structures. Their pharmacological effects are quite different even though the molecules are presumably unchanged in vivo vs. the solid state (Canepa et al., 1966). This implies that the portions which are not chemically and structurally in common are causing the observed variations in the inhibition of AChE. Similarly, there is a strong case for steric involvement in the mechanism of many, if not all, OP insecticides. Consequently, the precise distances afforded by X-ray crystallographic techniques will prove indispensable in the overall study of these processes.

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Supplementary Material Available. A listing of the observed and calculated structure factor amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.50 for photocopy or $2.50 for microfiche, referring to code number JAFC-75-811.

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An Improved Automated Determination of Riboflavine in Food Products

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A continuous flow scheme employing in-line permanganate oxidative cleanup has been developed in conjunction with a rapid sample preparation procedure for the automated determination of riboflavine in food products. It was shown that permanganate oxidation is required for accurate results. The automated procedure was compared with an accepted manual method for 61 different food products showing a correlation coefficient of 0.9869 and an overall standard error between methods of 0.23 mg/100 g (10.8% relative). The automated method showed a pooled relative standard deviation of 3.3% between duplicate preparation with riboflavine levels ranging from 0.05 to 43.6 mg/100 g. It was shown that an internal standard was not required in the automated method by obtaining recovery values with each sample and observing an average recovery of 100.7 ± 3.1%. The study demonstrated that the automated procedure allows rapid sample analysis without sacrificing accuracy on the majority of food products studied.

With the increased demand for nutrient analysis in food products, there exists a definite need for faster methods of analysis. Continuous flow automation is one approach to this problem.

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A semiautomated method for riboflavine in food products has been reported (Technicon Instrument Co., 1972); however, the method neglects permanganate treatment for the elimination of interfering fluorescent material and offers no justification for use of an external standard. The use of an internal standard has been recommended due to the possible effects of a complex matrix, such as a food hy-

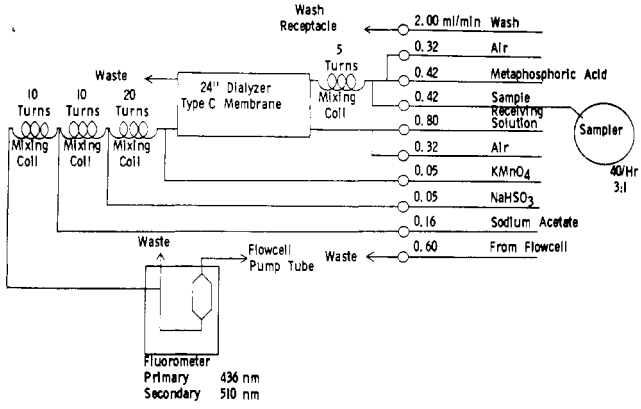

Figure 1. Flow scheme used for the determination of riboflavine. All transmission lines are glass.

drolysate, on riboflavine fluorescence (Strohecker and Henning, 1965; Association of Official Analytical Chemists, 1970).

This method (Technicon Instrument Co., 1972) was successfully employed for the analysis of riboflavine in milk but was reported to require manual permanganate oxidation in cereals and some selected vegetables (Kirk, 1974). However, no data were reported indicating the magnitude of the error encountered when permanganate treatment was omitted. Also, low recoveries were observed for certain cereals, carrots, and fresh tomatoes indicating that perhaps an internal standard was required in certain cases.

It was the intent of this project to: (1) determine if permanganate oxidation is required for accurate results, and if so, to incorporate this oxidative cleanup step into the flow scheme; (2) devise as simple a sample preparation as possible consonant with good results; (3) determine if an internal standard is required; (4) compare the automated method with accepted methodology on a broad spectrum of products thus placing the accuracy and generality on a firm basis; (5) ascertain if enzymatic hydrolysis is desirable in the sample preparation and compatible with the proposed flow scheme.

EXPERIMENTAL SECTION

Apparatus. The flow scheme shown in Figure 1 was constructed from the following Technicon AutoAnalyzer components: sampler IV; pump III; fluoronephelometer equipped with 436-nm excitation filter and 510-nm emission filter; and recorder. A Hewlett Packard Model 10 programmable calculator was used to obtain a best fit curve from the set of standards. A small diamter glass electrode (Corning No. 476050) was used to adjust pH in the volumetric flask. A dispensing unit (Oxford P-5058-1) was used for the rapid addition of base to the sample hydrolysate.

