Rapid Estimation of Vitamin A Using a Surface Active Agent

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An improved procedure employs a single-step extraction for determining naturally occurring vitamin A in chicken livers, synthetic vitamin A in fortified powdered formulas for infants, and stabilized vitamin A in animal feed supplements. Stabilized vitamin A is determined in the presence of *N,N'*-diphenyl-*p*-phenylenediamine by a simple modification of the procedure. The vitamin A is solubilized in the test material with the surfactant and extracted with a mixed solvent. Twenty-five minutes are required for one extraction and several may be run simultaneously. The procedure gives good recovery replication, and results comparable to those obtained by saponification.

THE DETERMINATION OF VITAMIN A in food products usually requires saponification (*2*), which in the case of milk may take as long as 3 hours (*3*), as well as numerous lengthy extractions. A more rapid analytical procedure is therefore desirable. Although surface active agents have been employed in vitamin analyses (*9*), their use has been confined to a preliminary step for separating the oil from milk. The present procedure is based on the solubilization of lipide material by a surfactant, and extraction of the vitamin A with a mixed solvent. Although the method was originally developed for determining vitamin A in powdered formulas for infants, with slight modifications it was equally well suited for similar determinations in other materials.

Analysis of Food

A 1.0- to 5.0-gram sample is weighed, transferred to a 100-ml. glass-stoppered cylinder containing 25 ml. of a surface active agent, and mixed thoroughly. The surface active agent consists of 12 grams of Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.) and 28 grams of sodium tripolyphosphate (Victor Chemical Works, Chicago, Ill.) dissolved in 800 ml. of water and diluted to 1000 ml. with methanol (*10*). Then 25 ml. of mixed solvent (petroleum ether, boiling point 30° to 60° C., and diethyl ether, 10 to 90 by volume) is added. The cylinder is placed on a mechanical shaker for 15 minutes. After the solvent layer has separated, a suitable aliquot is removed, placed in an Evelyn tube, and evaporated to dryness under vacuum while the tube is swirled in a hot water bath (*5*). The residue is dissolved in 2 ml. of chloroform. The chloroform solution is usually clear; if not, the evaporation is repeated. The vitamin A content of this solution is determined by the Carr-Price method, using an Evelyn photoelectric colorimeter.

Table I. Recovery of USP Standard Vitamin A Acetate Added to a Fortified Infant Formula Using Surfactant

Vitamin A Increment, γ	Vitamin A Measured, γ	Recovery, γ	Recovery, %
0.	2.52	...	...
1.20	3.62	1.10	92
1.80	4.26	1.74	97
2.40	4.92	2.40	100
3.00	5.52	3.00	100

Analysis of Feed Supplements

Feed supplement samples are extracted as follows: A 20-gram sample is placed in a 250-ml. glass-stoppered Erlenmeyer flask containing 30 ml. of surfactant; 10 ml. of methanol is added to aid in separation. The sample is mixed well and extracted by shaking vigorously for 15 minutes with 50 ml. of mixed solvent. An aliquot containing 50 γ vitamin A is evaporated to dryness, using mild heat and reduced pressure. The residue is taken up in 10 ml. of hexane (Skellysolve B). From this point the chromatographic and colorimetric steps are followed as in the AOAC procedure (*4*).

Feed supplement samples containing *N,N'* - diphenyl - *p* - phenylenediamine (DPPD) and fortified with the vitamin A embedded in a matrix (Rovimix, Hoffmann-La Roche, Nutley, N. J.) are given a preliminary washing with ethyl ether. After all traces of ether have been removed, the samples are extracted and chromatogrammed as above.

Results and Discussion

The recovery of various increments of vitamin A is tabulated and summarized in Table I. These results are based on six replicate samples of a powdered formula fortified with vitamin A palmitate. A known amount of USP standard A oil was added to each replicate sample and extracted as described in the experimental procedure. The table indicates that the percentage recoveries are well within the limits of error (*2*).

Table II lists replicate analysis of

samples containing various types of vitamin A. Liver and the powdered formula gave good replication of results, while the feed supplement containing a stabilized vitamin A (Rovimix) gave somewhat erratic results. This may be due to the method of sampling (*8*). Shaking the sample for 15 minutes with a single extraction appears to be satisfactory.

Tests using the surface active agent are compared with tests with the unsaponifiable fraction (*7*), calculated as milligrams of vitamin A per kilogram, in Tables III and IV. Results have been calculated from a standard curve and are not based on the use of an internal standard. There appears to be a good correlation between the result for unsaponifiable fraction and that obtained from the surfactant extraction.

