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

Acknowledgment

The authors wish to express their gratitude to H. W. Howard, The Borden Co., for encouragement and suggestions in the course of the study, and to S. R. Ames, Distillation Products Industries, for his constructive criticism.

Literature Cited

(1) Ames, S. R., Risley, H. A., Harris, P. L., *Anal. Chem.* **26,** 1378 (1954).
(2) Assoc. Vitamin Chemists, "Methods of Vitamin Assay," 2nd ed., pp. 25–35, Interscience, New York, 1951.
(3) Boyer, S. D., Spitger, R., Jensen, C., Phillips, P. H., *Ind. Eng. Chem., Anal. Ed.* **16,** 101 (1944).
(4) Christiansen, J. B., Koehn, R. C., *Anal. Chem.* **21,** 593 (1949).
(5) Cooley, M. L., *J. Assoc. Offic. Agr. Chemists* **34,** 371 (1951).
(6) Mitchell, H. R., Silker, R. E., J. AGR. FOOD CHEM. **1,** 1163 (1953).
(7) Oser, B. L., Melnick, D., Pader, M., *Ind. Eng. Chem., Anal. Ed.* **15,** 725 (1943).
(8) Parrish, D. B., Smith, H. A., *J. Assoc. Offic. Agr. Chemists* **39,** 126 (1956).
(9) Patton, S., *J. Dairy Sci.* **35,** 324–8 (1952).
(10) Sanders, G. P., Sager, O. S., *Can. Dairy Ice Cream J.* **32,** No. 3, 74–5, No. 4, 80 (1953).

Received for review August 18, 1955. Accepted March 8, 1956.

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.