

Photostability of Vitamin K_1 in its Dosage Form

The photostability of PY in its dosage form was also studied. Remarkable decreases in absorbance was observed as illumination time increased and analysis of the sample in its dosage form before and after illumination by HPLC was shown in Figures 9a and 9b. The rate of photodegradation of PY in its dosage form is 0.222 h^{-1} which is greater than in bulk powder. This is may be attributed to the interference of one or more of the excipients contributing to the increase of its degradation [6]. Excipients can initiate, propagate or participate in photochemical reactions [7,8]. So excipients used in PY preparation strongly influenced the photodegradation kinetics so the photostability testing for the final pharmaceutical products is very important.

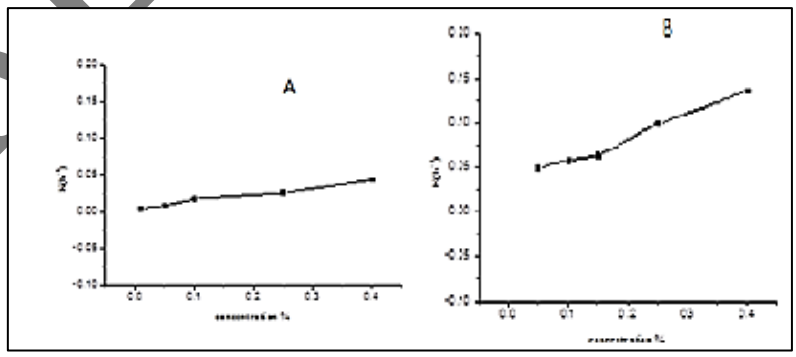


Figure 8: Rate of photodegradation of PY versus different concentration of A) PABA, B) propyl 4-hydroxybenzoate

The method was validated for parameters like linearity range, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy. The limit of detection (LOD) and limit of quantification (LOQ) were estimated from the standard calibration curve. LOD (the smallest concentration of the analyte that gives the measurable response was calculated using the following equation (1) :

$$LOD = 3.3\ (\sigma / S) \quad \text{.....(1)}$$

LOQ (the smallest concentration of the analyte, which gives a response that can be accurately quantified) was calculated using the following equation (2):

$$LOQ = 10 (\sigma / S) \quad \text{.....(2)}$$

Where σ = standard deviation of the response, and S = slope of calibration curve [9].

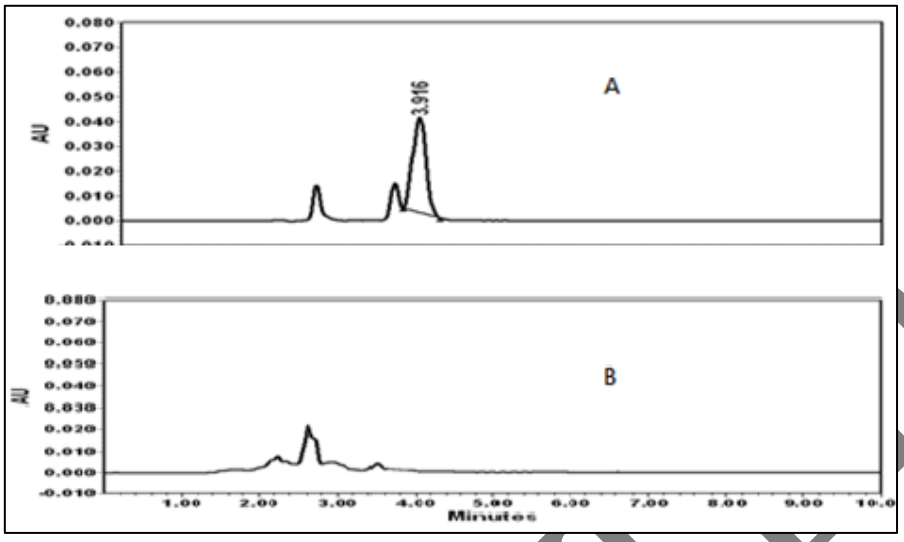


Figure 9: HPLC chromatogram of PY in its dosage form (A) Pre-exposure and (B) post-exposure to the illumination

