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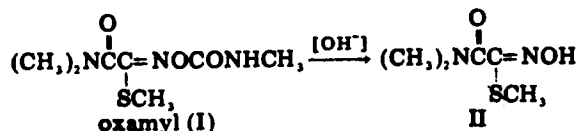
Determination of Oxamyl Residues Using Flame Photometric Gas Chromatography

Richard F. Holt and Harlan L. Pease*

Oxamyl (methyl *N,N'*-dimethyl-*N*-[(methylcarbamoyl)oxy]-1-thioxamimidate) residues in plant and animal tissues and in soil are determined by initial extraction with ethyl acetate, alkaline hydrolysis to the more volatile oximino fragment, and final determination by gas chromatography with sulfur-sensitive flame photometric detection. Method sensitivity is 0.02 ppm based on 25-g samples. Recoveries of added material average about 90% in the 0.02–10 ppm range.

Oxamyl is the approved common name for methyl *N,N'*-dimethyl-*N*-[(methylcarbamoyl)oxy]-1-thioxamimidate. This material was formerly known as DPX-1410 and is the active ingredient in Du Pont's "Vydate" Oxamyl Insecticide/Nematicide.

The residue method for oxamyl (I) is based on the gas chromatographic measurement of the corresponding oximino fragment II, methyl *N,N'*-dimethyl-*N*-hydroxy-1-thioxamimidate, after extraction of oxamyl from the substrate with ethyl acetate and subsequent alkaline hydrolysis to produce the more volatile, but stable derivative:



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EXPERIMENTAL SECTION

Apparatus and Reagents. The Perkin-Elmer Model 3920 gas chromatograph (Perkin-Elmer, Norwalk, Conn.) equipped with a flame photometric detector with interference filter for spectral isolation of sulfur emission at 394 m μ was used. The chromatographic column was 10% SP-1200/1% H₃PO₄ on 80–100 mesh Chromosorb W AW (Supelco, Inc., Bellefonte, Pa.), 3 ft glass, 0.25 in. o.d., 1/16 in. i.d.

Homogenization and extractions were conducted using a blender-centrifuge bottle and adapter base as shown in Figures 1 and 2. These items were designed in this laboratory. It is not necessary to construct this specialized equipment unless desired. Conventional blender bottles and centrifuge tubes may be used but are somewhat more time consuming. Centrifugation was carried out with an International Size 1, Type SB Centrifuge capable of accommodating the 250-ml bottle shown in Figure 1.

The reference standards of I and II were obtained from the Biochemicals Department, Agrichemicals Marketing Division, E. I. du Pont de Nemours & Co., Inc., Wilmington, Del. The solvents used were distilled-in-glass,

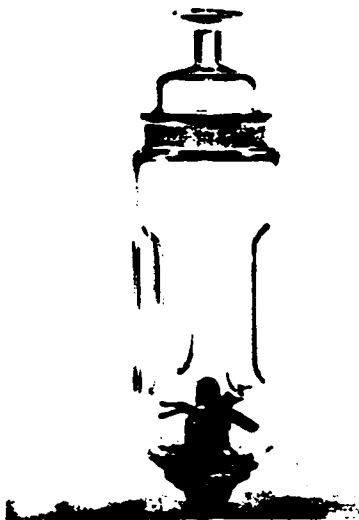


Figure 1. Blender-centrifuge bottle.

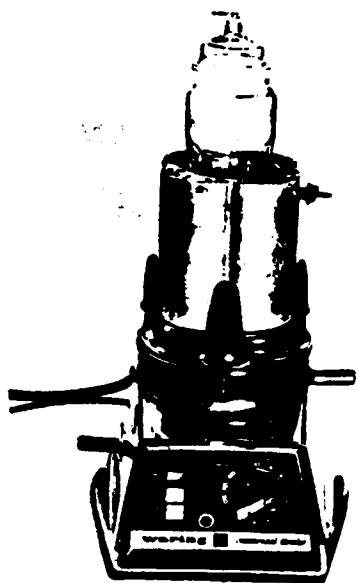


Figure 2. Combined extraction unit: Waring Blender base, adapter base, and blender-centrifuge bottle. Figures 1 and 2 (Pease and Holt, 1971) reprinted by permission of copyright owners.

purchased from Burdick & Jackson Laboratories, Inc., Muskegon, Mich.

