Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 295–301

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Development and validation of a dissolution test method for vitamin A in dietary supplement tablets

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ARTICLE INFO

Article history:
Received 21 January 2010
Received in revised form 25 March 2010
Accepted 26 March 2010
Available online 1 April 2010

Keywords:
Dissolution test
Vitamin A tablets
Retinyl acetate
Retinyl palmitate
Reverse phase HPLC
Dietary supplements

ABSTRACT

A dissolution test method and an analytical procedure by HPLC were developed and validated for evaluation of the dissolution behavior of dietary supplements tablets containing vitamin A in the forms of retinyl acetate or retinyl palmitate. Seven different commercially available products containing retinyl acetate or retinyl palmitate were selected for this study. A dissolution medium containing 1% (w/v) Octoxynol 9 (Triton X-100) and 1% (w/v) (+)-sodium α-ascorbate in 0.05 M phosphate buffer, pH 6.8, was found suitable to ensure sink conditions and chemical stability for both retinyl acetate and retinyl palmitate. Two rotation speeds, 50 and 75 rpm, were evaluated with USP Apparatus 2 and 900 ml dissolution medium. Dissolution profiles were generated over 120 min. Dissolution samples were analyzed with a reversed-phase HPLC method with UV detection at 325 nm. Each product was also assayed for vitamin A content according to *USP 32–NF 27*. The results from 45 min to the last time point of the dissolution tests performed at 75 rpm were consistent with the *Assay* results. The dissolution test described here could be proposed as a pharmacopeial standard to assess the performance of tablet formulations containing vitamin A as retinyl esters.

1. Introduction

Vitamin A is the collective name for a number of substances of structure related to all-*trans*-retinol and having similar biological activity (Fig. 1). In tablet dosage forms, the dietary ingredient vitamin A is generally used in the form of retinyl esters mostly as all-*trans*-retinyl acetate and all-*trans*-retinyl palmitate containing minor amounts of 13-*cis* and 9-*cis* isomers, incorporated in solid carriers or excipients. The activity of vitamin A is expressed in retinol equivalents (RE) or in International Units (IU). Initially, the activity of vitamin A in International Units (IU) was based on bioassays and international standard materials that are no longer available [1,2], therefore The United States Pharmacopeia (USP) discontinued the use of IU for potency of vitamin A. USP requires labeling in terms of mass (mg) or in terms of retinol equivalents (RE) only. However, even after discontinuation of the international reference material, many products in the market still continue to be labeled in international units, based on a redefined equivalency in molecular terms, as one IU of vitamin A being equivalent to the biological activity of 0.300 μg of the all-*trans*-isomer of retinol. The activity of the other all-*trans*-retinol esters is calculated stoichiometrically so that 1 IU of vitamin A is equivalent 0.344 μg of all-*trans*-retinyl acetate or 0.550 μg of all-*trans*-retinyl palmitate [2].

The quality of oral solid pharmaceutical dosage forms depends on their ability to release the active ingredients in aqueous media in a consistent and reproducible manner, making the active substances available for gastrointestinal absorption. Therefore, the performance of oral solid dosage forms should be verified preferably with an in vitro dissolution test before the product is released to the market. Currently, USP requires dissolution testing for almost every drug in oral solid dosage forms, but in the case of dietary supplements that contain vitamin A among other oil-soluble vitamins, disintegration is the only test required [3]. European, British, and Japanese pharmacopeias have general chapters for dissolution harmonized with the USP, but these pharmacopeias do not have monographs with specifications for vitamin A in tablets. However, disintegration is not a good predictor of how well the active ingredient will be released from a dosage form in vivo, because tablets can break apart in small pieces without releasing the active components. The results of the dissolution test are generally better correlated with the bioavailability of an active ingredient, and thus dissolution testing is a highly valuable tool to evaluate the in vitro performance of oral solid dosage forms as delivery systems. Since vitamin A is widely used in dietary supplement products, it is essential to have a suitable dissolution method as a quality descriptor for performance.