Reagents. The metaphosphoric acid solution was prepared by dissolving 20 g of metaphosphoric acid in 1 l. of distilled water and adjusting the pH to 1.9 with sodium acetate. Sodium chloride (30 g) was added along with 1 ml of Brij 35 solution [35% aqueous solution, surfactant (Atlas Chemical)]. The reagent was stored at room temperature and prepared fresh weekly.

The wash solution was prepared by adjusting the pH of 2 l. of 0.1 *N* HCl to 4.3 using a solution of 1.25 *N* sodium acetate. Dilute acetic acid (2 l., pH 4.3) was added to the solution followed by 4 ml of Brij solution. An equal volume mixture of the wash and the metaphosphoric acid solution was used as the dialysate receiving solution.

The sodium bisulfite solution was prepared by dissolving sodium bisulfite (1.1 g) in 50 ml of pH 7 phosphate buffer (4.55 g of KH_2PO_4 and 9.45 g of Na_2HPO_4 per l. of distilled water). If the recorder noise level exceeded 1.0% with the high riboflavine standard (0.15 μg/ml), the sodium bisulfite level was increased by 0.1-g increments until the noise was eliminated. This was done to ensure only a minimum excess of bisulfite. Once the proper bisulfite concentration was determined, it was not necessary to repeat this procedure until the pump tubes were changed. The solution was prepared fresh daily. The air oxidation of bisulfite was decreased by slowly bubbling nitrogen through the bisulfite solution while in use.

The 1% potassium permanganate solution was prepared fresh weekly and the sodium hydrosulfite solution was prepared just prior to use by dissolving 2.0 g of sodium hydrosulfite in 100 ml of 0.4% sodium acetate solution. The mixture was stable for about 2 hr when kept in an ice bath.

Procedure. The samples were ground to pass a 40 mesh screen. A portion of material containing about 7 μg of riboflavine was added to a 100-ml amber volumetric flask followed by 50 ml of 0.1 *N* hydrochloric acid, taking care to wash the sides of the flask. The samples were autoclaved for 30 min at 15 psi (exhausted slowly to prevent bumping) and allowed to cool to room temperature. A set of standards (1, 3, 5, 10, and 15 μg) was autoclaved with the samples after carefully adjusting the volume to 50 ml with 0.1 *N* HCl (same as samples). A sodium acetate solution (about 6.0 ml of 1.25 *N*, volume predetermined so that when added to 50 ml of 0.1 *N* HCl the final pH was 4.3) was dispensed into the samples and standards followed by the addition of about 35 ml of pH 4.3 acetic acid. Any sample differing from the standards by more than 0.1 pH unit was adjusted to the pH of the standards using a narrow glass electrode. The samples and standards were diluted to volume with pH 4.3 acetic acid and filtered through glass fiber filter paper (Whatman GF/A) after thorough mixing.

When enzyme digestion was employed, the samples were prepared as described above except 5 ml of Takadiastase solution (5% in pH 4.3 acetic acid) was added after the addition of the sodium acetate solution to both the standards and the samples. The mixture was heated at 37° overnight prior to final pH adjustment, diluted to volume, and filtered.

The standard and sample filtrates were pumped through the flow scheme shown in Figure 1 (40 samples/hr, sample to wash ratio, 3:1) after adjusting the 0.15 μg/ml standard to 100% recorder pen deflection. A 0.10 μg/ml standard was aspirated every 15 samples to correct for any system drift. Blank fluorescence was determined by replacing the dilute sodium acetate line with the hydrosulfite solution and pumping the samples through the system a second time.

A best fit second-order polynomial was obtained from the set of standards using a programmable calculator and the riboflavine concentration was determined by comparing the peak height minus the blank with the set of standards.

Duplicate analyses were conducted on different days and the results compared with an AOAC method (Association of Official Analytical Chemists, 1970). The duplicate analyses were separate sample preparations. With certain products, a Florisil adsorption–elution purification step was employed with the manual method (Association of Vitamin Chemists, 1947).

