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Received for review August 20, 1954. Accepted October 23, 1954.

INSECTICIDE RESIDUE ANALYSIS

Sodium Reduction Technique for Microdetermination Of Chlorine in Organic Insecticides

W. F. PHILLIPS and M. E. DeBENEDICTIS
Food Chemistry Laboratory, Beech-Nut Packing Co., Canajoharie, N. Y.

The sodium digestion technique has been modified for application to the determination of residues of organic chloride materials on food products. This technique offers the simplicity of the conventional sodium reduction method, with accuracy and precision comparable to those obtained with the combustion technique. This procedure could be useful in the study of residues where the spray history is known and in the quality control laboratory where such information may not be complete. The technique is particularly well suited for use as a screening test in the food packer's quality control laboratory.

ROUTINE CONTROL DETERMINATION of residual amounts of organic chlorides is a major problem faced by food processors as a result of the development and use of modern pesticides. There are specific methods for determining most of the compounds that fall in this category, but at best these methods are difficult to employ routinely in a quality control laboratory. It is not practical for the average control laboratory to perform a wide variety of specific tests on all fresh foodstuffs to be packaged; nevertheless, there is a definite need for specific methods. The practical approach is to employ a rapid, sensitive screening test that will indicate the presence or absence of chlorinated hydrocarbons, followed by colorimetric analysis when further identification is necessary.

The available methods for determining total organic chlorine were investigated, in order to select the one that would be most suitable for routine control analyses, and would permit participation in a collaborative study of the effects of canning procedures on dieldrin residues on peaches.

The most widely used methods of determining residues of organic chlorides are the sodium reduction (*3–5*) and combustion (*1, 2, 6*) techniques. The sodium reduction method, although readily adapted to large volume analyses, was not found satisfactory for estimating dieldrin, and its sensitivity, approximately 100γ of chlorine (*3*), was not adequate. The combustion technique described by Agazzi *et al.* (*2*) exhibits good precision, accuracy, and sensitivity, but would be difficult to employ as a routine procedure in the small food control laboratory.

The sodium digestion method was modified to improve its sensitivity and effectiveness. In brief, the procedure consists of six steps and the modifications described herein are concerned with steps 3, 5, and 6.

1. Removal of the organic residue from the surface of the raw material.
2. "Cleanup" of the strip solution and evaporation of the solvent.
3. Refluxing the residue with metallic sodium in isobutyl alcohol.
4. Elimination of excess sodium.
5. Preparation of the reflux mixture for amperometric titration with silver nitrate solution.
6. Titration and final calculation of results.

Special Apparatus

Amperometric Titration Assembly. The apparatus employed consisted of a sensitive current-measuring instrument (Fisher Elecdropode), a rotating platinum electrode, and a saturated calomel electrode. This equipment has been described and discussed (*1, 2, 6, 8*).

Büchner funnel, M porosity, 65 mm. in diameter.

Burets, 2-ml., graduated in 0.01-ml. units.

Flasks, 50-ml., round-bottomed, semi-ball joint 28/15.

Condensers, West type, length 16 inches, ball and socket joint No. 28/15.

Heating mantles, 50-ml. size.

Reagents

Acetone, C.P.

Acetone solution, equal parts by volume of acetone and distilled water.

Calcium carbonate, low in alkalies.

Celite No. 545 (Johns Manville Co.).

Filter-Cel (Johns Manville Co.).

Gelatin Solution, 1%. Dissolve 1 gram of C.P. gelatin in 100 ml. of hot distilled water and add 1 ml. of C.P. chloroform.

Isobutyl alcohol, boiling point 106–108° C.

Isopropyl Alcohol Solution. Mix equal parts by volume of 99% isopropyl alcohol and distilled water.

Mineral oil, white, chlorine-free.

Nitric Acid Solution. Mix 1 volume of concentrated acid with 1 volume of distilled water.

Table I. Titration of 10 Ml. of Chloride

Cl^-, N	γCl^-/Ml.	$AgNO_3$, N	Mean Titer, Ml.	Mean γCl^- Found	Standard Deviation, γCl	Mean % Recovery
0	0 (Blank)	0.0011	0.50[a]	19.50	0.70	...
2.82×10^{-6}	0.1	0.0011	0.026[b]	1.01	0.21	101
8.45×10^{-6}	0.3	0.0011	0.082	3.19	0.18	106
1.41×10^{-5}	0.5	0.0011	0.13	5.07	0.00	101
2.82×10^{-5}	1.0	0.0011	0.25	9.82	0.17	98
5.62×10^{-5}	2.0	0.0011	0.52	20.28	1.06	101
1.13×10^{-4}	4.0	0.0011	1.09	42.58	0.17	106
1.40×10^{-4}	5.0	0.0011	1.28	49.99	0.97	100
2.82×10^{-4}	10.0	0.0011	2.68	104.52	3.27	104
0	0	0.0101	0.05	17.93	...	...
7.05×10^{-4}	25.0	0.0101	0.68	243.81	3.39	97
1.41×10^{-3}	50.0	0.0101	1.42	509.14	1.60	102
2.82×10^{-3}	100.0	0.0101	2.79	1000.35	9.33	100

[a] Average of 25 titers.
[b] This, and all subsequent values, was corrected for reagent blank and represents average of five analyses. These data show a standard deviation of $\pm 3.2\gamma$ of Cl^- with $\pm 3.6\%$ precision.