Vitamin A tablets are formulated with retinyl esters of very low solubility in water incorporated into carriers and often are coated with protective substances to improve stability, and other physical properties of the powder to aid in the tablet manufacturing

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doi:10.1016/j.jpba.2010.03.036

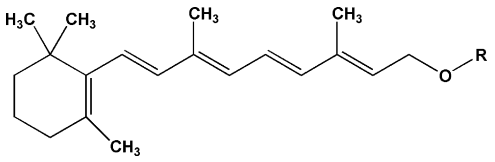

Fig. 1. Structure of vitamin A: R=H, all-*trans*-retinol; R=CO–CH_3, all-*trans*-retinyl acetate; R=CO–$C_{15}H_{31}$, all-*trans*-retinyl palmitate.

processes. Until now, the main reason to exempt tablets containing vitamin A from the dissolution requirements was the absence of a suitable aqueous dissolution medium for the test, in which the vitamin achieves both stability and sink conditions. Surfactants are commonly used in dissolution media to improve wetting and solubility of substances poorly soluble in water [4]. The susceptibility of vitamin A to oxidation [5] should be considered in the selection of an appropriate dissolution medium, which should adequately protect the dissolved vitamin from any possible degradation during the testing period.

In addition, adequate analytical procedures should be applied for the determination of the amount of vitamin A dissolved during dissolution testing. The method intended for this purpose should be sufficiently sensitive, selective in presence of excipients and other nutrients typically present in formulations, and robust and rapid in order to analyze the large number of samples generated from dissolution experiments. Various analytical methods for the quantitative determination of vitamin A in biological samples, food, and pharmaceutical dosage forms have been reported in the literature [6–10]. All these methods are based on liquid–liquid or solid-phase extraction of the vitamin with different organic solvents and subsequent measurement. No studies report direct HPLC analysis of vitamin A aqueous solutions without extra sample preparation procedures. The UV analytical procedure for vitamin A determination in European pharmacopeia is intended for the pure ingredient, and is not specific and sensitive enough for this purpose. HPLC procedures for multivitamin combinations in USP provide enough sensitivity and specificity, but they are normal phase procedures, which do not work well with aqueous samples like the ones taken during dissolution tests [2].

In this study, a dissolution test and a simple HPLC method were developed and validated to evaluate the dissolution performance of dietary supplement tablets containing retinyl acetate or retinyl palmitate.

2. Experimental

2.1. Reagents and materials

Dietary supplement tablets for this study were purchased from various stores in Rockville, MD, USA. Composition of the products according to the label claims is reported in Table 1. USP Vitamin A Reference Standard (RS) Lot W0F-126 (with a certified content of 30.0 mg of vitamin A acetate in 1 g of peanut oil solution) (Rockville, MD, USA) was used as the reference material. Retinol, Retinyl palmitate, Octoxynol 9 (Triton X-100), Polyoxyl 35 Castor Oil (Cremophor EL), L-ascorbic acid, (+)-sodium L-ascorbate, and 2-propanol (iPrOH, HPLC grade) were obtained from Sigma–Aldrich (Saint Louis, MO, USA). The exact vitamin A content in retinyl palmitate was determined by UV measurement at 325 nm according to the procedure presented in the *European Pharmacopoeia* [11]. Polysorbate 80 (Tween 80), methanol (MeOH, HPLC grade), potassium phosphate monobasic (ACS grade), sodium hydroxide solution (50%, w/w, ACS grade), syringe filters, 0.45-μm Millex-HV, Millex-PTFE, Millex-LCR, and Whatman PVDF were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Ultra-pure water (Milli-Q Advantage, Millipore, USA) was used for the dissolution medium and throughout analysis.

2.2. Equipment

2.2.1. Analysis of dissolution samples

A Hewlett Packard 1100 series (Agilent Technologies, Wilmington, DE, USA) HPLC system equipped with a quaternary pump, column heater, auto-sampler, and diode array detector was used. The data were collected and processed using Empower 2 software version 6.10.00.00 (Waters). Analysis of aqueous solutions of retinol, retinyl acetate and retinyl palmitate was performed on a Phenomenex Luna C18 (2), 10 cm × 4.6 mm, 3-μm analytical column using gradient elution of two solutions, A and B. Solution A was a mixture of methanol and water, 90:10 (v/v), and solution B consisted of a mixture of methanol and 2-propanol, 55:45 (v/v). The following gradient program was applied: from 100% A to 100% B in 8 min, 100% B for 5 min, from 100% B to 100% A in 0.1 min, and 100% A for 1.9 min. The flow rate was 1 ml/min, and total run time was 15 min. Column temperature was maintained at 25 °C. The injection volume was 50 μl with UV detection at 325 nm.