RESULTS AND DISCUSSION

The flow scheme shown in Figure 1 differs from the published method (Technicon Instrument Co., 1972) by incorporation of permanganate oxidation into the flow scheme. The problem that existed with this permanganate incorporation was the precipitation and build up of manganese dioxide in the coil where the excess permanganate was reduced with sodium bisulfite. This manganese dioxide formation caused a drift in the riboflavine response. Since the manganese dioxide formation originated with localized oxidation of manganous ion by unreacted permanganate, the problem was eliminated by the addition of a manganous sequestering ligand. Metaphosphoric acid proved to be the

Table I. Comparison of the Automated Method with and without Permanganate Treatment vs. the Manual AOAC Methods

Sample ready-to-eat cereal	Automated[a] (with $KMnO_4$) ± SD, mg/100 g	Automated method recovy, % (5 μg added to sample)	Automated[a] (without $KMnO_4$) ± SD, mg/100 g	Automated method recovy, % (5 μg added to sample)	Manual[a] AOAC ± SD, mg/100 g
1. Oat + corn (colored)	3.02 ± 0.04	101	3.72 ± 0.15	102	2.90 ± 0.15
2. Buckwheat + wheat	5.26 ± 0.09	102	5.67 ± 0.12	99	5.81 ± 0.28
3. Oat	2.59 ± 0.00	102	2.70 ± 0.13	98	2.71 ± 0.01
4. Corn (colored)	2.79 ± 0.04	101	3.46 ± 0.10	98	2.59 ± 0.27
5. Corn	7.50 ± 0.18	102	7.95 ± 0.56	93	7.89 ± 0.41
6. Oat + corn (colored)	3.18 ± 0.04	100	3.95 ± 0.07	99	3.11 ± 0.29
7. Corn	3.60 ± 0.03	101	4.11 ± 0.21	94	3.50 ± 0.14
8. Oat + corn (colored)	2.42 ± 0.04	99	3.45 ± 0.08	100	2.59 ± 0.27
9. Oat	2.59 ± 0.01	106	3.46 ± 0.19	105	2.61 ± 0.16
10. Corn (colored)	3.74 ± 0.09	103	4.51 ± 0.25	103	3.53 ± 0.24
11. Wheat	2.86	102	2.51	98	2.71 ± 0.02
12. Oat	0.13 ± 0.01	101	0.69	110	0.21
	Av, 3.31 mg/100 g Pooled SD, 0.07 mg/100 g Rel SD, 2.1%	Av ± SD, 101.6 ± 1.7%	Av, 3.85 mg/100 g Pooled SD, 0.23 mg/100 g Rel SD, 6.0%	Av ± SD, 99.9 ± 4.6%	Av, 3.35 mg/100 g Pooled SD, 0.23 mg/100 g Rel SD, 6.9%

[a] Standard deviation derived from duplicate sample preparation and when not shown only singlet analysis conducted.

most successful complexing agent since it did not interfere with the riboflavine fluorescence as did the other ligands tested (α,α-dipyridyl and 8-hydroxyquinoline). With this modification only a minor drift in the riboflavine response was observed and periodically corrected.

It was shown that there was no sample carry-over at 40 samples/hr and sample to wash ratio of 3 to 1. This was demonstrated by pumping ten replicates of a sample extract (0.11 μg/ml, containing Takadiastase) and eight consecutive replicates of a standard (0.15 μg/ml) through the system with no significant increase in fluorescence from the first to the last replicate (relative standard deviations of 0.37 and 0.12%, respectively).

The one-flash sample preparation procedure described in the Experimental Section was designed so as to keep the manual manipulation to a minimum and in this respect amounts to a substantial improvement over that previously reported (Association of Official Analytical Chemists, 1970). Direct hydrolysis in a volumetric flask eliminated quantitative transferring; however, frothing can be a problem unless proper precaution is exercised during autoclave exhausting. It was not a problem during the course of this study.

The riboflavine fluorescence is pH dependent and it was found that if the samples differed from the standards by more than ±0.1 pH unit, an unacceptable error was encountered (greater than 1.0%). By accurately adding (rapid dispensing unit) sodium acetate solution to the 50 ml of 0.1 *N* HCl which was accurately added to the samples and standards, the final pH rarely deviated from 4.3 ± 0.1 (isoelectric point of many proteins) and required pH adjustment only when the sample changed the pH. Of the 61 products used in this study only three required pH adjustment which was rapidly effected by dipping a narrow glass electrode directly into the volumetric flask.

