AGRICULTURAL MATERIALS

Sources of Error in Vitamin A Analysis

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Potential sources of error in analysis of vitamin A in animal feed and pet food are discussed. Errors arising during sample handling and preparation and those due to standards, calibration, sample size, hydrolysis, extraction, evaporation, detection, equipment calibration, and isomerization are addressed. Quality control practices are recommended, and safety considerations are outlined. A summary of systematic and random errors is provided.

Large variances are observed in the results of analysis of vitamin A in the American Association of Feed Control Officials (AAFCO) Feed Check Sample Program. The large between-laboratory variations suggest variations in the way laboratories perform the analysis. One objective of the Fat Soluble Vitamin Subcommittee of the AAFCO Laboratory Methods and Services Committee is to publish guidelines listing sources of error that may be causing the large variances. This document should supplement review articles (1–4) and references herein should assist vitamin chemists in reducing interlaboratory variation. The following recommendations apply to both AOAC Official Method **974.29** (5) and to liquid chromatographic (LC) methods.

Vitamin A in Animal Feed and Pet Food

Vitamin A is labile and losses its activity by formation of stereoisomers and by autoxidation. Loss of biological activity is initiated or catalyzed by hydroperoxides of unsaturated fats, air, light, heat, moisture, mineral acids, metal ions, and other pro-oxidants. Vitamin A in the form of mixed stereoisomers of fatty acid (predominantly palmitate) esters as found in fish liver oils was the original source of the vitamin in feeds. Currently the most common source of vitamin A added to feeds is synthetic all-*trans*-retinyl acetate (or less frequently retinyl palmitate). The esters are more stable than the vitamin A alcohol, retinol. To protect vitamin A from the feed matrix, moisture, and air, synthetic products are manufactured into stabilized powders or cross-linked beadlets that consist of a gelatin, pectin, or similar matrix (6). Even in the form of beadlets, the stability of vitamin A varies with the feed matrix and with manufacturing and storage conditions (7, 8).

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Sample Handling and Preparation

Feed samples taken in the field must be protected from sunlight, excessive heat, and moisture during transport and in the laboratory. Localization of vitamin A in beadlets makes it more difficult to disperse the vitamin evenly throughout the feed and, therefore, more difficult to obtain a representative subsample. Therefore, analysts should take as large a subsample as possible, using the most scientific method, to ensure that the subsample represents the whole. (9)

The initial sample must be as large as possible. It must be mixed before any reduction steps and reduced only with a gated or rotary riffle. Analysts should not assume the vitamin A has been dispersed uniformly throughout the sample. The ground sample must be handled in the same manner as an easily segregated unground sample. The ground sample should be remixed thoroughly after grinding and again before a sample test portion is weighed. AOAC Method **974.29 E(a)** (5) requires grinding all of a 600–800 g sample such that 95% passes a No. 20 sieve, mixing, regrinding, and remixing. Improved recoveries of vitamin A are obtained on mineral mixes if they are analyzed without grinding. If it is necessary to grind a mineral mix to obtain a homogeneous sample, do so immediately before analysis. Pet food products and other high-fat materials show rapid loss of vitamin A after grinding and should be ground immediately before analysis.

Grinding does not always reduce the vitamin A beadlets in modern supplements. Vitamin A materials that reduce on grinding yield a more homogeneous ground sample; however, such samples are more often subject to rapid decomposition. Vitamin A beadlets that are not subdivided by grinding may yield more stable ground samples, but they will make it more difficult to obtain a representative test portion.

Most ground feeds stored in tightly closed quart jars in a freezer are stable for at least one month (10, 11). Chilled jars should be warmed slowly to room temperature before they are opened to avoid condensation of moisture on the feed.

Standards

Accurate analytical results are impossible to obtain if the purity of the external standard is not known accurately. Inaccuracy or impurity of standards is one cause of poor reproducibility among laboratories performing vitamin A analyses. Commercial standards are not of equal quality and are very susceptible to deterioration. One laboratory found that standards purchased from different suppliers gave readings from 50% of stated value to 140% when measured against the United States Pharmacopeia (USP) standard retinyl acetate (12).