2.2.2. Analysis of vitamin A assay samples

Chromatographic analysis was carried out on a Waters Alliance HPLC system using Zorbax NH_2, 15 cm × 4.6 mm, 5-μm column. *n*-Hexane was used as mobile phase with a flow rate of 2 ml/min. Injection volume was 40 μl with UV detection at 325 nm.

2.3. Solubility studies

Amongst the retinyl esters usually found in dietary supplement tablets, retinyl palmitate is the substance with the lowest solubility in aqueous media. For this reason, retinyl palmitate was selected to perform the solubility studies in order to find an aqueous dissolution medium that ensures sink conditions during dissolution testing. Dissolution media containing 0.25%, 0.5%, 1%, and 2% of Polysorbate 80, Octoxynol 9, or Polyoxyl 35 Castor Oil in 0.05 M phosphate buffer, pH 6.8 were prepared. Potassium phosphate monobasic was used for buffer preparation. Sodium hydroxide solution (50%, w/w) was used for pH adjustment.

Accurately weighed amounts of retinyl palmitate equivalent to 4500 IU were placed into amber test tubes, and 20 ml of different dissolution media were added. The tubes were capped and placed into a shaking water thermostatic bath at 37 ± 0.5 °C. Samples were taken after 24 h, filtered through 0.45 μm PVDF syringe filters and analyzed by UV at 325 nm. The solubility in each medium was determined in triplicate.

2.4. Stability studies

Stability of retinyl acetate and retinyl palmitate in the dissolution medium containing 1% (w/v) Octoxynol 9 in 0.05 M phosphate buffer, pH 6.8, and also with addition of 1%, 2%, 5%, 10% of L-ascorbic acid and 0.5%, 1%, 2% of (+)-sodium L-ascorbate was evaluated on standard and sample solutions. The solutions for the stability experiments were maintained under typical dissolution testing conditions over 24 h. The samples were analyzed by the HPLC procedure for dissolution samples described above.

2.5. Dissolution testing

Dissolution testing was performed using a qualified VanKel VK 7010 (Varian Inc., Palo Alto, CA, USA) dissolution tester operated as USP Apparatus 2 (paddle) at 50 rpm or 75 rpm and at 37 ± 0.5 °C. Nine hundred milliliters of 1% (w/v) Octoxynol 9 and 1% (w/v) (+)-sodium L-ascorbate in 0.05 M phosphate buffer (pH 6.8) was used

Table 1
Dietary supplement label claims per tablet.