It has been reported that riboflavine adsorption is eliminated with ashless filter paper (Association of Official Analytical Chemists, 1970). We observed, however, an average decrease of 5% when 100 ml of riboflavine standards (0.03–0.4 μg/ml) was filtered through Whatman 44. The problem was circumvented by the use of Whatman GF/A glass fiber filter paper.

A best fit second-order polynomial was obtained (programmable calculator) from the set of standards to account for the slight nonlinearity observed. The blank fluorescence was determined by pumping the samples through the system a second time with the hydrosulfite solution replacing the dilute sodium acetate solution. The riboflavine concentration of the samples was determined by relating the decrease in fluorescence to the riboflavine standards.

A set of 12 ready-to-eat (RTE) cereals was analyzed using the automated method with permanganate oxidation in the flow scheme and without the oxidative cleanup. The flow scheme shown in Figure 1 was modified by removing the permanganate and bisulfite to obtain this comparative data. The same hydrolysate was analyzed with both flow schemes. Duplicate preparations of most samples were analyzed on separate days and a recovery was determined on one of the duplicates by adding a known amount of riboflavine prior to autoclaving. The results were compared with a manual riboflavine method employing permanganate oxidation and an internal standard. The results are shown in Table I. When permanganate oxidation is not used, the set of values are systematically higher (student's *t* test, 99% confidence) than the manual. This establishes that the oxidation of interfering fluorescent material is necessary to preclude artificially high results for some products. There was no significant systematic difference between the automated method employing automated permanganate treatment and an external standard and the manual method

Table II. Comparison of the Automated Method with and without Enzymatic Hydrolysis vs. the Manual AOAC Method

Sample	Without enzyme[a] automated ± SD, mg/100 g	Automated method recovery, % (5 μg added to sample)	With enzyme automated ± SD, mg/100 g	Automated method recovy, % (5 μg added to sample)	Manual AOAC[a] ± SD, mg/100 g	Manual AOAC with Florisil column cleanup, mg/100 g
1. Wheat flour	0.31 ± 0.01	101			0.30 ± 0	
2. RTE cereal (wheat)	2.74 ± 0.08	102	2.80	108	2.71 ± 0.02	
3. RTE cereal (oat–corn)	1.23 ± 0.08	103	1.24	105	1.30 ± 0.22	
4. Enriched salt	43.59 ± 0.45	97	43.60	98	43.54	
5. Cocoa	0.62 ± 0.01	91	0.61	77	1.48 ± 0.18	0.44 ± 0.01
6. Powdered milk	2.17 ± 0.01	101	2.30	102	2.18	1.73 ± 0.04
7. Powdered egg white	3.50 ± 0.29	103	3.61	103	3.03 ± 0.18	2.44
8. Powdered egg	1.09 ± 0.03	101	1.15	102	1.16 ± 0.06	1.12
9. Dried dates	0.23 ± 0.01	93	0.27	95	0.74 ± 0.02	0.23 ± 0.01
10. Almonds	0.86 ± 0.02	105	1.00	100	0.94 ± 0.08	0.68
11. Corn snack	0.92 ± 0.02	99	0.95	100	0.96	
12. Tomato spice mix	0.58 ± 0.03	97	0.58	102	0.65	
13. Tomato powder	0.76 ± 0	101	0.78	101	1.55	0.69 ± 0.07
14. Sour cream sauce	0.20 ± 0.01	101	0.22	99	0.23	
15. Cheese blend	9.23 ± 0.08	97	9.26	94	9.59	
16. Textured soy flour	0.99 ± 0.03	101	1.14	98	1.20	
17. Spice mix	1.68 ± 0	95	1.79	98	2.62	1.76
18. Spice mix	1.37 ± 0.01	102	1.54	98		1.36
19. Spice mix	0.99 ± 0	101	1.06	101	1.91 ± 0.08	1.20 ± 0.23
20. Wheat cereal	0.37 ± 0.03	99	0.41	95	0.39	
21. Dried yeast	3.65 ± 0.01	102	3.70	96	4.54 ± 0.12	
22. RTE cereal (graham)	4.71 ± 0.17	97	4.92	91	4.81 ± 0.55	
23. Oats	0.13 ± 0	97	0.15	99	0.20 ± 0.04	
24. Wheat	0.11 ± 0.02	98	0.11	99	0.18 ± 0.05	
25. Chocolate pudding	0.13 ± 0.01	101	0.14	106	0.12 ± 0.01	
26. Banana pudding	0.13 ± 0	101	0.14	102	0.15	
27. Cooked ham	0.21 ± 0.01	111	0.19	101	0.25 ● 0	
28. Bologna	0.14 ± 0	104	0.13	107	0.15 ± 0.01	
	Av, 2.95 mg/100 g Pooled SD, 0.09 mg/100 g Rel SD, 3.0%	Av ± SD, 100.0 ± 3.8%	Av, 2.99 mg/100 g	Av ± SD, 99.1 ± 5.9%	Av 2.96 mg/100 g[b] Pooled SD, 0.17 mg/100 g Rel SD, 5.3%	