Phenolphthalein, 1% Solution. Dissolve 1 gram in 60 ml. of ethyl alcohol and dilute to 100 ml. with distilled water.

Skellysolve B (a commercial C_6 petroleum fraction, Skelly Oil Co.).

Silver Nitrate Solution. Prepare $0.01N$ and $0.001N$ solutions and standardize reagents as indicated in the procedure. Store in dark bottles wrapped with aluminum foil.

Sodium, C.P. Cut in cubes of ca. 3 mm. and store in mineral oil.

Sodium Chloride Solution. Prepare standard solutions of sodium chloride containing a chlorine concentration of 100, 50, 5, and 1γ per ml.

Sodium sulfate, C.P., anhydrous.

Procedure

A 1- to 2-kg. weighed sample and 500 ml. of Skellysolve B are placed in a wide-mouthed glass jar. The jar is rolled for a minimum of 5 minutes and the solvent decanted into a 500-ml. Erlenmeyer flask containing ca. 20 grams of anhydrous sodium sulfate and 4 grams of Filter-Cel. The mixture is shaken and filtered through coarse filter paper and the recovered volume is recorded. The filtrate is then eluted through a fritted-glass Büchner funnel packed to within 1.0 inch from the top with a mixture of equal parts by weight of calcium carbonate and Celite No. 545. The funnel is washed with 200 ml. of fresh Skellysolve B and the combined eluate and washings are evaporated over a steam bath to 5 to 10 ml.

The solution is quantitatively transferred to a 50-ml. round-bottomed flask fitted with a 28/15 socket joint and evaporated to dryness on the steam bath. Ten milliliters of isobutyl alcohol is pipetted into the flask and 1 ± 0.2 gram of metallic sodium is added. This mixture is refluxed for 60 minutes and then the mantle is lowered. When cool, the excess sodium is eliminated by adding 10 ml. of the isopropyl alcohol solution through the condenser and the contents of the flask are refluxed again for 5 minutes. When reflux has subsided, the flask is disconnected from the condenser and placed in a cold water bath (ca. 16° C.). The flask is removed and 5 ml. of acetone is introduced to effect a uniform mixture. Three drops of phenolphthalein are added and the solution is titrated to the end point with the (1 to 1) nitric acid solution and then transferred to a 50-ml. volumetric flask. The reaction flask is rinsed successively with 2 ml. of nitric acid solution, 4 ml. of the gelatin solution, and the (1 to 1) acetone solution. The washings are added to the volumetric flask and brought to the mark with the acetone solution. The mixture is adjusted to room temperature and a 10-ml. aliquot in a 50-ml. beaker is amperometrically titrated with silver nitrate at the rotating platinum electrode.

The choice of the proper silver nitrate solution is dependent upon the concentration of chloride ion in the solution to be titrated. In attempting to establish the sensitivity limit, it was observed that standardizing the silver nitrate reagent against a chloride solution, at a concentration which was previously established as the point where the standard deviation and accuracy were best, resulted in obtaining a sensitivity on the order of 0.1γ of chlorine per ml.

The data shown in Table I indicate that concentrations in the range of 2.8×10^{-6} to $2.8 \times 10^{-4}N$ (0.1 to 10.0γ of chloride per ml.) should be titrated with approximately $0.001N$ silver nitrate standardized against a known sodium chloride solution at the indicated concentration. Solutions containing quantities of chlorine greater than 2.8×10^{-4} N (10γ of chloride per ml.) should be titrated with $0.01N$ silver nitrate up to $2.8 \times 10^{-3}N$ (100γ of chloride per ml.).

Table II. Recovery of Chlorine from 1 Mg. of Dieldrin

Alcohol	Reflux Temp., ° C.	Reflux Time, Min.	Chlorine Found, Mg.	% Recovery
Isopropyl	89–97	60	0.04	7
		180	0.37	67
Isobutyl	108–110	15	0.37	67
		30	0.55	100
		45	0.55	100
		60	0.56	102

Table III. Recovery of Known Quantities of Dieldrin

Dieldrin Added, γ	Equivalent Chlorine, γ	Chlorine, N	Recovered[a], γ	% Recovery
950	523	2.9×10^{-4}	530	101
731	402	2.3×10^{-4}	401	100
475	262	1.4×10^{-4}	264	100
238	131	7.4×10^{-5}	125	95
143	78.6	4.4×10^{-5}	82.5	105
95	52.3	2.9×10^{-5}	55.0	105
48	26.4	1.5×10^{-5}	25.8	98
24	13.2	7.3×10^{-6}	13.6	103
9.5	5.2	2.9×10^{-6}	5.6	108
4.8	2.6	1.5×10^{-6}	2.7	104

[a] Each value is average of three analyses.