Ingredients/Samples	A (blank)	B	C	D	E	F	G	H
Retinyl acetate (IU)		2485	2485	2000	5000			
Retinyl palmitate (IU)						10,000	10,000	5000
Beta carotene (IU)	4000	1015	1015	500				
Vitamin C (mg)	120	90	120	60				10
Biotin (mcg)		30	30					
Vitamin B12 (mcg)	8	6	6	6	3			
Folic acid (mcg)	800	500	500	400	400			
Niacin (mg)	20	20	20	10	16.7			
Panthotenic acid (mg)		10	10	5				
Vitamin B6 (mg)	2.6	2	2	2				
Riboflavin (vit. B2) (mg)	1.7	1.7	1.7	1.7	1.8			
Thiamine (vit. B1) (mg)	1.8	1.5	1.5	1.5	1.5			
Vitamin K (mcg)		25	25					
Vitamin D (IU)	400	400	600	400				
Vitamin E (IU)	30	30	60	30	5			
Calcium (mg)	200	200	200	450	250			55
Iron (mg)	28	18	18	18				
Phosphorus (mg)		109	109					
Iodine (mcg)		150	150					
Selenium (mcg)		55	55					
Copper (mg)		0.9	1					
Magnesium (mg)		100	100	50				
Manganese (mg)		2.3	2.5					
Chromium (mcg)		35	40					
Molybdenum (mcg)		45	750					
Chloride (mg)		72	72					
Potassium (mg)		80	80					
Zinc (mg)	25	11	22.5	15				
Boron (mcg)		150	150					
Nickel (mcg)		5	5					
Silicon (mg)		2	2					
Tin (mcg)		10	11					
Vanadium (mcg)		10	10					
Lycopene (mcg)		300	300					
Lutein (mcg)		250	275					
Other components								
Whey					x			
Stearic acid	x			x	x	x	x	
Magnesium stareate	x			x	x	x		x
Croscarmelose sodium	x		x	x	x			
Silicon dioxide	x	x	x	x	x	x	x	x
Cellulose	x	x	x	x		x	x	x
Corn starch	x	x	x	x				
Crospovidone	x	x	x	x				
Maltodextrin	x	x	x	x				
Acacia	x	x	x	x				
Hypromellose	x	x	x	x				
Dextrin	x			x				
Gelatin	x	x	x	x				
Titanium dioxide	x	x		x				
Polyethylene glycol	x	x	x	x				
Red 40 lake	x			x				
Dextrose	x	x	x	x				
Soy lecithin	x			x				
Yellow 6 lake	x	x		x				
Blue 2 lake	x			x				
Hydrogenated palm oil		x						
Polyvinyl alcohol		x	x					
Anhydrous citric acid		x						
Sodium ascorbate		x	x					
Sodium benzoate		x						
Sodium citrate		x						
Polysorbate 80			x					
Talc		x	x					
Sorbic acid		x						

x—indicates the presence of the particular component in the tablet composition.

as the dissolution medium that was prepared freshly each day of use. Each dissolution test was performed with 6 tablets. Sample aliquots were collected at 10, 20, 30, 45, 60, and 120 min, filtered through 0.45-μm Millex-HV syringe filters, and analyzed by the HPLC procedure for dissolution samples described above.

2.6. Preparation of standard solutions

2.6.1. Standard stock solution

Standard stock solution containing a mixture of retinyl acetate with a final concentration of 200 IU/ml and retinyl palmitate with

a final concentration of 400 IU/ml in isopropanol was freshly prepared each day of use.

2.6.2. Calibration standard solutions

Calibration standard stock solution was prepared by dilution of the standard stock solution with dissolution medium to yield a concentration of 10.0 IU/ml for retinyl acetate and 20.0 IU/ml for retinyl palmitate. Five additional calibration points were prepared from calibration standard stock solution by serial dilution with dissolution medium to yield concentrations of 5.00, 2.50, 1.25, 0.67, and 0.33 IU/ml for vitamin A acetate and 10.0, 5.00, 2.50, 1.25, and 0.67 IU/ml for vitamin A palmitate. These solutions were also prepared each day of use.

2.7. Preparation of dissolution samples

2.7.1. Blank sample preparation

Tablets of the sample A (Table 1) were used as the blank samples to rule out possible interferences from the typical dietary ingredients and excipients in the HPLC system. One tablet was placed in the dissolution vessel containing 900 ml of the dissolution medium at 37 ± 0.5 °C and stirred for 1 h at 75 rpm using USP Apparatus 2. An aliquot was collected, filtered through a 0.45-μm Millex-HV syringe filter, and analyzed by HPLC, showing no interference at the locus of the analytes of interest.

2.7.2. Dissolution control samples

Control samples at high (Hi), medium (Me) and low (Lo) concentrations of retinyl acetate and retinyl palmitate were prepared both in a dissolution medium and in a blank sample solution to evaluate the accuracy and precision of the method. High-concentration dissolution control sample contained 10.0 IU/ml of retinyl acetate and 19.4 IU/ml of retinyl palmitate, medium dissolution control sample contained 4.4 IU/ml of retinyl acetate and 8.9 IU/ml of retinyl palmitate, and low dissolution control sample contained 0.3 IU/ml of retinyl acetate and 1.2 IU/ml of retinyl palmitate. Different types of syringe filters were evaluated for sample recovery.

Intraday and interday precision and accuracy were established based on the 3 replicates each of low-, medium-, and high-concentration dissolution control samples. The experiments were repeated on 2 separate days.