[a] Standard deviation derived from duplicate sample preparation and when not shown only singlet analysis conducted. [b] Florisil column cleanup values used.

employing permanganate treatment and an internal standard.

It has been reported that enzymatic hydrolysis aids in riboflavine extraction for some products (Strohecker and Henning, 1965). Also, it was of interest to determine if enzymatic hydrolysis would be compatible with this procedure, since the common extract could then be used for thiamine analysis as well.

Twenty-seven samples of food products were analyzed by the automated method both with enzymatic hydrolysis (singlet analysis) and without enzymatic hydrolysis (duplicate preparation). Recoveries were determined on one preparation of each sample by the addition of riboflavine prior to autoclaving and the results are shown in Table II. The same amount of enzyme added to the samples was added to the set of standards; hence, any error caused by the riboflavine found in the Takadiastase (0.85 μg/g) was eliminated. The small but statistically significant difference between means (0.04 mg/100 g, t test, 99% confidence) could be explained by more efficient extraction of the riboflavine when enzymatic hydrolysis was employed in addition to acid hydrolysis. Thus, it was demonstrated that enzymatic hydrolysis is desirable and compatible with the automated system.

The question of whether or not an internal standard is required was resolved by obtaining a recovery value for every product studied. An average recovery of 100.7 ± 3.1% (based on single assay) and the comparison with the manual method employing an internal standard established that the external standard used with the automated method gives sufficiently accurate results. However, some products did give troublesome recoveries, presumably due to matrix effects (see cocoa, dates, and ham in Table II).

As shown in Tables I–III, the automated method compared favorably with the manual method for most products studied. A correlation coefficient of 0.9869 existed between the automated and the manual method. The three high level products, 7.5 mg/100 g (Table I) and 43.59 and 9.23

Table III. Comparison of the Automated Method with the Manual AOAC Method for Some Low Level Products

Sample	Automated ± SD,[a] mg/100 g	Automated method recovy, % (5 μg added to sample)	Manual AOAC[a] ± SD, mg/100 g	Manual AOAC with Florisil column cleanup, mg/100 g
1. Potato chips (flavored)	0.11 ± 0.01	101	0.23 ± 0.05	0.14
2. Potato chips	0.27 ± 0.03	103	0.23	
3. Bisquick mix	0.29 ± 0	102	0.27 ● 0.02	
4. Textured soy product	0.56 ± 0.01	105	0.55	
5. Textured soy + egg product	0.30 ± 0.01	103	0.33	
6. Textured soy + ham product	0.12 ± 0.01	99	0.11	
7. Textured soy + beef product	0.18 ± 0.01	105	0.26 ± 0.01	
8. Corn	0.05 ± 0.01	103	0.07 ± 0.01	
9. Cake mix	0.30 ± 0	99	0.34	
10. Cake mix	0.08 ± 0	99	0.15 ± 0.01	
11. Cake mix	0.18 ± 0	98	0.21 ± 0.4	
12. Frosting mix	0.11 ± 0	97	0.30 ± 0.02	0.11
13. Enriched flour	0.33 ± 0.01	97	0.33 ± 0.05	
14. Bread	0.34 ± 0.01	103	0.38 ± 0.02	
15. Bread	0.57 ± 0.01	101	0.58 ± 0.04	
16. Dehydrated peas	0.15 ± 0.01	101	0.22 ± 0.01	0.20
17. Baby food, veal and vegetable	0.11 ± 0	102	0.15 ± 0.07	0.10
18. Macaroni noodles	0.55 ± 0.01	102	0.55 ± 0.04	
19. Egg substitute	0.46 ± 0.01	103	0.47 ± 0.04	
20. Enriched rice	0.26 ± 0.01	97	0.22 ± 0.01	
21. Dried potatoes	0.11 ± 0.01	103	0.15 ± 0.02	
	Av, 0.26 mg/100 g Pooled SD, 0.01 mg/100 g Rel SD, 3.9%	Av ± SD, 101.1 ± 2.5%	Av, 0.27 mg/100 g[b] Pooled SD, 0.03 mg/100 g Rel SD, 10.3%	