Isolation. Weigh 25 g of a representative solid crop or tissue sample into the blender-centrifuge bottle, add 100 ml of ethyl acetate, cover, place in the adapter base, and blend at high speed for about 5 min. Centrifuge at 1500 rpm for 10–15 min and carefully decant the ethyl acetate through a 1.5-in. bed of anhydrous sodium sulfate contained in a 45° funnel and collect in a 500-ml round-bottomed flask. Repeat the extraction two more times each time using 100 ml of ethyl acetate. Add 50 ml of water to the combined extracts and evaporate the ethyl acetate in a vacuum rotary evaporator at 60°C.

When analyzing soil, weigh 25 g into a 250-ml glass-stoppered Erlenmeyer flask, add 100 ml of ethyl acetate and 25 ml of water, stopper, and shake on a wrist-action shaker for 15 min. Allow to settle, and filter the extract through cotton into a 500-ml round-bottomed flask. Repeat the extraction two more times each time using 100 ml of ethyl acetate. Add 50 ml of water to the combined

extract and evaporate the ethyl acetate in a vacuum rotary evaporator at 60°C.

For analysis of milk or other liquids, transfer a 50-g sample into a 250-ml separatory funnel, add 100 ml of *n*-hexane, and shake gently for 2 min. Allow the phases to separate (centrifuge if necessary to obtain clean separation). Discard the hexane wash. Extract the aqueous phase with three 100-ml portions of ethyl acetate using 2-min shaking periods for each extraction. Allow the phases to separate and filter the ethyl acetate through a 1.5-in. bed of anhydrous sodium sulfate contained in a 45° funnel into a 500-ml round-bottomed flask. Add 50 ml of water to the combined extracts and evaporate the ethyl acetate in a vacuum rotary evaporator at 60°C.

For all samples, transfer the remaining water (~40 ml) from the 500-ml flask to a 250-ml separatory funnel using several small volumes of water as wash. Dilute to approximately 50 ml with water. Add 50 ml of *n*-hexane to the separatory funnel, shake gently for about 1 min, and allow the phases to separate. Centrifuge, if necessary, to obtain a clean separation. Discard the hexane layer. Repeat the hexane wash two more times using additional 50-ml portions of solvent. Discard the hexane after each wash.

Adjust the pH to about 12 by adding 3 ml of 1 *N* NaOH (check with pHydron paper; add more if necessary) and add 50 ml of chloroform. Shake gently for about 1 min and allow the phases to separate. Discard the chloroform layer. Repeat the chloroform wash one more time using 50 ml of chloroform. Discard the chloroform.

Heat the aqueous phase on a steam bath with occasional stirring to remove the residual chloroform. Cover and continue to heat on the steam bath for an additional 15 min to convert I to II.

Cool and quantitatively transfer to a 250-ml separatory funnel, using several small water washes. Add 50 ml of chloroform, shake gently for about 1 min, and allow the phases to separate. Discard the chloroform layer. Repeat the wash using a second 50-ml portion of chloroform. Discard the chloroform.

Saturate the aqueous phase by adding 15 g of NaCl (add more if needed) and extract with four 50-ml portions of ethyl acetate-methanol (90:10, v/v) using 2-min shaking periods for each extraction. Allow the phases to separate and filter the ethyl acetate-methanol phase through a 1.5-in. bed of anhydrous sodium sulfate into a 250-ml round-bottomed flask.