2.8. Preparation of vitamin A assay samples

The vitamin A content in the dietary supplement tablets was determined using the *USP 32–NF 27* Assay for vitamin A, *Method 1* [2]. Accurately weighed portions of the powder quivalent to 5 tablets were dissolved in 20 ml of dimethyl sulfoxide and extracted 4 times with 15 ml of *n*-hexane. The extracts were diluted with *n*-hexane to a final concentration of 50 IU/ml.

3. Results and discussion

3.1. Solubility studies

According to the Food and Nutrition Board of the Institute of Medicine and FDA, the vitamin A in dietary supplement products should not exceed 10,000 IU (5.5 mg retinyl palmitate) [12]. Therefore, 5.5 mg of retinyl palmitate per tablet was considered to be the highest dose and was used to establish sink conditions in this study. According to the definition for sink conditions (i.e., a solubility of at least 3–5 times the highest dose to be evaluated in the specified volume of dissolution medium), and considering 900 ml as the specified volume, the solubility of retinyl palmitate in the dissolution medium should be at least 33.3 IU/ml (18.3 μg/ml).

The solubility of retinyl palmitate in 0.05 M phosphate buffer (pH 6.8) containing different concentrations of Polysorbate 80, Octoxynol 9, and Polyoxyl 35 Castor Oil after 24 h at 37 ± 0.5 °C is shown in Table 2. Retinyl palmitate showed insufficient solubility to attain sink conditions in dissolution media containing Polysorbate 80 and Polyoxyl 35 Castor Oil. Medium containing 1% (w/v) Octoxynol 9 was selected for further studies because it ensured sink conditions for retinyl palmitate even if the limit for sink conditions is set at a solubility of 5 times the highest dose in the specified volume.

3.2. Stability studies

Vitamin A was found to be not stable in dissolution medium containing 1% (w/v) Octoxynol 9 in 0.05 M phosphate buffer (pH 6.8) when the samples were kept in the HPLC system in the amber vials at room temperature. The content of retinyl palmitate decreased by 2.6% in the standard solution and by up to 23% in the sample solutions within 10 h. The addition of ascorbic acid to the dissolution medium improved the stability of retinyl acetate and retinyl plamitate in the dissolution samples. The large amount of ascorbic acid, not less than 10% (w/v), and additional readjustment of the pH required to prevent losses of retinyl palmitate during a 12 h. The use of 1% sodium L-ascorbate instead of ascorbic acid further improved the stability of vitamin A, and pH adjustment was not necessary. Sample solutions of retinyl acetate and retinyl palmitate were stable over a period of 15 h in the dissolution medium composed of 1% (w/v) Octoxynol 9 and 1% (w/v) (+)-sodium L-ascorbate in 0.05 M phosphate buffer (pH 6.8).

3.3. Analysis of dissolution samples

3.3.1. HPLC method

In general, the analytical methods for analysis of sample solutions generated during tablet dissolution testing should be simple, specific, rapid, and, preferably, without extra sample preparation procedures. As mentioned earlier, the existing HPLC methods for the determination of vitamin A often include complicated sample preparations [2,6–10]. In this study a sensitive, selective, robust and rapid chromatographic method for analysis of retinol, retinyl acetate and retinyl palmitate in dissolution samples by direct injection of aqueous solutions was developed and validated. All three peaks were successfully separated within 15 min run time. A typical chromatogram of standard solution, representing retinol, retinyl acetate, and retinyl palmitate in dissolution medium is shown in Fig. 2(a).

3.3.2. Specificity

HPLC chromatograms of a blank solution, blank solution spiked with mixture of retinyl acetate and retinyl palmitate, and typical sample solutions obtained from dissolution testing of the dietary supplement are shown in Fig. 2(b–e). No interferences from the excipients or other vitamins and minerals with the peaks of interest were observed.

3.3.3. Linearity

Calibration curves were created at 6 different concentrations ranging from 0.3 to 20 IU/ml to cover the range from approximately 15% of the lower labeled dose strength (2000 IU/tablet for retinyl acetate in 900 ml) and up to 185% of the highest labeled amount (10,000 IU/tablet for retinyl palmitate in 900 ml). The highest concentration was chosen based on the USP requirements for vitamin A tablets, which allow to contain not more than 165.0% of the labeled amounts of vitamin A [2]. Hydrolysis of the ester forms was not observed in the study and therefore retinol determination was not necessary and linearity of retinol was not evaluated.