USP vitamin A acetate is a consistently accurate standard material in the authors' laboratories. Therefore, USP retinyl acetate (ca 100 000 IU/g cottonseed oil with ca 1/4 g oil per ampule) standard material is recommended for use as external standard for chemical analyses of vitamin A and as a primary standard against which materials obtained from other sources are compared. The USP standard material is supplied as a relatively stable oil solution in soft ampules of a size suitable for a single day's analysis. Twenty-four such ampules are supplied with each bottle of standard material. If refrigerated and protected from light, the standard is stable as long as that lot of USP vitamin A acetate is listed in the USP catalog.

A method for determining the concentration of vitamin A ester in oily concentrates reported by a group of experts (13) is outlined below. The authors believe the method should not be used for determining vitamin A ester concentrations below ca 500 000 IU/g oil (ca 15% retinyl ester); however, the technique has been used successfully for quality control to verify the level of vitamin A in the USP standard oil (11). The $F_{1/a}$ factor (*see Quality Control*) can be used to indicate deterioration of vitamin A in the standard material. In addition, a UV scan (or absorbance ratios) of the standard as received can be used for comparison with spectra (or ratios) obtained at a later date to indicate standard deterioration. Even though examination of absorbance ratios of pure vitamin A was not recommended for vitamin A in oil at the level of the USP standard (13), these ratios can be determined and compared with theoretical values to indicate purity of the USP standard material. An LC method that allows separation of *cis* isomers of retinol from the more stable and biologically active all-*trans* isomer can be used to evaluate the quality of a vitamin A standard solution and to compare a secondary standard with the primary standard USP material.

Vitamin A as retinol, retinyl acetate, and retinyl palmitate are commercially available. Vitamin A purities listed in catalogs range from 70 to 99%; however, actual concentrations can vary from those stated. Even when the original material is of excellent purity, it may deteriorate as a result of improper packaging. The concentration at such high levels (15% or 500 000 IU vitamin A/g) can be determined by procedures reported by Bolliger et al. (13) and outlined below. Because of the susceptibility of vitamin A, especially retinol, to rapid deterioration through autoxidation, these materials should be divided and carefully resealed under vacuum or nitrogen immediately after they are opened and stored cold. Or a high-purity vitamin A ester may be dissolved in degassed oil (e.g., cottonseed oil)—some warming may be required for dissolution—and refrigerated in small, completely full vials with air-tight covers (e.g., autosampler vials). A new vial must be opened for each day's assay set.

Some synthetic vitamin A is sold dispersed in a cornstarch–gelatin matrix. Vitamin A in such a matrix should be more stable. However, the concentration of vitamin A in these products cannot be determined by the procedure suggested below. Instead, these materials should be saponified and the resulting retinol extract compared with a primary standard by the measurement technique used for analysis.

Calibration (wavelength and absorbance) of spectrophotometers used for determining standard concentration is essential. A high-quality narrow-band-pass spectrophotometer, calibrated for wavelength and absorbance accuracy, is necessary to obtain accurate results. The wavelength and absorbance accuracy of a spectrophotometer can be determined by using National Institute of Standards and Technology (NIST) standard test glass or by a glass traceable to NIST standard glass (e.g., Milton Roy No. 33-31-50; Fisher Science 14-385-335). Potassium chromate and dichromate solutions also may be used for spectrophotometer calibration (1). Solutions of potassium chromate and cobalt ammonium sulfate for spectrophotometric wavelength calibration in the visible and UV ranges are available commercially (Fisher Science 14-377-190). (Listing commercial products, or sources of such products, is not intended to be an endorsement of either but is presented to show that such products are available.)

If the result of the procedure is to be used for defining a standard, at least 3 replicates should be analyzed. 2-Propanol is a better solvent than ethanol for dissolving vitamin A standards in an oil matrix.

Procedure for Determining Concentration of Standard Vitamin A Solutions

Note: To avoid direct light, use actinic glassware or perform the procedure in low-UV (yellow) light.

