Assay of Vitamin A Oils

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EDITOR'S NOTE. *This article, published by Nature on February 8, 1958 (vol. 181, p. 395), is accepted for publication in our journal also because so many members of the American Oil Chemists' Society, in the U.S.A. and abroad, work with vitamin A in marine oils, margarine, feeds, etc. Furthermore considerable impetus was given to development of the present "official" methods for vitamin A analysis through suggestions by the A.O.C.S. vitamin committee (Oil and Soap, 23, 275–276 [1946]).*

The quotations below from an announcement dated December 27, 1957, from the United States Pharmacopeia show that the U.S.P. method is being brought into close agreement to the above recommendations for category 1.

The following U.S.P. XV interim revision has been approved, to become effective as of April 1, 1958:

It should be noted that this revision provides specifically for only two of the three possible situations arising from applying the familiar Morton-Stubbs correction to the observed ultraviolet absorbance of a vitamin A-containing material: a) where the correction is not more than ± 3%; b) where the correction exceeds 3% in the direction of reducing the observed absorbance at 325 mμ; and c) where the correction, if applied, would increase the observed absorbance by more than 3%. In the first-named situation the correction is disregarded as being within the experimental error, and in the second situation the correction is applied as giving a more nearly correct indication of the true content of vitamin A. No provisions are made for the last-named case since it is interpreted as indicating the presence of some constituent that makes the material under assay unsuitable for pharmaceutical purposes. A result of this kind constitutes evidence that the sample fails to meet the requirements of the assay as revised.

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Vitamin A Assay, p. 941. Delete the section, Calculation, and substitute therefor the following:

Calculate the vitamin A content as follows:

$$\text{Vitamin A content (in mg.)} = 0.549\left(\frac{A_{325}}{L \times C}\right)$$

in which A_{325} is the observed absorbance at 325 mμ, L is the length, in cm., of the absorption cell and C is the amount of sample expressed as g., capsule, or tablet in each 100 ml. of the final isopropyl alcohol solution, provided that A_{325} has a value not less than A_{325}(corr.)/1.030 and not more than A_{325}(corr.)/0.970 where

$$A_{325}(\text{corr.}) = 6.815\ A_{325} - 2.555\ A_{310} - 4.260\ A_{334}$$

in which A designates the absorbance of the wavelengths indicated by the subscripts.

Where A_{325}(corr.) has a value less than $A_{325}/1.030$ the following holds:

$$\text{Vitamin A content (in mg.)} = 0.549\left(\frac{A_{325}(\text{corr.})}{L \times C}\right)$$

in which the values are as defined above.

Since 1 U.S.P. unit of vitamin A is represented by 0.003 mg. of vitamin A alcohol, the vitamin A content (in U.S.P. units) may be calculated by substituting 1,830 for 0.549 in the equations given above.

IN 1954 a Vitamin Assay Commission was set up by the Food Division of the Applied Chemistry Section of the International Union of Pure and Applied Chemistry. The official members of the Vitamin Assay Commission are: E. Brunius (Sweden, chairman), W. F. J. Cuthbertson (Great Britain), M. Kofler (Switzerland), B. L. Oser (United States) and H. Simonnet (France). The Commission has held four conferences: in London 1954, Zurich 1955, Amsterdam 1956, and Paris 1957. In all the conferences, besides members of the commission, the following participated: D. W. Kent-Jones (Great Britain, honorary secretary of the Food Division), R. Nicolaysen (Norway, member of the Food Division), E. Hayes (Great Britain), and R. J. Taylor (Great Britain). In addition the following were present at the Amsterdam conference: J. Straub (The Netherlands, member of the Food Division), and C. Engel (The Netherlands), and at the Paris conference: K. Dürrenmatt (Switzerland, chairman of the Food Division), J. Brüggemann (Germany), C. Engel (The Netherlands), A. Francois (France), and J. Tiews (Germany).

One of the Commission's tasks was to develop a standardized method for the assay of vitamin A oils which would be acceptable in international trade.