Table 2
Solubility of retinyl palmitate in different dissolution media (IU/ml) ± SD.

Surfactant/Surfactant conc.	0.25%	0.5%	1.0%	2.0%
Octoxynol 9	41.8 ± 3.8	44.7 ± 4.7	53.5 ± 4.2	58.2 ± 2.9
Polysorbate 80	–	1.6 ± 0.5	2.0 ± 0.2	2.2 ± 0.7
Polyoxyl 35 Castor Oil	–	2.4 ± 0.36	2.2 ± 0.7	2.9 ± 0.4

A linear relationship was observed between peak areas of retinyl acetate and retinyl palmitate and their nominal concentration with correlation coefficients of 0.9999 for both compounds. The percentages of *y*-intercept bias were in the ranges from 0.1% to 0.6% for retinyl acetate with nominal concentration of 2.5 IU/ml, and from 0.2% to 0.3% for retinyl palmitate with nominal concentration of 5.0 IU/ml. The results showed good correlation between the peak areas and concentration of both compounds.

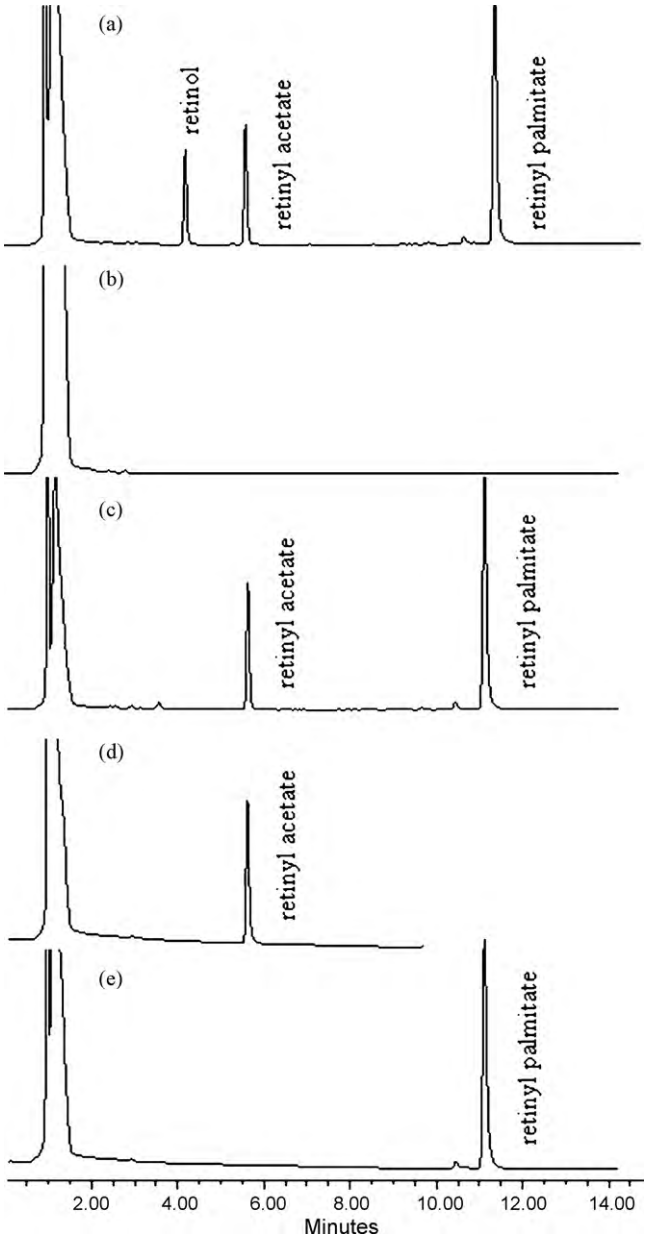

Fig. 2. A typical chromatograms of: (a) standard solution containing retinol, 2.5 IU/ml, retinyl acetate, 2.75 IU/ml, and retinyl palmitate, 9.0 IU/ml, in dissolution medium; (b) blank sample (product A); (c) blank sample (product A) spiked with mixture of retinyl acetate (2.5 IU/ml) and retinyl palmitate (5.5 IU/ml); (d) dietary supplement tablets containing retinyl acetate (product C); and (e) dietary supplement tablets containing retinyl palmitate (product H).

