

Note

Determination of oxine-copper in orange by gas chromatography

H. KOBAYASHI, O. MATANO and S. GOTO

Chemistry Division, The Institute of Environmental Toxicology, Uchimoriya-cho 4321, Mitsukaido-shi, Ibaraki 302-02 (Japan)

(First received December 12th, 1988; revised manuscript received March 23rd, 1989)

Oxine-copper [bis(quinolin-8-olato)copper(I)] is commonly used as a fungicide for controlling crop diseases. Several analytical methods have been reported for its determination. Bromometric titration¹ and a spectrophotometric method² have been developed for the determination of 8-hydroxyquinoline which is a decomposition product of oxine-copper. Oxine-copper has also been determined by coupling with *p*-nitrobenzenediazonium fluoroborate after being distilled with steam³. However, there is a limitation to these colorimetric methods in either the sensitivity or selectivity when a trace amount of oxine-copper is to be determined. In addition, the clean-up procedure is still not satisfactory. Some samples cause foams during steam distillation. For such samples, the steam distillation is not pertinent. For residue analysis of oxine-copper in crops, recently a derivative spectrophotometric method⁴ and a high-performance liquid chromatographic (HPLC) method⁵ using a fluorimetric detector were applied. In the latter method, 8-hydroxyquinoline was determined as an aluminium chelate. Although these are selective methods suitable for the determination of oxine-copper, there still remain some shortcomings in respect of the sensitivity and clean-up procedure.

In this paper, we describe a simple and effective clean-up procedure in which an Extrelut column is used instead of steam distillation, and a sensitive gas chromatographic (GC) determination of oxine-copper after derivatization with pentafluorobenzyl bromide (PFB-Br).

EXPERIMENTAL

Materials

Oxine-copper and 8-hydroxyquinoline were obtained from Kanesho Chemical (Tokyo, Japan) and Wako Pure Chem. (Tokyo, Japan), respectively. Aluminium nitrate and PFB-Br were obtained from Kanto Chem. (Tokyo, Japan) and Aldrich (Milwaukee, WI, U.S.A.), respectively. Extrelut-20 columns were from E. Merck (Darmstadt, F.R.G.). Oranges used in this study were obtained from The Agricultural Center of Mie Prefecture.

The identification of the PFB derivative of oxine-copper was verified by gas chromatography-mass spectrometry (GC-MS) (JEOL JMS-DX 300 GC-MS equipped with a JMA-DA 5000 GC-MS data system).

Instrumentation

Gas chromatography was performed using an Hewlett-Packard 5890-A instrument equipped with a nitrogen-phosphorus flame ionization detector. A column (4 ft. \times 3 mm I.D.) packed with 1% PEG-HT on Chromosorb W AW DMCS (80-100 mesh) was used. The column, injection port and detector temperatures were maintained at 220, 250 and 300°C, respectively. Helium at 30 ml/min was used as the carrier gas. The hydrogen and air flow-rates were 3 and 100 ml/min, respectively.

HPLC was carried out on a Tri Rotar SP chromatograph (Japan Spectroscopic, Tokyo, Japan) connected to a Hitachi 650-10S fluorescence spectrophotometer with excitation at 380 nm and emission at 520 nm. The Pyrex column (500 mm \times 3 mm I.D.) was packed with Hitachi gel No. 3030. The mobile phase consisted of 1% aluminium nitrate in methanol, and the flow-rate was 1.2 ml/min. All operations were carried out at an ambient temperature.

Sample extraction

A 5-ml volume of 1 M hydrochloric acid and 150 ml of acetone were added to 50 g of homogenized orange (flesh). The mixture was shaken vigorously for 30 min at room temperature, then filtered *in vacuo*. After the residue was washed with 50 ml of acetone, the pooled filtrates were concentrated to approximately 5 ml. To the concentrated solution, 70 ml of distilled water were added. The mixture was acidified to pH 1 by adding 1 M hydrochloric acid and washed with 50 ml of *n*-hexane. The aqueous phase was concentrated to approximately 5 ml, and adjusted to pH 13 by adding 10% sodium hydroxide. To this mixture, 1 ml of methanol and 10 ml of distilled water were added. After being stirred, the solution was charged on an Extrelut-20 column for approximately 20 min. Oxine-copper was eluted from the column with 100 ml of dichloromethane. The effluent was evaporated and the residue obtained was dissolved in 18 ml of acetone.

Pentafluorobenzylation

To an 18-ml volume of the extract containing the oxine-copper, 2 ml of 1% PFB-Br in acetone solution and 1 ml of 50% potassium carbonate solution were added. The mixture was heated at 100°C for 3 h, then allowed to cool. Distilled water (30 ml) was added and extracted twice with 10 ml of benzene. The pooled organic layers were dried over anhydrous sodium sulphate, then evaporated *in vacuo*. The residue obtained was dissolved in an appropriate volume of acetone and a 2- μ l aliquot of the solution was injected into the gas chromatograph.