[a] Standard deviation derived from duplicate sample preparation and when not shown only a singlet analysis conducted. [b] Florisil column cleanup values used.

mg/100 g (Table II), were omitted from the correlation coefficient calculation to prevent calculating an artificially high value (column cleanup values used for manual method where available).

In Table I, the systematic difference between the means (0.04 mg/100 g, 1.2% relative) is not significant and the standard error between methods (0.24 mg/100 g, 7.3% relative) is not large in view of the variance within the manual method. In Table II, the difference between the means (0.01 mg/100 g, 0.3% relative) is not significant; however, the standard error between methods (0.30 mg/100 g, 10.2% relative) is large, indicating poor correlation between methods for some products (cocoa, milk, yeast, and oats). In Table III, the difference between means (0.01, 3.8% relative) is statistically significant (95% confidence) and the standard error between methods (0.036 mg/100 g, 13.6% relative) is somewhat high. Again, this suggests poor correlation between methods for some products (textured soy product).

Although there is good correlation with most products studied, these data suggest that there is a difference between this automated method and the manual AOAC method for certain products. The magnitude of these product differences can only be ascertained by comparing more samples of the problem products.

The relative accuracy of the automated vs. the manual fluorometric method could be assessed by comparing these methods with a more specific technique such as a microbiological or chromatographic method. The difference between the manual method values obtained with and without Florisil column cleanup (Tables II and III) demonstrates that high results can be a problem with certain products.

It has been reported that column cleanup is desirable for certain products which contain an appreciable amount of interfering fluorescing material (Association of Vitamin Chemists, 1947). When certain products showed a high result with the manual method, they were analyzed again employing a Florisil column cleanup step. That the automated method actually correlates better with the manual method employing column cleanup is evidenced by the fact that the standard error between the automated and manual methods for the problem products shown in Tables II and III is 0.55 mg/100 g without column cleanup in the manual method and 0.33 mg/100 g when the Florisil column was employed. Presumably the dialysis in the automated method removes impurities also eliminated by the Florisil column. In view of these results, it appears as if the automated method gives a better determination of the riboflavine level for certain products than the manual method without column cleanup.

As expected, the automated method was more reproducible than the manual method showing a pooled relative standard deviation of 3.3% (60 degrees of freedom) and 7.0% (43 degrees of freedom), respectively. Since each duplicate analysis was conducted on a different day using different sample preparations, these values represent the variance of the total method (sampling, extraction, and analysis).

The results of this study demonstrate that the automat-

ed procedure is more precise and at least as specific as the tedious manual method for a broad spectrum of food products. This procedure appears to be the method of choice when sample through-put is a prime consideration and when specificity no better than the fluorometric method is required.

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COMMUNICATIONS

Method to Determine Ronnel and Its Oxygen Analog in Eggs

A simple method to determine ronnel (*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate) and its oxygen analog (dimethyl 2,4,5-trichlorophenyl phosphate) in chicken eggs is presented. With this method, lower limits of detectability of ronnel and its oxygen analog were found to be 0.5 and 2.0 ppb, respectively. Recoveries of the compounds from eggs fortified at 1–80 ppb were in the range of 70–75%.