Standardization of Silver Nitrate

Five reagent blanks are prepared by refluxing 1 gram of sodium with 10 ml. of isobutyl alcohol and making the solution up to 50 ml., as previously described. Two sets of five 10-ml. aliquots, one aliquot from each of the five samples, are titrated with the 0.001*N* and 0.01*N* silver nitrate and the average titer for each set constitutes the reagent blank. To three sets of five 9-ml. aliquots, taken in the same manner, are added 1 ml. of the sodium chloride solution that will provide a chlorine concentration of 0.1, 0.5, and 1.0γ per ml. for the respective sets. These aliquots are titrated with 0.001*N* silver nitrate. The normality of the silver nitrate solution is then calculated, using the set showing the best accuracy and least standard deviation.

The concentration of chlorine is not as critical in the standardization of 0.01*N* as with 0.001*N* silver nitrate at low concentrations of chlorine. One milliliter of sodium chloride solution, containing 0.5 mg. of chloride per ml., is added to another set of five 9-ml. aliquots, and titrated with 0.01*N* silver nitrate. The normality is calculated from the titers thus obtained. In order to be within the limits described in Tables I, III, and IV, titers should not exceed a volume of 3 ml.

Discussion

The major differences between the technique described and other sodium reduction methods are the reaction temperature and the composition of the titration mixture of the inorganic chloride. A comparison of the relative effectiveness of refluxing dieldrin with sodium in isopropyl and isobutyl alcohol is shown in Table II.

The recommended medium (*2, 7, 8*) for the amperometric titration of chloride is a 50% solution of acetone in water. The sensitivity of titrating in this solution was found to be in the vicinity of 0.2γ of chlorine per ml. (*2*). The titrating solution, described above, exhibits approximately the same sensitivity.

As dieldrin was the most stable to reduction of the organic chlorides encountered, this insecticide was evaluated at several levels of concentration and these data are shown in Table III.

Several other organic insecticides containing chlorine have been analyzed by this technique and the results were found satisfactory in each case. The data in Table IV show the results obtained with benzene hexachloride, DDT, and aldrin.

Table V summarizes the results obtained in a cooperative study conducted with the Shell Development Co. to observe the effect of canning procedures on residues of dieldrin on peaches and to compare analytical data obtained by different methods.

In this experiment dieldrin was applied to fresh peaches in a manner calculated to leave a deposit of 1 to 2 p.p.m., a residue far greater than that normally encountered when the material is applied according to recommendations. All the results tabulated have been corrected for the chlorine found in the check sample.

The method described was found applicable for analyses of residues on peaches, apples, celery, and white potatoes.

When natural inorganic halides are evident, it is essential to eliminate them. Additional cleanup steps are necessary when the extractive to be refluxed with sodium in isobutyl alcohol exceeds 100 mg. Most of the results reported herein were obtained on samples containing approximately 50 mg. or less of plant extractives. However, apples and celery have been satisfactorily analyzed when the weight of waxes exceeded 100 mg.

Acknowledgment

The authors wish to extend their sincere appreciation for the wholehearted cooperation of Louis Lykken, Shell Chemical Co., and to thank the members of the Beech-Nut Packing Co. laboratory staff for their assistance on this project.

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Received for review March 15, 1954. Accepted October 18, 1954. Presented before the Division of Agricultural and Food Chemistry at the 125th Meeting of the AMERICAN CHEMICAL SOCIETY, *Kansas City, Mo.*

Table IV. Evaluation of Method on Aldrin, Benzene Hexachloride, and DDT

Insecticide	Added, Mg.	Equivalent Chlorine, Mg.	Mean Titer, Ml.[a] 0.0011N $AgNO_3$	Mean Chlorine Recovered, Mg.	Mean % Recovery
Aldrin	0.960	0.560	2.85	0.555	
	0.480	0.280	1.35	0.264	
	0.096	0.056	0.28	0.055	97
γ-BHC	1.00	0.732	3.79	0.740	
	0.50	0.366	1.86	0.363	
	0.10	0.073	0.38	0.074	100
DDT	1.00	0.500	2.70	0.527	
	0.50	0.250	1.23	0.240	
	0.10	0.050	0.29	0.056	101

[a] All values corrected for blank and each is average of 3 analyses.

Table V. Effect of Canning Procedure on Residues of Dieldrin on Peaches

Sample Treatment	Insecticide	Dieldrin Found, P.P.M. Beech-Nut Lab., total chloride	Residue Analysis Laboratory, Shell Development Co. Colorimetric	Total chloride
Unpeeled, whole, unprocessed	None	0.06[a]	0.02[a]	0.15[a]
Unpeeled, whole, unprocessed	Dieldrin	2.2	1.4	1.6
Peeled, whole, unprocessed	None	0.0	0.02	0.20
Peeled, whole, unprocessed	Dieldrin	<0.1	<0.1	<0.1
Unpeeled, pureed, processed	None	0.04	0.0	0.19
Unpeeled, pureed, processed	Dieldrin	1.4	1.5	1.1
Peeled, pureed, processed	None	0.0	0.02	0.15
Peeled, pureed, processed[b]	Dieldrin	<0.1	<0.1	<0.1

[a] Apparent dieldrin.
[b] Also analyzed by bioassay, <0.1 p.p.m. obtained.