Concentrate the combined ethyl acetate-methanol extracts to about 10 ml in a vacuum rotary evaporator at 60°C. Quantitatively transfer the concentrated extract to a 30-ml beaker, using ethyl acetate as wash (if traces of water are present, filter through sodium sulfate). Continue to concentrate the solution to about 0.5 ml by evaporation at room temperature in a well-ventilated hood. Transfer the concentrated extract to a 1-ml volumetric flask using a dropper with a fine tip and several small washes of ethyl acetate. Dilute to volume with ethyl acetate and mix thoroughly.

Gas Chromatographic Calibration. Equilibrate the chromatograph under the following conditions: inlet temperature, 230°C; detector temperature, 200°C; column temperature, 180°C; helium carrier gas flow, 70 cm³/min; oxygen flow, 20 cm³/min; air flow, 40 cm³/min; hydrogen flow, 180 cm³/min. After conditioning the chromatographic column by maintaining the temperature at 180°C with carrier gas flowing for at least 24 hr, set the initial column temperature at 100°C. Inject aliquots (1 to 3 μl) of a standard solution of II prepared in ethyl acetate to

Table I. Oxamyl Recovery Data

Crop	Residue, ppm	No. of detns.	Recovery, %	
			Av	Range
Peanut, nut	0.02-0.80	6	103	73-120
Peanut, hull	0.10-5.0	8	99	82-120
Peanut, foliage	0.05-10	12	93	70-113
Tobacco	0.02-5.0	19	93	72-110
Apples	0.04-0.40	3	98	94-100
Turf grass	0.02-4.0	6	94	76-105
Peaches	0.04-2.0	4	76	54-105
Lettuce	0.04-1.0	4	92	81-99
Oranges	0.04-2.0	11	83	70-90
Cottonseed	0.02-0.20	3	95	84-102
Grapefruit	0.08-0.40	9	79	72-85
Coffee beans	0.02-2.0	2	98	96-100
Grapes	0.02-2.0	3	85	70-94
Potatoes	0.02-4.0	9	91	76-120
Tomatoes	0.02-2.0	11	83	71-109
Celery	0.04-2.0	12	84	70-109
Peppers	0.02-1.0	3	91	84-100
Carrots	0.02-0.20	6	83	79-96
Soil	0.04-6.6	21	94	74-120
Urine	0.40-4.0	4	100	75-130
Feces	0.20-2.0	4	83	72-100
Liver	0.04-0.40	6	90	75-102
Kidney	0.04-0.40	6	89	82-100
Lean meat	0.04-0.40	6	107	85-114
Fat	0.04-0.40	6	98	80-130
Milk ^a	0.02-0.20	6	83	70-110

^a Used 50-g sample.

contain 0.5, 1, 2, 3, 5, and 10 $\mu\text{g}/\text{ml}$ so that the peak will not exceed full scale deflection. Program the column temperature at 16°C per minute to a maximum of 200°C. Hold the column at this temperature for about 8 min. The retention time for II is about 7 min. Operate the flame photometric detector according to instructions furnished by the manufacturer. Construct calibration curves for the different attenuations by plotting micrograms of II injected vs. peak height. Use log-log paper to obtain a straight line. Chromatograph one or more calibration solutions daily to ensure that the calibration curve remains accurate.

Gas Chromatographic Analyses. Equilibrate the instrument and chromatograph aliquots of the residue extracts as described under Gas Chromatographic Calibration. Measure the peak height of II and determine the micrograms of this material in the aliquot, using the calibration curve previously prepared. Calculate the amount of oxamyl in parts per million by dividing the micrograms of II found, corrected for the molecular weight conversion (1.35), aliquot, and recovery factors, by the sample weight in grams.