3.3.4. Accuracy and precision

The accuracy of the method was demonstrated by the recovery of spiked sample solutions where dissolution medium containing blank formulation was spiked with retinyl acetate and retinyl palmitate at about 15% (Lo), 100% (Me), and 185% (Hi) of the labeled amount. The mean recovery percentages ranged from 98% to 101% for both, retinyl acetate and retinyl palmitate. No issues regarding filter absorption were found.

The instrumental precision of the method was determined by making 5 consecutive injections of standard solutions of retinyl acetate or retinyl palmitate and calculating the response factors. The relative standard deviations (RSDs) were lower than 2.0%. The intraday precision of the method was evaluated at 3 different concentration levels of spiked samples on 2 different days. The results of intraday and interday accuracy and precision are reported in Table 3.

3.3.5. Robustness

The robustness of the method was determined by analyzing the standard solution with the following deliberate changes to the chromatographic conditions: column temperature ±4%, flow rate ±5%, and content of methanol in mobile phase ±3%. The calculated response factors of the peaks of interest and the performance of the chromatographic system were not influenced by the modified operational parameters.

3.4. Dissolution profiles and sample analysis

Seven dietary supplement products containing vitamin A in the form of retinyl acetate or retinyl palmitate with different strengths and from 7 different manufacturers were used for the dissolution study (Table 1). Two different rotation speeds, 50 and 75 rpm, were evaluated using USP Apparatus 2 and 900 ml of dissolution medium. The dissolution medium was a 0.05 M phosphate buffer solution (pH 6.8) containing 1% (w/v) Octoxynol 9 and 1% (w/v) (+)-sodium L-ascorbate. Dissolution experiments performed at 50 rpm showed incomplete dissolution of the vitamin A, which could indicate that stirring was not satisfactory. A higher amount of dissolved vitamin A was achieved by increasing the rotation to 75 rpm. Fig. 3

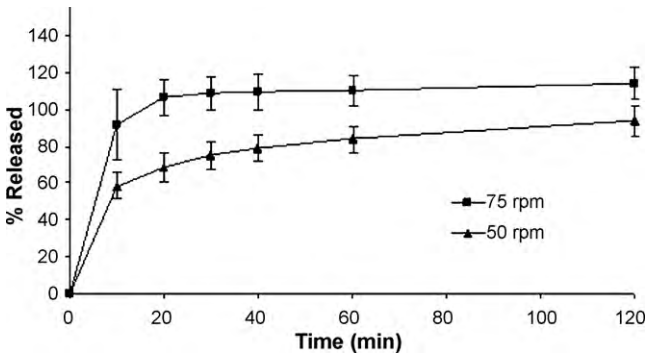

Fig. 3. Retinyl acetate dissolution profiles of dietary supplement tablets (product B) at different rotation speeds: 50 and 75 rpm.

Table 3
Intraday and interday accuracy and precision results obtained by spiking retinyl acetate and retinyl palmitate with dissolution media containing placebo formulation.

Vitamin A	Level	Concentration added (IU/ml)	Concentration found (IU/ml)	Recovery (%)	%RSD
Intraday ($N=3$)					
Retinyl acetate	Lo	0.33	0.33	101.0	1.0
	Me	4.44	4.41	99.3	1.5
	Hi	10.00	10.07	100.7	1.5
Retinyl palmitate	Lo	1.22	1.21	99.3	0.6
	Me	8.89	8.77	98.7	0.6
	Hi	19.44	19.38	98.3	1.2
Interday ($N=6$)					
Retinyl acetate	Lo	0.33	0.32	98.0	2.7
	Me	4.44	4.36	98.1	1.2
	Hi	10.00	9.82	98.2	2.4
Retinyl palmitate	Lo	1.22	1.22	99.8	0.4
	Me	8.89	8.62	98.0	1.0
	Hi	19.44	19.13	98.4	1.5

Table 4
Comparison of the results obtained from the dissolution samples and the *Assay* results.