Current vitamin A assay methods are based on the recommendations made by the subcommittee on fat-soluble vitamins of the World Health Organization. In its report (1) International Unit of vitamin A potency is defined in terms of the activity of crystalline all-*trans* vitamin A acetate, and the conditions for the spectrophotometric assay of the vitamin are broadly set down. The elaboration of methods in different countries however has led to divergences in the assessment of vitamin A potency, and this has given rise to difficulties in the international trade in vitamin A oils. It was therefore considered an urgent task for the Commission to attempt to develop a standardized method of assay.

Vitamin A oils vary widely in quality and potency. At the outset the Commission decided, on the evidence available, to consider them in two categories depending on whether their spectrophotometric characteristics approached those of pure vitamin A or not. For oils in the first category (comprising most fish-liver oils and vitamin A concentrates) a correction procedure based on the Morton-Stubbs principle (2) was considered sufficient. For oils in the second category (comprising whale-liver oils and other oils having a high proportion of irrelevantly light-absorbing materials) a chromatographic step should precede the determination of extinction and the application of correction. All measurements should be made on the unsaponifiable matter of the oils. It was suggested that this should be isolated by the method described in the United States Pharmacopeia.

The Commission organized three collaborative experiments, in which laboratories in Canada, Europe, Japan, and the United States cooperated. Thirty-seven laboratories participated in the first experiment, 34 in the second, and nine in the third.

The main purposes of the experiments were: a) to ascertain the loss of vitamin A in isolating unsaponifiable matter of oils according to the method of the United States Pharmacopeia; b) to investigate whether isopropyl alcohol and absolute ethanol could be used as alternative solvents in measuring extinction; c) to determine the constants for a correction formula based on the Morton-Stubbs principle (2); d) to ascertain the loss of vitamin A in a chromatographic method suggested for samples in the second category; e) to investigate the selectivity of this method; f) to compare two techniques for following the movement of vitamin A through the chromatographic column; and g) to estimate the experimental errors in the vitamin A potencies of oils in both categories as assayed by the procedure suggested.

A vitamin A reference standard containing all-*trans* vitamin A acetate in vegetable oil (nominal potency 100,000 I.U. per g.) and samples of oils belonging in the second category were used as test materials.

In the chromatography the unsaponifiable matter of the sample was passed through a column of weakened alumina, and the vitamin was eluted with mixtures of light petroleum and ether. The movement of the vitamin was followed by testing aliquots of the eluate with antimony trichloride solution and, in the third experiment, alternatively by observing the column in weak ultraviolet light. Two different samples of alumina were used by most participants.

A statistical screening procedure, based on Chauvenet's criterion (3), was applied to the results.

The main outcome of the experiments can be summarized as follows. The average loss of vitamin A in isolating the unsaponifiable matter of the vitamin A reference standard was found to be 1.2% in the first experiment and 1.2–1.5% in the second. Isopropyl alcohol and absolute ethanol could not be recommended as alternative solvents because

the respective E_{310}/E_{325} and E_{334}/E_{325} ratios differed significantly in the two solvents, which gave rise to systematic errors when correction was applied. The correction formula derived from isopropyl alcohol from the results of the first and second experiments was:

$$E_{325} \text{ corr.} = 6.815\ E_{325} - 2.555\ E_{310} - 4.260\ E_{334}$$

The average loss of vitamin A in chromatographing the unsaponifiable matter of the vitamin A reference standard amounted to 2.4% irrespective of the kind of alumina used. The average total loss of vitamin A, that is, the losses in isolating and chromatographing the unsaponifiable matter, was 3.5–4%. After chromatography the samples of oils belonging in the second category readily met the specifications laid down for oils in the first category. The two procedures for following the movement of vitamin A through the column were equally efficient and could thus be recommended as alternative techniques. The interlaboratory error(s) at $P = 0.95$ for a duplicate assay of vitamin A potency was estimated at 6–8% for an oil in the first category and at 10–14% for an oil in the second category. The error of the mean of assays performed at n different laboratories—each of them making a duplicate assay—will accordingly be $s/\sqrt{n}$.