RESULTS AND DISCUSSION

The gas chromatographic method described is sensitive to about 0.5 μg of oxamyl or 0.02 ppm based on a 25-g sample. Since oxamyl is difficult to chromatograph at the levels required for this sensitivity, the analytical procedure is based on the gas chromatographic measurement of the characteristic oximino fragment, methyl *N,N'*-dimethyl-*N*-hydroxy-1-thioxamimidate (II). This derivative is easily formed by simple alkaline hydrolysis in aqueous solution.

The applicability of the method for determining oxamyl residues has been demonstrated on a variety of substrates. Recovery of this compound added to untreated control samples is essentially quantitative as is shown in Table I. The recoveries were conducted by adding known amounts of oxamyl to the samples contained in the Blender-Centrifuge Bottle. After evaporation of the solvent, analyses were then initiated by addition of the first portion of ethyl acetate.

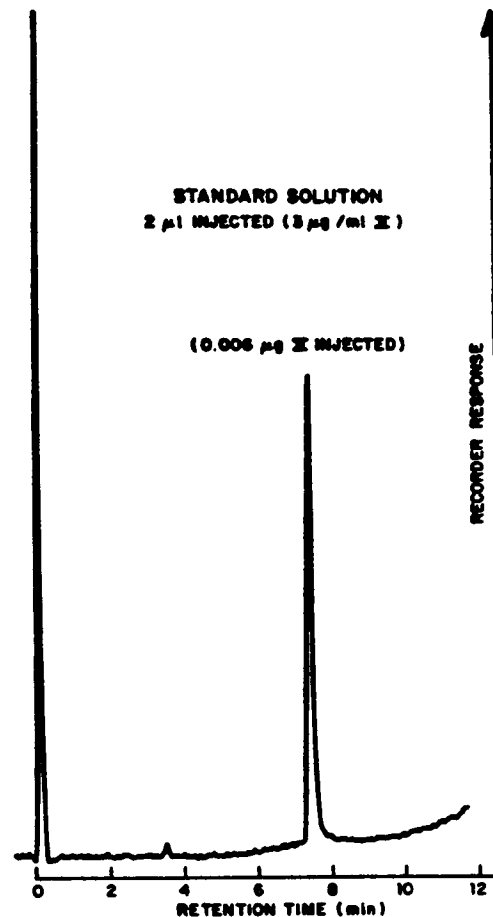


Figure 3. Standard solution of II.

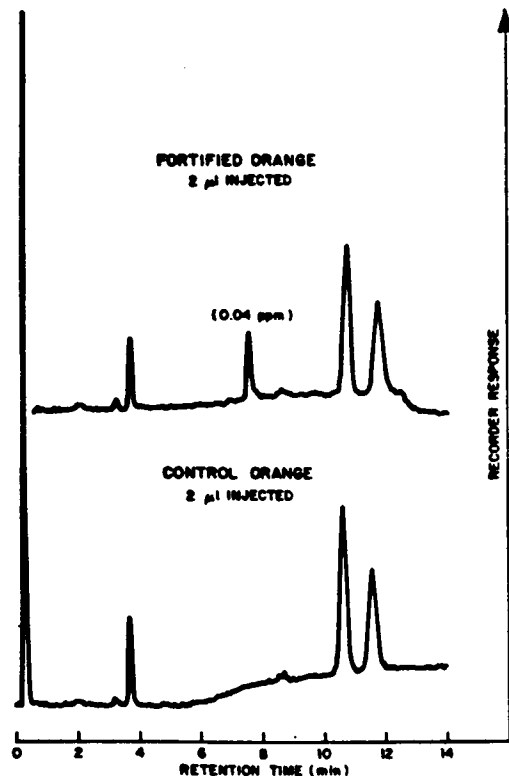


Figure 4. Extracts of oranges.

During the analysis of highly acidic substrates, i.e. oranges, grapefruit, and peaches, somewhat lower recoveries were noted. This was attributed to the detrimental effect of heating oxamyl and the oximino fragment in the acidic media which results from the initial extractions of fruit

crops. Somewhat higher recoveries can be obtained by concentrating these extracts over 1 N NaOH instead of distilled water (see section on Isolation, paragraph 1) thereby neutralizing the extract.