Product	Label claim (IU) per tablet	Dissolution test (%released ± SD, $N=6$)	*Assay* results (%claimed)
B	2485 as retinyl acetate	110 ± 9.5	134.4
C	2485 as retinyl acetate	123 ± 12.6	104.6
D	2000 as retinyl acetate	84 ± 19.7	72.2
E	5000 as retinyl acetate	0	7.0
F	10,000 as retinyl palmitate	59 ± 8.5	48.5
G	10,000 as retinyl palmitate	42 ± 0.9	37.5
H	5000 as retinyl palmitate	101 ± 5.4	119.6

displays examples of the dissolution profiles from dietary supplement tablet, product B, recorded over 120 min at 50 and 75 rpm. Average data from 6 tablets were used to display each dissolution profile. The mean dissolution profiles ($N=6$) of retinyl acetate in products B–D and retinyl palmitate in products F–H using USP apparatus 2 at 75 rpm are shown in Fig. 4(a) and (b), respectively.

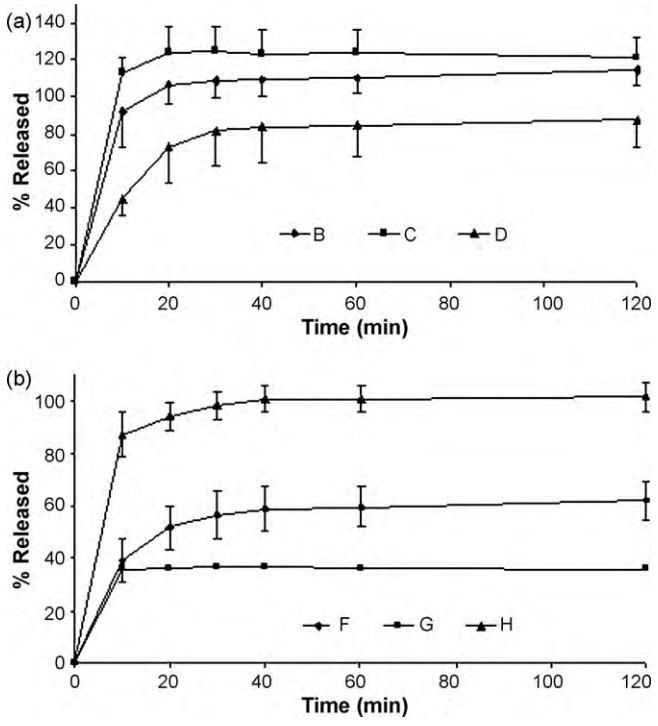

Fig. 4. Mean dissolution profiles ($N=6$) of retinyl acetate in products B–D (a) and retinyl palmitate in products F–H (b) using USP Apparatus 2 at 75 rpm.

The dissolution of retinyl acetate after 45 min reached 110% of the labeled claim in product B, 123% in product C, and 84% in product D. For products F, G, and H containing retinyl palmitate, the dissolution after 45 min was 54%, 37%, and 102% of the labeled claim. Product E, which was labeled as containing 5000 IU of vitamin A as retinyl acetate, did not show any vitamin A released in 45 min. The results from the dissolution tests were consistent with the *Assay* results (Table 4).

4. Conclusion

A novel dissolution test and HPLC methods were developed and validated to evaluate performance of dietary supplement tablets containing vitamin A. Complete dissolution of vitamin A in dietary supplement tablets could be achieved after 45 min using USP Apparatus 2 at 75 rpm in 900 ml of dissolution medium containing 1% (w/v) Octoxynol 9 and 1% (w/v) (+)-sodium L-ascorbate in 0.05 M phosphate buffer (pH 6.8). A reversed-phase HPLC method was shown to be accurate, precise, linear, and specific for the determination of vitamin A in dissolution samples. The new dissolution test can be proposed as a pharmacopeial standard to assess the performance of commercially available products, or in the process of development of new tablet formulations containing retinyl acetate or retinyl palmitate.

Acknowledgments

The authors would like to thank Junjie Hao, Zarema Kassymbek, and Pallavi Nithyanandan for their contributions to this work.

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