Liver storage of vitamin A has attracted much attention, because biological activity of vitamin A in foods can be determined in less time by measurement of liver storage than by rat growth. The surfactant extraction procedure is applicable to liver assay. It has been demonstrated that vitamin A can be readily extracted from liver without saponification, if the tissue is dried with anhydrous sodium sulfate prior to the ether extraction (*1*). The surfactant extraction eliminates the need for drying the tissue. Phase separation is rapid and complete.

The feed supplement samples listed in Tables III and IV are fortified with a stabilized vitamin A (Rovimix). The AOAC method of analysis (*4*) is satisfactory for many feedstuffs, but with some feeds the insolubility of stabilized vitamin A in organic solvents necessitates a different type of extraction (*8*). The extraction procedure described in this paper allows chromatographic separation of stabilized vitamin A in feed supplements.

The presence of *N,N'*-diphenyl-*p*-phenylenediamine in feed supplements necessitates an additional purification step. As the stabilized vitamin A (Rovimix) is insoluble in organic solvents, and *N,N'*-diphenyl-*p*-phenylenediamine is soluble, a preliminary washing of the sample with ether can effectively remove most of the *N,N'*-diphenyl-*p*-phenylenediamine from the sample.

Inasmuch as the vitamin A ester is eluted before carotene (*4*) and carotene is eluted before *N,N'*diphenyl-*p*-phenylenediamine (*6*), vitamin A can be separated from *N,N'*-diphenyl-*p*-phenylenediamine if left in the ester form. The vitamin A can be extracted in the ester form by the above procedure. Results of tests of a feed supplement containing various levels of vitamin A and *N,N'*-diphenyl-*p*-phenylenediamine are listed in Table IV. These results were obtained by the experimental procedure.

Table II. Replicate Vitamin A Estimations on Various Materials

Sample	Absorbance	Vitamin A, Mg./Kg.
Chicken liver	0.1788	53.4
	0.1707	51.1
	0.1659	49.3
	0.1643	48.7
	0.1723	51.6
	0.1707	51.1
Mean 50.9		
S.D. 1.69 (or 3.3%)		
S.E. 0.69 (or 1.35%)		
Fortified powdered infant formula	0.325	12.3
	0.328	12.4
	0.330	12.5
	0.323	12.2
	0.323	12.2
	0.319	12.1
Mean 12.3		
S.D. 0.148 (or 1.2%)		
S.E. 0.060 (or 0.49%)		
Animal feed supplement	0.2499	6.3[a]
	0.2460	6.2[a]
	0.2756	7.0
	0.328	8.3
	0.297	7.5
	0.2656	6.7
	0.314	7.9
	0.303	7.6
	0.2656	6.7[b]
	0.2756	7.0[c]
Mean 7.1		
S.D. 0.690 (or 9.6%)		
S.E. 0.22 (or 3.1%)		

[a] Shaken 7 minutes.
[b] Shaken 20 minutes.
[c] Extracted three times.

Table III. Comparison of Surfactant Extraction and Saponification Procedures

Sample	Vitamin A, Mg./Kg. Unsap.	Vitamin A, Mg./Kg. Surfactant	100 × Surfactant / Unsap.
Fortified powdered infant formula	10.9	10.9	100
	12.0	11.7	98
Chicken liver	13.2	13.0	99
	24.0	23.7	99
	37.5	37.8	101
	45.0	47.7	106
	83.0	84.0	101
Animal feed supplement	3.45	3.42	99
	8.64	8.58	99
	24.8	24.2	98
	48.0	48.7	102
Animal feed premix	126	132	105
	180	180	100
	210	205	98

Table IV. Extraction of Feed Supplement Fortified with Stabilized Vitamin A and Containing *N,N'*-Diphenyl-*p*-phenylenediamine

Calculated Potency, Mg./Kg.	Measured Potency, Mg./Kg. 0% DPPD	0.25% DPPD	0.50% DPPD
67.0	62.6	63.4	64.3
39.6	40.7	42.0	41.1
20.2	20.2	19.3	20.4
6.7	6.7	6.7	6.5

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Correction

In the article on "Presence of Levoglucosan in Cornstarch Hydrolyzates," [Ough and Rohwer, J. AGR. FOOD CHEM. **4,** 269 (1956)] Figure 2 contains an error. The labels for Hydrolysis 5 and Hydrolysis 6 were reversed.