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FLUOROMETRIC DETERMINATION OF VITAMIN A IN STOCKFOOD CONCENTRATES AND PREMIXES

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SUMMARY

Concentrates and premixes with a wide range of retinol (vitamin A) concentrations were analysed by both the A.O.A.C. method and a rapid fluorometric method. Results from the two methods compare very favourably but the fluorometric method has limitations on some samples.

I. INTRODUCTION

The method for retinol in the "Official Methods of Analysis of the Association of Official Agricultural Chemists" (Association of Official Agricultural Chemists 1965, p. 755) for mixed feeds involves reaction of retinol with antimony trichloride in the Carr Price reaction. This method is tedious and difficult to use on an intermittent basis. Shortcomings have been listed by Ames (1966), by Parrish and Aguilar (1969) and in "Methods of Vitamin Assay" (Association of Vitamin Chemists Inc. 1966).

When suitably irradiated, retinol isomers exhibit a strong blue-green fluorescence whose excitation and emission wavelengths (uncorrected) are 330–340 nm and 480 nm respectively.

This fluorescence is intense and a detection limit of 0·001 ug/ml has been reported by Aaron and Winefordner (1972). Fluorimetry has been applied to the analysis of retinol in blood and tissue by Drujan, Castillon and Guerrero (1968) and by Garry, Pollack and Owen (1970), but reports of its use for retinol analysis of stockfood concentrates have not been found.

II. MATERIALS AND METHODS

Twenty-three commercial concentrates and premixes representing a wide range of retinol concentrations and sample types were selected. These were analysed by the A.O.A.C. method and by the fluorometric method described below.

An estimation of the precision of the fluorometric method was made by analysing one sample on five successive days.

As an additional check on the procedure, two samples containing quite different retinol levels were selected. To the appropriate analytical sample weight of each of these premixes was added a known weight of standard retinol oil containing approximately the same amount of retinol as had been found by prior analysis to be present in the samples. These mixtures were then analysed by the proposed fluorometric method.

Fluorometric Method

(a) *Reagents and Apparatus*

Reagents used included distilled ethanol (aldehyde free), distilled hexane, saturated aqueous potassium hydroxide and anhydrous sodium sulphate prewashed with distilled chloroform. All glassware was carefully washed to remove traces of detergent or grease.

The spectrofluorimeter used was a Baird Fluorispec SF 100E. Standard retinol was U.S.P. Vitamin A—Reference Standard Capsules—supplied by Roche Products Pty. Ltd.

(b) *Sample Analysis*

Sample preparation.—Grind a representative portion of the sample *finely,* rapidly remix and sub-sample for analysis.

Saponification and extraction of retinol.—Carry out as in "Official Methods of Analysis of the Association of Official Agricultural Chemists (Association of Official Agricultural Chemists 1965, p. 757). Dilute an aliquot of the final retinol/hexane solution to give a concentration of less than 5 i.u./ml for reading on the fluorimeter.

(c) *Saponification and Extraction of Standard Retinol*

A standard retinol/hexane solution of known concentration is prepared by the saponification and extraction procedure used on the sample. A reagent blank omitting only the retinol standard should also be carried through the method.

(d) *Reading on the Fluorimeter*

Dilute a suitable aliquot of the standard retinol/hexane solution to give a solution of known concentration ($\leqslant$ 5 i.u./ml) close to that of the diluted sample solution. Dilute the reagent blank in a similar way.

Set the fluorimeter up as follows (uncorrected spectra):—

Excitation wavelength 330–340 nm

Emission wavelength 480 nm

Adjust gain and slit settings to give a midscale reading for the sample. Using the same excitation, emission, gain and slit settings for standard, sample and reagent blank, read the solutions on the fluorimeter in order—

standard, sample, standard, reagent blank.

From the readings and dilution factors determine the retinol concentration of the premix.

III. RESULTS AND DISCUSSIONS

Table 1 lists results of samples analysed by both methods. From the mean ratio of the A.O.A.C. to fluorometric methods of 0·996 it is apparent that for these samples both methods give similar results.

TABLE 1

RETINOL ANALYSIS OF STOCKFOOD PREMIXES AND CONCENTRATES
i.u./lb x 10^{-3}

No.	A.O.A.C.	Fluorometric	A.O.A.C. / Fluorometric
1	181·55	185·04	0·981
2	93·31	93·21	1·001
3	115·56	114·86	1·006
4	1451·8	1577·62	0·920
5	2115·17	2056·36	1·028
6	1319·43	1379·37	0·956
7	539·31	519·53	1·038
8	175·57	179·47	0·978
9	826·68	830·32	0·995
10	686·1	691·80	0·992
11	1700·0	*..	..
12	1582·0	*..	..
13	835·73	845·73	0·988
14	8649·95	8819·95	0·981
15	9437·44	9511·79	0·992
16	3334·73	3166·56	1·053
17	994·23	1008·22	0·986
18	22340·99	22202·29	1·006
19	6167·33	5829·62	1·057
20	5323·61	5409·89	0·984
21	2025·86	2143·06	0·945
22	2576·93	2550·03	1·010
23	2164·21	2124·98	1·018
			Mean=0·996 S.D.=0·0329 S.E. of mean=0·0072

* Marked fluorometric interferences—method not applicable.

The confidence intervals for this mean ratio were calculated to be $\pm$ ·015 (P = ·05) and $\pm$ ·020 (P = ·01).

Results for one of the samples analysed five times by the fluorometric method together with results of the analysis of the spiked samples are presented in Table 2. The precision compares satisfactorily with that of the A.O.A.C. method as reported in "Methods of Vitamin Assay" (Association of Vitamin Chemists Inc. 1966) and with that reported by Katz, Fassbender and Dorfman (1971) for the fluorometric determination of chlortetracycline in mixed feeds present at similar levels to the retinol.

TABLE 2

FLUOROMETRIC ANALYSIS OF SAMPLE 1

1. 193·37 (i.u./lb x 10^{-3})
2. 185·04
3. 184·12
4. 190·75
5. 183·27

MEAN = 187·31
S.D. = 4·478
C.V. = 2·39%

RESULTS OF ANALYSES OF THE SPIKED SAMPLES

Sample	Nominal Retinol Content	% Retinol Recovered
A	185,000 i.u./lb	102·5
B	3,200,000 i.u./lb	96·8

Vitamin A is commonly present in feed premixes in small starch/gelatin beadlets. This coating is not completely soluble in the ethanolic potassium hydroxide saponification mixture and thus it is necessary to assist this extraction by fine grinding of the analytical sample immediately before analysis. Exposure of retinol to light and oxygen should be minimized during the analysis.

The concentration of the final retinol/hexane solution to be read on the fluorimeter should be kept to less than 5 i.u./ml. At higher concentrations quenching leads to non-linearity and a shift of the excitation peak.

Excitation and emission peaks for standard and sample should occur at similar wavelengths. Any marked departure from these settings by the sample probably results from the presence of interfering fluorescent substances in the sample. The method is not applicable to such a sample.

Interfering fluorescent substances may also arise by extraction from cotton wool, filter paper, etc. by organic solvents.

Limitations of the fluorometric method are its inapplicability to samples containing fluorescent interferences and its lack of allowance for fluorescence quenching by ultraviolet absorbing species other than retinol.

Work is continuing to extend the method to samples containing interfering substances, although in its present form it is rapid, convenient for intermittent use and applicable to a wide range of feed premixes.

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