Ronnel (*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate) is an organophosphorus insecticide that is used to control external parasites such as ticks, flies, lice, and mosquitoes on livestock. One of the more recent methods developed to determine ronnel and its oxygen analog (dimethyl 2,4,5-trichlorophenyl phosphate) in tissues of cattle was by Ivey and Claborn (1971). Their method works well on a variety of tissues of other species also, but consistent recovery is not obtained when this method is used to determine residues of ronnel and its oxygen analog in chicken eggs. The purpose of this present research was to develop a method adequate for the consistent extraction, cleanup, and analysis to determine ronnel and its oxygen analog in eggs.

EXPERIMENTAL SECTION

All chemicals were reagent grade. All solvents used—acetonitrile, dichloromethane, and hexane (Skellysolve B)—were redistilled in glass. The silicic acid (100 mesh analytical reagent grade powder obtained from Mallinckrodt) used in the cleanup columns was heated 16 hr at 225°, cooled to room temperature, had 20% water added, and then was allowed to equilibrate prior to use (Ivey and Claborn, 1971). The gas chromatograph, Micro-Tek Model 160, was equipped with a flame photometric detector and a 4 mm i.d. × 1.22 m borosilicate glass column packed with Gas-Chrom Q (80–100 mesh) coated with 5% DC-200. Carrier gas was prepurified nitrogen at 75 ml/min. The column was operated at 200°, injector at 240°, and detector at 170°, operating in the phosphorus mode. A Polytron Homogenizer was used to blend the egg samples with the drying agent and the extraction solvent.

Extraction of Ronnel and Its Oxygen Analog from Eggs. Egg samples used in the study were from pooled samples (yolk and albumin) that had been mixed and frozen until analysis. While still frozen, the outer part of the pooled eggs was shaved off to reduce the possibility of obtaining freezer-burned samples. Five-gram samples of the eggs (semi-thawed) were weighed in duplicate into 100-ml beakers. After the samples were allowed to thaw, 5.0 g of anhydrous sodium sulfate was added to each beaker and mixed well. A 25-ml aliquot of acetonitrile was added, and the mixture was blended for 2 min with the Polytron Homogenizer at medium speed (setting of 3); care was taken to loosen the mixture from the bottom or sides of the beaker. An additional 25 ml of acetonitrile was added, and the mixture was blended for 2 more min. After the egg residue was allowed to settle, the supernatant liquid was filtered through folded Whatman No. 1 filter paper into a 125-ml erlenmeyer flask. Another 25-ml aliquot of acetonitrile was added to the egg residue, and it was blended for an additional 1 min. The entire contents of the beaker were transferred to the filter paper, and the generator of the homogenizer and the beaker were rinsed several times with small aliquots of acetonitrile. These washings were poured over the egg residue on the filter paper. After filtration was complete, a three-ball Snyder column was attached to the flask containing the supernatant liquid, and the acetonitrile was evaporated to ca. 5–10 ml. Twenty-five milliliters of hexane was added to the flask and the solution was again evaporated to a low volume. [Hexane and acetonitrile form an azeotropic mixture. The mixture boils at 54.4° when the quantity of acetonitrile is about 26% of total (Horsley, 1947).] The addition of hexane followed by evaporation was repeated twice more or until all traces of acetonitrile were removed.

Cleanup and Quantitation. The concentrated sample was then passed through a cleanup column as described by Ivey and Claborn (1971). The chromatographic column was prepared by adding, in order, a glass wool plug, 2.5 cm of sodium sulfate, 12 g of silicic acid, another 2.5 cm of sodium sulfate, and a glass wool plug. The column was prewashed with hexane. The ronnel was eluted with hexane and the oxygen analog with a mixture of dichloromethane–hexane (3:1). The solvents were again evaporated to about 3 ml with a Snyder column and hot plate. The rest of the solvent was removed at ambient temperature by a filtered dry air stream. The residues of ronnel and its oxygen analog were dissolved in an appropriate volume of hexane (0.5–2.0 ml) and were subjected to separate gas chromatographic analyses. The gas chromatographic conditions have been described previously (Ivey and Claborn, 1971). Extracts of unknowns were compared with known standards for accurate quantitation.