(a) *Preparation of standard solutions (use class A glassware).*—Depending on the expected concentration of vitamin A in the standard, accurately prepare a working solution of the oil in 2-propanol to contain ca 10 IU vitamin A/mL. If purity of the standard material is to be calculated, record the weight (m) of the material to the nearest 0.1 mg and the total dilution volume (V) to the nearest 0.01 mL. If concentration of an intermediate stock solution is to be determined, record to the nearest 0.01 mL the volume of stock solution (v_1) used to prepare the working solution. Choose volumetric glassware for dilutions to minimize relative error and optimize precision (14).

(b) *Spectrophotometric measurement.*—The purity of retinyl ester in 2-propanol may be checked as follows: Measure and record absorbance of test solution at 300 (A_{300}), 326 (A_{326}), 350 (A_{350}), and 370 (A_{370}) nm in quartz cuvettes of 1 cm optical path length against 2-propanol. The vitamin A ester can be considered sufficiently pure if $(A_{300})/(A_{326}) < 0.593$, $(A_{350})/(A_{326}) < 0.537$, and $(A_{370})/(A_{326}) < 0.142$ (13). These ratios have been determined from pure retinyl acetate.

(c) *Calculations.*—The concentration of vitamin A is calculated with the following equations. Equation 1 is used to calculate the concentration in the working solution (IU/mL). Equation 2 is used to calculate the concentration in the stock standard (IU/mL). Equation 3 is used to calculate the concentration in the standard material (IU/g). An example calculation is shown in the appendix.

$$\text{Vitamin A, IU/mL} = (A_{326})\,(F) \quad (1)$$

$$\text{Vitamin A, IU/mL} = \frac{(A_{326})\,(V)\,(F)}{(v_{1})} \quad (2)$$

$$\text{Vitamin A, IU/mL} = \frac{(A_{326})\,(V)\,(F)}{(m)} \quad (3)$$

where m = mass of standard material (g), V = total dilution volume of working standard (mL), v_1 = volume of stock standard to prepare working standard (mL), and F = factor for converting absorbance to IU vitamin A = 18.27 for retinol and 19.09 for retinyl acetate and retinyl palmitate (*see* Appendix). Using the traditional rounded calculation factors of 19 instead of 19.09 and 18.3 instead of 18.27 makes little difference in calculation of results; however, use of rounded values can lead to confusion when calculations involving other constants (e.g. IU/mg and absorptivity) are involved. (Derivation of the equations and factors is explained in the appendix.)

The same factor F = 18.27 is used to calculate the concentration of retinol in 2-propanol, ethanol, or light petroleum. For retinyl acetate, the absorptivity, and therefore the calculation factor, changes with solvent: F = 18.66 for ethanol and F = 18.28 for light petroleum.

Calibration (Colorimetric Methods)

In most laboratories, calibration solutions are run with each sample set. Typically one standard is dissolved to a given volume of solvent and dilutions are prepared to generate the standard curve. This daily calibration is important to show system linearity and to verify between-day stability of the spectrophotometer or other measurement system. If the spectrophotometer is stable, it is more accurate to use a composite of daily calibration data to calculate results than to use results of a single calibration standard. Normally, more variability is associated with weighing and diluting one standard capsule, even if multiple hydrolyses and readings are made, than with day-to-day repeatability of the spectrophotometer. If a calibration curve is obtained each day, an ongoing average and standard deviation of the calibration slope and intercept must be maintained and sets that fall outside control limits must be discarded. The slope and intercept are calculated through measured points; do not force the zero intercept.

Instrument stability is not usually a major problem with current instruments. Spectrophotometer stability can be monitored by reading 2 solutions of methylene blue in 2-propanol before and after each run. Solutions with high (ca 0.70) and low (ca 0.35) absorbances are read. The solution(s) will be stable for several weeks. The spectrophotometer should rarely vary by more than 0.01 absolute units during runs or between days. Shifts in absorbance or a noisy recorder baseline indicate a bad lamp or detector.