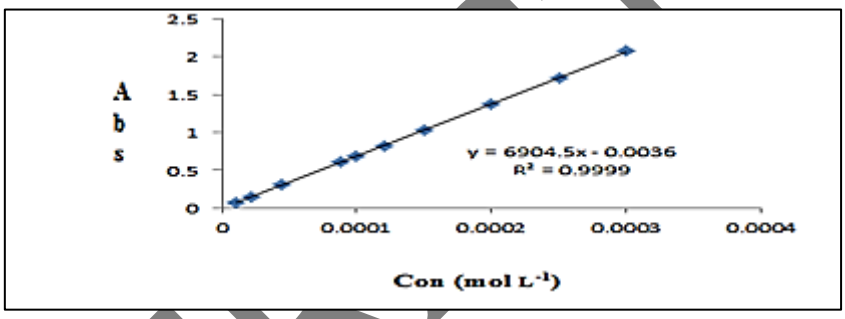


Figure 10: Calibration curve for PY in the concentration range ($30.0\times10^{-5} – 1.1\times10^{-5}$ mol L^{-1})

Method Validation for the Stabilization of Vitamin K_1 in Presence of (0.01%) PABA

Linearity study:

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range [10,11]. Linearity was evaluated by preparing standard concentrations in the range of (1.1×10^{-5} - 30.0×10^{-5}) mol L^{-1} of PY at constant concentration of PABA 0.01%. Three independent solutions for each concentration were prepared the solutions were scanned on a spectrophotometer in the UV range 200–400 nm. The spectrum was recorded at 325 nm after 10 h. The calibration curve was constructed by plotting the absorbance verses concentration (Figure 10). The regression equation was computed. The linearity parameters for the determination of PY in presence of PABA (Table 3).

Table 3: Linearity parameters for the stability of PY in present of (0.01%) PABA

Parameters	Results observed PY
Slope	6904.5
Intercept	0.0036
Correlation coefficient (R^2)	0.9999
LOD	2.42×10^{-6}
LOQ	7.35×10^{-6}

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. To confirm the accuracy of this method, the recovery test was performed following the ICH guideline recommendations. This was determined by adding known amounts 0.68, 0.56, 0.45, 0.34, 0.22, 0.2, 0.1, 0.05 and 0.025 mL of PY(4.43×10^{-3} mol L^{-1}) to series of 100 mL volumetric flask with 0.01 g PABA in each volumetric flask and completed to the mark with the cyclohexane to get final concentrations (1.1, 2.2, 4.4, 8.8, 10, 15, 20, 25, 30)×

10^{-5} mol L^{-1} for PY and 0.01% PABA. The resulting solutions were analyzed and the recovered percentages were calculated (Table 4). Moreover, the accuracy of the method was also determined by the standard addition method. Known amounts of PY were added to a pre-quantified sample solution, and the amount of PY was estimated by measuring the absorbance and by fitting these values to the straight-line equation of calibration curve (Table 5).

Table 4: Data of standard calibration curve for the stability of PY in presence of (0.01%) PABA

No	Therotical Concentration of PY $\times10^{-5}$ mol L^{-1}	Absorbance means± SD(n=3)	Concentration found of PY $\times10^{-5}$ mol L^{-1}	Recovery %
1	30	2.08±0.01	28.18	93.95
2	25	1.72±0.002	23.29	93.14
3	20	1.37 ±0.003	18.58	92.94
4	15	1.03±0.003	14.02	93.46
5	12	0.82±0.004	11.22	93.55
6	10	0.68 ± 0.004	9.33	93.3
7	8.8	0.61±0.003	8.39	95.4
8	4.4	0.30±0.001	4.22	95.99
9	2.2	0.14 ±0.001	2.06	93.95
10	1.1	0.06±0.002	1.05	95.84
Means±SD				94.15±1.15

Table 5: Application of standard addition method on K chewable tablets for the determination of PY in present of (0.01%) PABA

Drugs	PY			
	Label claim ($\times10^{-5}$ mol L^{-1})	Amount of standard added ($\times10^{-5}$ mol L^{-1})	Amount Found ($\times10^{-5}$ mol L^{-1})	Recovery (%)[a]
K chewable tablets	4.4	30	29.8	99.35
	4.4	20	19.91	99.58
	4.4	10	9.78	97.82
	4.4	4.4	4.35	98.9
	4.4	2.2	2.15	97.89
	Mean ± SD			98.71 ± 0.81
	RSD (%)			0.81

Precision:

Precision is the measure of the degree of repeatability of a method under normal operation, and is normally expressed as the percent relative standard deviation (RSD) for a statistically significant number of samples. Precision may be performed at different levels: repeatability and intermediate precision [12].