For vitamin A oils showing negligible amounts of irrelevant light absorption, the corrected E value should theoretically be identical with the uncorrected E value but, because of the experimental error due to the correction procedure, positive corrections must sometimes be encountered. About half the assays on the vitamin A reference standard in the first and second experiments showed positive corrections. In the first experiment 14 out of 15 positive corrections did not exceed 2.5% and in the second 29 out of 30 positive corrections did not exceed 3.5%.

The Commission now makes the following recommendations for the assay of vitamin A oils.

1. All oils shall be saponified, and the assay made on the unsaponifiable matter.

2. The unsaponifiable matter shall be isolated by the method of the United States Pharmacopeia XV (4).

3. All spectrophotometric measurements shall be made in isopropyl alcohol as solvent.

4. Oils shall be considered in two categories:

Category 1: those oils, the unsaponifiable matter of which has an absorption maximum lying within the region 323–327 mμ and an extinction ratio E_{300}/E_{325} not exceeding 0.73.

Category 2: those oils, the unsaponifiable matter of which has an absorption maximum lying outside the region 323–327 mμ or an extinction ratio E_{300}/E_{325} exceeding 0.73.

5. For oils in category 1 the E_{310}, E_{325} and E_{334} values of the unsaponifiable matter shall be measured and a correction applied according to the formula:

$$E_{325} \text{ corr.} = 6.815\ E_{325} - 2.555\ E_{310} - 4.260\ E_{334}$$

6. For oils in category 2 the unsaponifiable matter shall be chromatographed according to a prescribed procedure. Provided the chromatographed unsaponifiable matter satisfies the criteria for category 1 oils, its E_{310}, E_{325} and E_{334} values shall be measured and a correction applied as directed under 5.

7. Corrected E_{325} value shall be used for calculating vitamin A potency, except in the cases mentioned under 8.

8. A corrected E_{325} value which lies within $\pm$ 3.0% of the uncorrected value shall be regarded as equal to the uncorrected value for the purpose of evaluation, and the uncorrected value shall be used for calculating vitamin A potency. A corrected E_{325} value which is greater than 1.030 times the uncorrected value shall be re-determined and, if confirmed, treated as a special case between buyer and seller.

9. The E_{325} value is converted into $E^{1\%}_{1cm.}$ 325 mμ. The vitamin A potency of the sample, expressed in I.U. per gram, is obtained by multiplying the $E^{1\%}_{1cm.}$ 325 mμ value by 1830 (5), that is, the factor of the reference quoted, taken to three significant figures.

Detailed reports of the Commission's collaborative work and of the recommended procedure for assay will be published elsewhere.

REFERENCES

1. Report of the Subcommittee on Fat-Soluble Vitamins, WHO/BS/65 Rev. 1 (1949); World Health Org. Techn. Rep. Ser., No. 3 (1950).
2. Morton, R. A., and Stubbs, A. L., Analyst, *71*, 348 (1946).
3. Chauvenet, "Theoretical and Practical Astronomy;" 2. Quoted from Brunt, D., "The Combination of Observations," 130 (University Press, Cambridge, 1923).
4. "The Pharmacopeia of the United States of America," Fifteenth revision, 941 (Mack Publishing Co., Easton, Pa., 1955).
5. Boldingh, J., *et al.*, Nature, *168*, 598 (1951).

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Industry Items . . .

Davis and Bennett Inc., Worcester, Mass., a 35-year-old organization of consulting chemists, chemical engineers, and biologists, was recently acquired by FOSTER D. SNELL INC., New York, N. Y.

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E. H. SARGENT AND COMPANY, Chicago, Ill., manufacturer and distributor of laboratory equipment and supplies, has started construction of a plant in Springfield, N. J., to house its new eastern division. The 106-year-old firm recently acquired the glass-blowing company of Otto R. Greiner, Newark, N. J., which will be moved to the Springfield location.

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A new company, California Chemical International Inc., has been formed to take over the expanding export market of ORONITE CHEMICAL COMPANY, San Francisco, Calif., a subsidiary of the Standard Oil Company of California.

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Great Lakes Carbon Corporation has closed its diatomite plant at Walteria, Calif., and has moved the equipment to Lompoc, Calif., thereby consolidating two plants of the DICALITE DEPARTMENT, Mining and Mineral Products Division.