Sample Size

Avoid the temptation to cut weights and/or volumes to increase sample throughput and reduce chemical usage. Do not miniaturize the method by using smaller sample weights and volumes. Use AOAC Method **974.29** (5) sample weights as minimums. These weights may represent less than 100 vitamin A-containing beadlets for some concentrations of vitamin A. Measuring a test portion of the sample to an exact weight may lead to a nonrepresentative portion because of segregation. To avoid segregation, accurately weigh grab portions of the sample to an approximate target weight.

Hydrolysis

Antioxidants and stabilizers minimize degradation of vitamin A during analysis. Suggested antioxidants are ascorbic acid and pyrogallic acid. Fresh peanut or cottonseed oil is added to low-fat (<5% fat) samples, and soybean meal may be added to mineral mixes to aid hydrolysis. However, excessive amounts of peanut or cottonseed oil will create extraction problems. Glycerol can be added to all samples to aid solubilization of gelatin capsules. Use of glycerol may be especially important if methanol is used in place of ethanol, because methanol is not as good a solvent for fat as ethanol.

In saponification of dry feeds and oil samples, alcohol is added to the sample in the reflux flask before addition of KOH; however, for liquid feed samples (e.g., viscous molasses) the following protocol should be followed:

(*1*) Mix weighed sample thoroughly with a volume of warm H_2O approximately equal to sample volume.

(*2*) Add slowly, with mixing, a volume of KOH solution equal to the sample weight.

(*3*) Add slowly, with mixing, a volume of alcohol (mL) 4 times the sample weight (g).

"Feed particles tend to gelatinize and stick during the digestion. Therefore, several times during digestion the flasks should be swirled, or shaken hard until complete dispersion is obtained. This seems to be a critical step" (15). Some laboratories use magnetic stirrers to provide gentle, continuous mixing during digestion. Others add magnetic stirring bars to the saponification flask during manual shaking to help disperse nonsoluble material. Use of freshly prepared KOH saponification solutions is recommended because such solutions may deteriorate in a few days.

After saponification, cool the flasks rapidly to room temperature by partial submersion in running tap water or an ice bath.

The dilution volume is affected by solids remaining after hydrolysis and may lead to a positive bias. No filtering is needed if residue volume is relatively small. However, inaccurate dilution factors may be a problem if a large sample size is used that significantly increases residue volume and reduces solvent volume in the hydrolysis flask. To avoid dilution error when large samples are used, filter the solids from the hydrolysate, rinse thoroughly, and take to an exact volume. Filtration

should be performed as quickly as possible and with care to minimize exposure of the filtrate to light and heat.

Extraction

Actinic (red) glassware or special low-UV (yellow) lighting should be used to minimize photochemical degradation of vitamin A.

"When appreciable amounts of carotenoids are present in the solution after hydrolysis and vitamin A content is low, a single extraction does not appear to remove vitamin A adequately, and two or more extractions may be required" (16). When the procedure gives a low bias, the digestion aliquot should be extracted a second time with a second volume of hexane, and the extract must be run as a separate sample. If the second extract contains more than 5% of the vitamin A found in the first hexane aliquot, additional extractions are needed.

In high-fat samples (>1 g fat/sample weight), extra soaps formed during saponification will change the partition coefficient in favor of the aqueous-alcohol phase. In these cases, more extractions are required to partition the retinol into hexane.

The quality of hexane used for extractions is critical. Recently, several laboratories traced problems with poor recovery of vitamin A to problems with hexane quality. If low bias or rapid color decay is experienced, try a different source of hexane.

The intensity of mixing and/or shaking while extracting with hexane is critical for complete recovery, especially if the extraction is done in centrifuge tubes. If recoveries are low, try shaking more vigorously for a more thorough extraction. Analysts should optimize their partitioning technique by measuring the relative recovery on various matrixes while varying the shaking time and technique.

Evaporation

Vitamin A solutions can degrade during solvent evaporation if high temperatures are used. Temperatures must be kept below 40°C. Vitamin A residue must not be exposed to air. It must be redissolved in appropriate solvent immediately upon drying. Chemists should optimize evaporation temperature and technique. A bad solvent lot also can cause loss of vitamin A during evaporation.