The use of the sulfur-sensitive flame photometric detector provides for a highly selective measurement of the desired compound and no interference was encountered in the majority of the untreated controls analyzed. For illustration, Figures 3 and 4 show typical chromatograms. Figure 3 shows a standard chromatogram for the oximino fragment (II). Figure 4 shows chromatograms obtained on extracts of oranges, the upper curve obtained on a sample fortified with 0.04 ppm of oxamyl, the lower curve

representing a control orange extract.

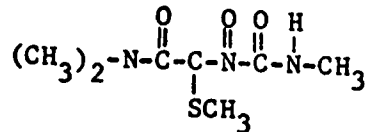
Some of the initial chromatography for this method was conducted using a column packed with 30% OV-101 on Gas-Chrom Q. However, the 10% SP 1200/1% H₃PO₄ column has proven to be more precise and selective. The column life is equivalent. In addition, the new column requires less conditioning than the earlier OV-101 column.

LITERATURE CITED

Pease, H. L., Holt, R. F., *J. Assoc. Off. Anal. Chem.* **54**, 1399 (1971).

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N', N' -DIMETHYL-N [(METHYLCARBAMOYL)OXY]-1-THIOOXAMINIDATE



Determination of Oxamyl Residues Using Flame Photometric Gas Chromatography

methyl N', N' -dimethyl-N- [(methylcarbamoyl)oxy] -1-thiooxamidate, thioxamyl, dioxamyl, DuPont 1410, Vydate

- I. E.I. du Pont de Nemours & Co., Inc., Wilmington, DE 19898 - Method submitted with Pesticide Petition 5F1650. R.F. Holt and H.L. Pease (1976) J. Agric. Food Chem. 24, 263-266.

Oxamyl residues in plant and animal tissues and in soil are extracted with ethyl acetate; the extract is subjected to alkaline hydrolyses to yield the more volatile, but stable oximino fragment, methyl N', N' -dimethyl-N-hydroxy-1-thiooxaminidate. The aqueous phase is extracted with ethyl acetate-methanol (90+10) and the organic phase is concentrated. The oximo fragment is determined by flame photometric GLC, using a detector sensitive to sulfur at 394 nm and a 3' x 0.25" od (1/16" id) glass column packed with 10% SP-1200/1% phosphoric acid on 80-100 mesh acid-washed Chromosorb W. The limit of detection of the method is 0.02 ppm, based on 25 g samples. Recoveries of oxamyl added to the following substrates at levels of 0.02-10 ppm averaged about 90%: apples, carrots, celery, coffee beans, cottonseed, fat, feces, grapefruit, grapes, kidney, lean meat, lettuce, liver, milk, oranges, peaches, peanut foliage and hulls, peanuts, peppers, potatoes, soil, tobacco, tomatoes, turf grass, urine.

The method submitted with Pesticide Petition 5F1650 is essentially that described above; however, the GLC column specified was a 4' x 0.25" od (3/16" id) glass column packed with 30% OV-101 on 80-100 mesh Gas-Chrom Q at a programming rate of 15 °C/min to a maximum of 225°C. Ethyl acetate alone was used to extract the oximino derivative from the aqueous phase.

In a method tryout, EPA obtained recoveries of 77 and 81% and 81 and 82% from duplicate celery samples fortified at 3 and 6 ppm oxamyl, respectively. Control samples showed apparent residues of <0.02 ppm. EPA used an Omni-Mixer for sample extraction and noted that centrifugation is not required. If necessary, the Omni-Mixer cup can be used for centrifugation. The only other change in the method tryout was the use of nitrogen as carrier gas rather than helium.

Product application: celery

Sensitivity: 0.02 ppm

FORM FD 297

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1605

TRANSMITTAL NO. 77-3 (10/77)