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Spectrophotometric determination of vitamin A in oily capsules using first derivative curves

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Abstract: The first derivative curve (D_1) of the absorption spectrum of vitamin A acetate in cyclohexane possesses a trough (D_{11}) at 348 nm and a maximum (D_{12}) at 306 nm. D_{11}, D_{12}, and ΔD_1 (= $D_{11} - D_{12}$) are linearly related to concentration over a range of 5–25 i.u. ml^{-1}. When a tangent is drawn between D_1 at 400 nm and D_1, at 306 nm, the amplitude at 348 nm (D_1 corr.) is linearly related to concentration. Ratios of $|D_{11}|/|D_{12}|$ and $D_1(\text{corr.})/|\Delta D_1|$ are independent of concentration, and have been used to reveal the presence of interferences in the D_1 curves of vitamin A in oily capsules. Vitamin A in two oily preparations has been assayed using ΔD_1 and D_1(corr.). The potency found was within ±2% from that obtained by using the B.P. method. The method is rapid, precise and accurate.

Keywords: *First derivative spectrophotometry; assay of vitamin A; vitamin capsules.*

Introduction

The three-point spectrophotometric assay of vitamin A [1] receives official recognition in the B.P. (1980) [2]. The method is designed to correct for linear irrelevant absorption under the absorption band of vitamin A. The direct recording of first-order derivative absorption curves (D_1) eliminates a constant irrelevant absorption, a difference between two readings ($D_{11} - D_{12}$) measured at λ_1 and λ_2, respectively, eliminates a linear irrelevant absorption [3–8] and a tangent or baseline applied to the D_1 curve eliminates a quadratic irrelevant absorption [9] in the sample. This paper describes the assay of vitamin A in two oily preparations by techniques based upon first derivative spectrophotometry.

Experimental

Apparatus

A Varian DMS 90 spectrophotometer with 1 cm quartz cuvettes was used.

First derivative spectra were recorded in the range 450–200 nm at a scan speed of 100 nm min^{-1}. The spectral bandwidth was 1 nm and the ordinate settings were ±0.10.

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The wavelength scale of the spectrophotometer was checked using the maxima of samarium perchlorate solution at 305.3, 317.6, 331.7, 344.6, 354.7, 362.4, 374.4 and 390.4 nm.

Materials

Vitamin A acetate. Crystalline vitamin A acetate was supplied in amber glass ampoules by Roche Laboratories (Basel, Switzerland). It was found to comply with pharmacopoeial specifications.

Vitamin A-containing oils. (1) "A-Vit", Wander, labelled to contain 50,000 i.u./capsule. (2) "Seven Seas", capsules (0.3 ml), British Cod-Liver Oil Ltd, labelled to contain: vitamin A, 600 i.u.; vitamin D, 60 i.u.; and vitamin E, 0.3 i.u./capsule.

Solvents. Spectroscopic grade cyclohexane (Koch-Light Lab. Ltd, Bucks, England) and analytical grade propan-2-ol (Winlab Ltd, UK) were used.

Determination of vitamin A in capsules

A solution of "A-Vit" or "Seven Seas" oil in cyclohexane was prepared to contain 10–25 i.u. of vitamin A ml^{-1} and the first derivative curve was recorded. D_{11} and D_{12} were read at 348 and 306 nm, respectively. D_1(corr.) was measured at 348 nm between D_1 and the point of intersection with the baseline (tangent) drawn at 400 and 306 nm (Fig. 1). From calibration graphs relating the different parameters to concentration of vitamin A acetate in cyclohexane, the concentration in units/capsule was computed using D_{11}, $\Delta D_1 (= D_{11} - D_{12})$ and D_1(corr.) for "A-Vit" and ΔD_1 and D_1(corr.) for "Seven Seas" preparations.

Results and Discussion

The first derivative curve of vitamin A acetate absorption spectrum in cyclohexane (Fig. 1) possesses a trough, D_{11} at 348 nm and a maximum, D_{12} at 306 nm which correspond with the highest slopes of the vitamin A acetate absorption curve.