At this point in the assay, retinol in sample extracts may be more stable than that in standard extracts because of the protective action of components of the unsaponifiable fraction in sample residue. A greater loss of retinol in the standard extract relative to that in sample extracts will result in a high bias.

Detection (Colorimetric)

The AOAC-approved trifluoroacetic acid (TFA) method is easier to use than the antimony trichloride method. TFA is less corrosive, dissolves immediately, develops a more stable color, and cleans up much more easily (17, 18).

Equipment

Routinely calibrate all relevant equipment: spectrophotometers, balances, pipettors, and dispensers. Flasks used for saponification must be accurately calibrated if the saponified material is to be diluted to volume in them. Flasks should be recalibrated periodically to ensure that the original calibration is accurate.

Isomerization

Five geometric *cis* isomers of the more biologically active all-*trans* vitamin A are known. The most common is 13-*cis*-retinol, which has 75% of the activity of all-*trans*-retinol. The 11-*cis*-retinol has ca 23%, 9-*cis*-retinol has ca 22%, 9,13-di-*cis*-retinol has ca 24%, and 11,13-di-*cis*-retinol has ca 15% of the activity of all-*trans*-retinol (19). The *cis* isomers are less likely to occur in stabilized, synthetic vitamin A esters now commonly used than in the marine oils used as vitamin A sources in the past. An equilibrium mixture of *cis* and *trans* isomers will form with time when any one of the isomers is in solution. The isomerization process is very slow for retinyl ester in solution in antioxidant-stabilized oil; for example, it is slow in the USP standard material. However, isomerization is more rapid after dissolution in common solvents and especially after saponification of retinyl ester to retinol.

Loss of biopotency due to presence of retinol isomers can be estimated by the maleic anhydride procedure (5). Many LC methods do not separate the vitamin A isomers. Normal-phase LC and some reversed-phase systems may resolve the major *cis* isomers from the all-*trans*-vitamin A. With LC methods, identification of a peak as a *cis* isomer should be positively verified. Some *cis* isomers may be found in the USP standard extract. If more than a few percent of the vitamin A solution prepared from this material is observed as *cis* isomer, the procedure for preparing standard solution and the chromatographic process should be examined for factors contributing to formation of the *cis* isomer from all-*trans*-vitamin A.

Quality Control

Sound quality control (QC) practices should be established to ensure that the analyst will be aware any time the method is not functioning properly (out of control). Each run should include a reagent blank, a spiked sample, at least one set of duplicates, and one or more QC samples. QC samples must be chosen by matching their concentration and matrix to those of the samples in the run. For example, if a run consists of a mixture of high-concentration mineral mix and low-concentration complete feed samples, the run must include one of each type of QC sample. Results of QC samples should be examined for trends and for being within control limits. If samples fall out of control limits, the run should be discarded.

The factor $F_{1/a}$ should be a constant. When calculated for each run, it can serve as a check on the standard carried through each sample set and on the measurement system. Use of this factor as a check requires use of the same size cuvettes for each sample set. The factor is based on the inverse of the absorptivity ($a = A/bc$; if b is constant, $F_{1/a} = 1/a = c/A$), which is constant. The inverse is used for convenience, because it yields results greater than one and avoids use of fractions:

$$F_{1/a} = \frac{c}{A_{620}}$$

where c = concentration of the standard (IU/mL) and A_{620} is absorbance of the standard at 620 nm.

A plot of the mean of this value with suitable limits can be used with QC sample results to show that the assay is in a state of control.

Safety Considerations

The mixture of alcohol and KOH used for hydrolysis is corrosive. Gloves must be worn while handling these reagents to avoid contact with the skin. The solvents used in vitamin A analysis (hexane, ethanol, methanol, and isopropyl alcohol) are flammable. Open flames should be avoided while handling solvents, which must be used in ventilated areas. Antimony trichloride and trifluoroacetic acid are extremely corrosive. Gloves must be worn to avoid contact with the skin while handling these reagents. The reagents must not be inhaled. Chloroform and methylene chloride are potential carcinogens. Analysts must avoid inhaling them and must handle them with care in a hood.