Precision was assessed as RSD % at different levels. Repeatability (intraday) was evaluated by the analysis of three different concentrations of pure drugs 6.6, 2.2 and 4.4 mol $L^{-1}$of PY with 0.01% PABA each in triplicates in the same day, while intermediate precision interday was evaluated by repeating the analysis of the same concentrations for three times on three consecutive days. The results of intraday and interday precision are indicated in (Table 6). From the data obtained, the stability of PY in presence of (0.01%) PABA given precise and results are revealed high precision (RSD% <2) for this method.

Table 6: Intraday and interday precision for the stability of PY in present of (0.01%) PABA

Chemical	Concentration ($\times10^{-5}$ mol L^{-1})	Intra-day precision		Mean recovery % ±SD	Mean RSD%
		Amount Found ($\times10^{-5}$ mol L^{-1})	Recovery %		
PY	2.2	2.07	94.23	95.16 ± 1.08	1.08
	4.4	4.23	96.36		
	6.6	6.26	94.9		
Inter-day precision					
PY	2.2	2.07	94.1	95.00 ± 0.98	0.98
	4.4	4.22	96.05		
	6.6	6.26	94.85		

Effect of 0.01% PABA and 0.05 % 0f Propyl 4-Hydroxybenzoate in the Rate of Photodegradation of Vitamin K_1 in its Dosage Form

It was found the addition of 0.01% PABA improves the photostability of PY in its dosage form by 25 fold while 0.05% of propyl 4-hydroxybenzoate improves its photostability by 5 fold its dosage form **(**Figure 11 and Table 7).

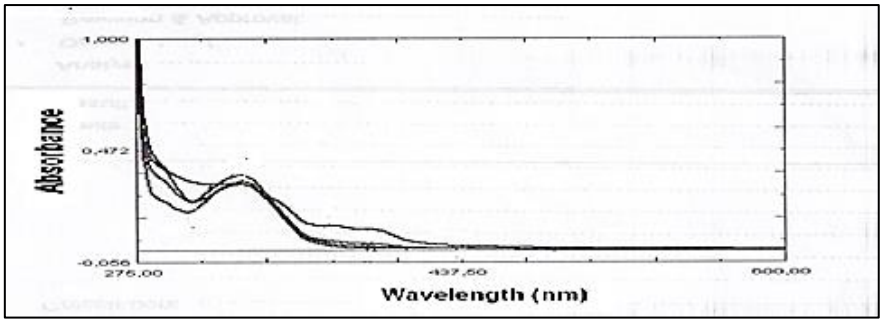


Figure 11: Change in absorption spectra of PY in doses form in present of (0.01%) PABA

Table 7: Rates of photodegradation of PY and its Half-life times in its dosge form in the presence of PABA, propyl 4-hyroxybenzoate

Vitamin K_1(PY)	Rate of photodegradation ($\times 10^{-3} h^{-1}$)	Half-life times $t_{1/2}$(h)	Percentage remaining (%)
In Bulk form	185.84	5.39	14.64
In dosage form	222.13	4.5	10.03
In dosage form in presence of PAPA	8.81	113.5	89.35
PY in dosage form with propyl 4-hydroxybenzoate	40.72	24.55	64.13

CONCLUSION

Vitamin K_1 is photolabile, its rate of photodegradation obeys first order kinetic law. Its rate of phtodegradation in its dosage form is higher than in bulk powder. Attempts were done to increase its photostability by addition of different photoprotective agents. It was found that 0.01% PABA was the best photoprotective agent which improves the photostability of vitamin K_1 by 46 fold in bluk powder and by 25 fold in its dosage form.

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