Graphs of $|D_{11}|$, $|D_{12}|$, $|\Delta D_1| (= D_{11} - D_{12})$ and D_1(corr.) versus concentrations were found to be linear and to have the following equations

$$|D_{11}| = + 0.0005 + 0.03782\, c$$

$$|D_{12}| = + 0.0004 + 0.03802\, c$$

$$|\Delta D_1| = + 0.0007 + 0.07585\, c$$

$$D_1(\text{corr.}) = + 0.0003 + 0.06180\, c$$

where c is the concentration in mg/100 ml.

The correlation coefficients were found to be 0.9993, 0.9994, 0.9997 and 0.9991, respectively, indicating excellent linearities. Furthermore, D_{11}(1%, 1 cm), D_{12}(1%, 1 cm), ΔD_1(1%, 1 cm), and D_1(corr.)(1%, 1 cm) were found to be equal to 37.77, 38.01, 75.79, 61.8, and reproducible ($n = 7$) with relative standard deviations of 0.89, 0.85, 0.57 and 1.20%, respectively.

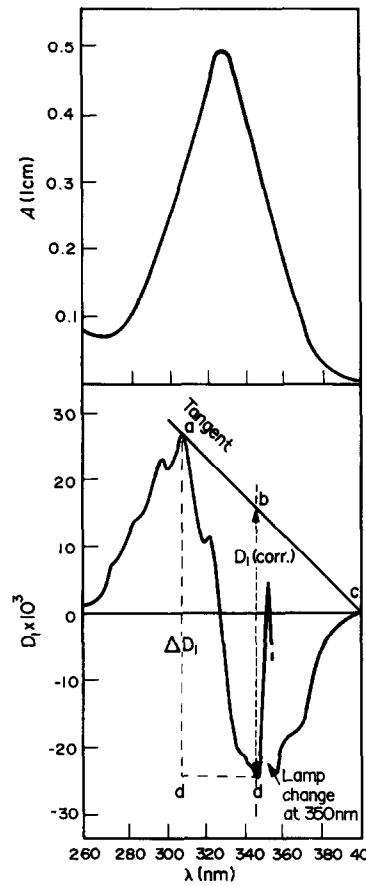

Figure 1
Absorption spectrum of 0.00066% w/v vitamin A acetate in cyclohexane and its first order derivative curve. [D_1 = dc, ΔD_1 = da, D_1(corr.) = bd]

Ratios of first derivative extrema [10]

Ratios of $|D_{11}|/|D_{12}|$, $|\Delta D_1|/|D_{11}|$, and $D_1(\text{corr.})/|\Delta D_1|$ for vitamin A acetate in cyclohexane were calculated for seven solutions in the concentration range 0.4–1 mg/100 ml. These ratios were found to be independent of concentration and equal to 0.996, 2.004 and 0.816, respectively, and to have relative standard deviations of 0.90, 0.68 and 1.23%, respectively. Each ratio was used as a criterion to test the validity of applying first derivative curves to the assay of vitamin A in oily preparations.

Spectrophotometric determination of vitamin A in the preparations using the B.P. method

Solutions of "A-Vit" and "Seven Seas" oil in cyclohexane were prepared to contain 9–15 i.u. of vitamin A ml^{-1}. The relative absorbances for "A-Vit" solutions indicated that the correction formula at 328 nm should be applied to obtain the potency of vitamin A. The linear irrelevant absorption which was observed to be present could have originated from the oil used or from any added antioxidant. The relative absorbances for "Seven Seas" solutions deviated by more than 0.02 of those specified by the B.P. Furthermore, the maximum absorbance was found to occur at 323 nm. Consequently, this sample was dealt with as described under "Other Vitamin A" in the official method.

This large deviation in relative absorbances and λ_{max} was attributed to the fact that the potency of the preparation was relatively low (600 i.u./capsule, 0.3 ml) and therefore, the contribution of the oil, any antioxidant and other constituents present in the preparation increased the absorbances at wavelengths shorter than A_{max}. Solutions of the unsaponifiable matter of "Seven Seas" oil in propan-2-ol were found to comply with the pharmacopoeial requirements with regard to λ_{max} and absorbance ratio. The relevant three-point equation was used to obtain the potency of vitamin A in this preparation.