Summary of Sources of Errors

Errors in analytical measurements are generally of 3 types: human errors, errors due to bias, and errors due to random causes (20, 21). Human errors are intermittent, occur at random, and vary in their effect on analytical results. These include errors such as misreading equipment or instruments, transposing digits when recording or entering data, and misreading method protocols. Errors due to bias, called systematic errors, are characterized by always being in the same direction (either positive or negative). Bias errors usually can be identified or characterized and then eliminated. Following is a list of frequent bias errors in vitamin A analysis.

(**a**) *Systematic errors producing a positive bias.*—(*1*) Presence of high levels of carotenoids in sample (AOAC colorimetric); (*2*) Presence of interfering substances such a phenothiazine, ethoxyquin, or Rabon (AOAC colorimetric); (*3*) Excessive solids remaining after hydrolysis, affecting dilution volume (AOAC colorimetric and LC); (*4*) Less than complete recovery of standard material carried through partitioning step of extraction procedure (AOAC colorimetric and LC); (*5*) Excessive standing of the standard solution in hexane after partitioning step before evaporation (AOAC colorimetric and LC); (*6*) Failure to remove standards from evaporators immediately after removal of hexane (AOAC colorimetric and LC); (*7*) Moisture in cuvettes (AOAC antimony trichloride colorimetric); (*8*) Cloudy solutions in cuvette (AOAC colorimetric); and (*9*) Standard concentration actually lower than value used in calculations (impure or degraded standard solution; AOAC colorimetric and LC).

(**b**) *Systematic errors producing a negative bias in LC and colorimetric methods.*—(*1*) Allowing samples to sit too long, sit in heat, be exposed to light, or be exposed to oxidants; (*2*) Grinding of mineral mix samples; (*3*) Failure to completely solubilize vitamin during saponification; (*4*) Failure to add antioxidant or stabilizer(s) during hydrolysis; (*5*) Failure to completely disperse sample during hydrolysis; (*6*) Old (more than a few days) KOH solution; (*7*) Failure to work with low actinic glassware and/or in subdued lighting; (*8*) Incomplete partitioning of vitamin A from hydrolysate into organic solvent; (*9*) Inclusion of Na_2SO_4 or some of particulates from digest when taking aliquots; (*10*) Problems with hexane quality; (*11*) Failure to remove samples from evaporators immediately after removal of hexane; (*12*) Moisture in pipets or cuvettes; and (*13*) Standard concentration actually higher than value used in calculations.

Random errors produce variability between replicates in analysis and are unpredictable fluctuations. Random errors (precision) include such things as noise in instrument response, sample heterogeneity, and variations in time of refluxing and extracting. Following is a list of random errors typical in vitamin A analysis.

(**c**) *Random errors producing poor precision in LC and colorimetric methods.*—(*1*) Failure to thoroughly mix sample before weighing; (*2*) Failure to properly reduce sample (riffle); (*3*) Use of insufficient sample weight to ensure a representative sample; (*4*) Use of a single standard reading from a single day to make calculations; and (*5*) Unstable spectrophotometer or detector.

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Appendix: Vitamin A Calculation Factors

The calculations presented are based on Beers Law: $A = abC$, where A is spectrophotometric absorbance at a specific wavelength; a is absorptivity constant, which is specific for a wavelength and compound; and C is concentration (mg/mL). The path length of the light passing through the solution containing the compound, b, is assumed to be 1 cm.

$$C, \text{ mg/mL} = \frac{A}{a}$$

$$C, \text{ IU/mL} = \left(\frac{A}{a}\right) \times \text{IU/mg unit conversion factor}$$

The absorptivity and unit conversion factors frequently are combined into a single calculation factor (constant): calculation factor (F) = IU/mg unit conversion factor/a, so that in equation 1 the concentration of vitamin A in the diluted solution is determined as shown:

$$\text{Vitamin A, IU/mL} = (A_{326}) \times (F) \qquad (1)$$

Multiplying equation 1 by the amount the stock standard was diluted, equal to the total dilution volume (V), and dividing by the volume of the stock standard solution (v_1) yields equation 2:

$$\text{Vitamin A, IU/mL} = \frac{(A_{326}) \times (V) \times (F)}{(v_1)} \qquad (2)$$

To calculate vitamin A activity in the standard material, multiply equation 1 by the total dilution (V) and divide the equation by the mass (m) in grams weighed:

$$\text{Vitamin A, IU/mL} = \frac{(A_{326}) \times (V) \times (F)}{(m)} \qquad (3)$$

Table 1 contains constants and calculation factors frequently found in the vitamin A literature. The notes associated with the table and the discussion in the Appendix explain how the tabulated values were obtained.

Example Calculations

(*1*) To determine the concentration of vitamin A as retinyl acetate in the stock standard solution where standard material was dissolved in 100 mL 2-propanol and then diluted 5/100 for measurement in a 1 cm cell, $A_{326} = 0.530$, and $V = 100 \times 100/5 = 2000$. According to equation 2:

$$\text{Vitamin A, IU/mL in stock solution} = \frac{0.530 \times 2000 \times 19.09}{100} = 202 \text{ IU/mL}$$

Table 1. Vitamin A constants and calculation factors in 2-propanol

Vitamin A form	IU/mg (USP)	*a* (absorptivity)[a]	Calculation factor (F)[b]
Retinol (R)	3333[c]	182.4[d]	18.27
Retinyl acetate (RA)	2907[e]	152.3[d]	19.09
Retinyl palmitate (RP)	1819[e]	95.3[f]	19.09[f]

[a] Absorptivity (a) = E(1%, 1 cm)/10.
[b] F includes constants for absorptivity and unit conversion. That is, F = IU/mg/a, to 4 significant figures. The use of E(1%, 1 cm), based on concentration in g/100 mL, resulted in calculation factors such as 1830 (3 significant figures) and 1900 (2 significant figures) in traditional literature. Based on Beer's Law, the Fs here are 1/100 of traditional values.
[c] Reference 22; 3300 and/or 3330 IU vitamin A/mg retinol used prior to 1985.
[d] Reference 23; Absorptivities (R and RA) were calculated from the mean ϵ_{max} values determined in this paper: ϵ_{max}/MW = a (MW = molecular weight). Small differences between values calculated here and in paper apparently result from using different significant figures in calculation. The authors state that the calculation factor based on the extinction coefficient (1%, 1 cm) of 1906 was rounded to 1900 in World Health Organization (1960) literature.
[e] IU vitamin A/mg ester = 3333 IU A/mg R $\times$ MW_R/MW_{ester}. If 3330 IU A/mg R is used in calculation, the frequently quoted values of 2904 IU/mg RA and 1817 IU/mg RP are obtained.
[f] Reference 13 authors use the same rounded value, 1900, to calculate IU vitamin A/g RA or RP. This yields a calculated E(1%, 1 cm) of 957 (95.7 as an absorptivity). The absorptivity of 95.3 is equal to 1819/19.09.

(2) To determine the concentration of vitamin A in an oil matrix expected to contain ca 100 000 IU/g according to equation 3, weigh 0.2000 g (m) standard retinyl acetate in cottonseed oil (i.e., ca 20 000 IU vitamin A) into a 100 mL class A volumetric flask. Dissolve in 2-propanol and dilute to volume. Dilute 5.00 mL to 100.00 mL with 2-propanol (V = 2000 mL). Transfer to a 1 cm quartz cuvette and determine absorbance vs 2-propanol in a calibrated spectrophotometer. If an absorbance of 0.530 is obtained, what is the concentration of vitamin A activity in the USP standard?

$$\text{Vitamin A, IU/mL in standard} = \frac{0.530 \times 19.09 \times 2000}{0.2000} =$$

$$101\ 177 \approx 101\ 000 \text{ IU vitamin A/g oil}$$