Ratios of first derivative extrema for "A-Vit" and "Seven Seas" oil in cyclohexane

The first derivative curves for seven solutions of each of "A-Vit" and "Seven Seas" oil of suitable concentrations (10–25 i.u. ml^{-1}) in cyclohexane were recorded. Ratios of $|D_{11}|/|D_{12}|$, $|\Delta D_1|/|D_{11}|$, and $D_1(corr.)/|\Delta D_1|$ were calculated and compared with the mean reference ratio obtained above for pure vitamin A acetate.

In the case of "A-Vit", the percentage deviation of the ratio $|D_{11}|/|D_{12}|$ from the reference ratio was between +6.93 and +10.44. This means that the irrelevant absorption present contributed to both $|D_{11}|$ and $|D_{12}|$ or to only one of them. This can further be investigated by examination of the ratio $|\Delta D_1|/|D_{11}|$. This ratio showed percentage deviations from the reference ratio between −3.24 and −5.19. Although these deviations were lower than those obtained using the ratio $|D_{11}|/|D_{12}|$, it cannot be concluded that $|\Delta D_1|$ suffered from irrelevant absorption. The third ratio, i.e. $D_1(corr.)/|\Delta D_1|$, showed relatively small deviations from the reference ratio of +0.61 to +2.94%. Therefore, it was concluded that vitamin A ester in this this preparation was accompanied by a linear irrelevant absorption which could be corrected by using either ΔD_1 or $D_1(corr.)$. Thus, it was expected that results in good agreement with the official method would be obtained by the measurement of ΔD_1 and $D_1(corr.)$.

In the case of "Seven Seas" oil, the percentage deviation of the ratio $|D_{11}|/|D_{12}|$ from the reference ratio was between +39.6 and 50.6. This means that the irrelevant absorption present markedly affects the first derivative readings at λ_1 and λ_2. Moreover, the ratio $|\Delta D_1|/|D_{11}|$ showed deviations from the reference ratio by −13.7 to −16.7%. Such a large deviation means that the irrelevant absorption still contributes either to both readings or to only one of them, i.e. D_{11}. The ratio $D_1(corr.)/|\Delta D_1|$ was found to be in good agreement with the reference ratio, the deviation being between −1.23 and +1.47%. These results suggest that by using $|\Delta D_1|$ and/or $D_1(corr.)$, the potency of vitamin A in "Seven Seas" capsules can be accurately determined, *without saponification.*

The potency of vitamin A in "A-Vit" capsules was determined by the official B.P. three-point method (without saponification) and by the first derivative spectrophotometric methods. The mean potency obtained by using the B.P. method was found to be 50,690 i.u./capsule with a relative standard deviation (RSD) of 1.12%. The first derivative spectrophotometric methods using D_1, ΔD_1 and $D_1(corr.)$ gave results that were 100.9% ± 1.02 (RSD), 99.0% ± 0.93, and 99.3% ± 0.91, respectively, of that obtained by using the B.P. method. These results show that the type of irrelevant absorption was a constant to a linear function of wavelength and that its contribution was eliminated using ΔD_1 or $D_1(corr.)$.

The potency of vitamin A in "Seven Seas" capsules was determined by the official B.P. method after saponification and by the first derivative spectrophotometric methods *without* saponification. The mean potency obtained by using the B.P. method was found to be 676.1 i.u./capsule with an RSD of 1.87%. The first derivative spectrophotometric

methods using D_1, ΔD_1, and D_1(corr.) gave results that were 115.9% ± 2.31, 99.6% ± 1.59, and 99.1% ± 0.90, respectively, of that obtained by using the B.P. method. The high results obtained by using D_1 were considered to be unsatisfactory, whereas the other two results were satisfactory. This means that the irrelevant absorption present in "Seven Seas" oil was a linear function of wavelength of high contribution. This linear interference changed to a constant when the first derivative curve was recorded. The resultant constant curve was corrected by using ΔD_1 or D_1(corr.).

It is noteworthy that the official method for "Seven Seas" oil takes about 3 h to complete. The present method requires only about 10 min to record a first derivative curve and to compute the potency using the proper equations as there is no need for the saponification step. The method is rapid, versatile, accurate and easy to automate for routine analysis. Unlike the B.P. method, the present method is not affected by overall shifts in the wavelength scale or inaccurate wavelength calibration because measurements are made on extrema, difference between extrema or amplitude at the trough with respect to the tangent or baseline method. The method warrants further studies on other vitamin A-containing preparations to investigate the general application of the method.

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