Determination

The oxine-copper was determined by comparing peak heights of samples to a calibration graph prepared by plotting the peak height *versus* the amounts of oxine-copper injected.

RESULTS AND DISCUSSION

Effect of pH on elution of oxine-copper from an Extrelut column

An Extrelut column has effectively been used for the clean-up of water-soluble compounds. It was therefore applied as a simple clean-up method for oxine-copper.

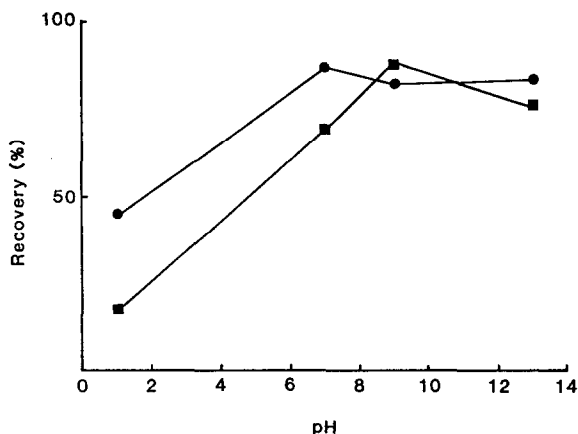


Fig. 1. Effect of pH on the elution of oxine-copper (●) and 8-hydroxyquinoline (■) from an Extrelut column. Values are duplicate determinations. Amounts of oxine-copper and 8-hydroxyquinoline: 100 μg . These data were determined by HPLC.

An 100- μg amount of oxine-copper or 8-hydroxyquinoline in acetone was adjusted to pH 1, 7, 9 or 13 with 1% sodium hydroxide or 1 *M* hydrochloric acid, and then applied to an Extrelut column and eluted with dichloromethane. The recoveries were directly determined by HPLC. As shown in Fig. 1, the recoveries from an Extrelut column were maximal in the ranges pH 7–13 and 9–13 for oxine-copper and 8-hydroxyquinoline, respectively. Then, the method was applied to the recovery test of oxine-copper from orange. The results are listed in Table I and compared with those obtained by the steam distillation method. The recoveries at pH 13 obtained by the Extrelut method were slightly better than those of the steam distillation method.

Thus, the method using an Extrelut column is effective for clean-up of oxine-copper.

TABLE I

RECOVERY (%) OF OXINE-COPPER AND 8-HYDROXYQUINOLINE FROM ORANGE

Values are the means of duplicate experiments. The sample solution was adjusted to pH 13 or 9 with 10% NaOH prior to being charged on an Extrelut column.

Compound	Added (ppm)	HPLC ^a			GC ^a
		Extrelut		Steam distillation	Extrelut pH 13
		pH 13	pH 9		
Oxine-copper	0.2	83.0	81.8	83.0	84.0
	0.4				90.4
	0.5	97.5	89.7	80.5	87.8
	2.0	88.3	88.5	88.2	92.4
8-Hydroxy-quinoline	2.0	98.6	95.1		

^a Method of detection.

Effect of reaction temperature, reaction time and amount of potassium carbonate on the PFB derivatization of oxine-copper

In general, phenols react with PFB-Br to form PFB derivatives. 8-Hydroxyquinoline which is a decomposition product from oxine-copper also gave a PFB derivative with PFB-Br. Consequently, an examination of the hydrolysis in alkaline media and of the derivatization with PFB-Br of oxine-copper was simultaneously carried out.

The effects of the reaction temperature and reaction time on the PFB derivatization of oxine-copper were examined. A 100- μ g amount of oxine-copper was dissolved in 18 ml of acetone and 20 mg of potassium carbonate followed by 2 ml of 1% PFB-Br in acetone were added. The mixture was heated for 1, 3 and 5 h at 70 or 100°C. The PFB derivative of 8-hydroxyquinoline heated at 100°C for 3 h was used as a control compound for the determination of the PFB derivative of oxine-copper. Table II shows that the derivatization of oxine-copper reached equilibrium after heating at 100°C for 3 h. However, the yield was only 1.8%. It can be assumed that the amount of potassium carbonate was not sufficient for the reaction.

To examine the effect of the amount of potassium carbonate, several amounts of potassium carbonate were added to the acetone solution containing 100 μ g of oxine-copper and the mixture was heated at 100°C for 3 h. The results are listed in Tables II and III. They indicate that the preparation of the PFB derivative of oxine-copper was strongly affected by the concentration of potassium carbonate. Table II shows that the yield was less than 26% in the presence of 20, 50, 100, 200 and 300 mg of potassium carbonate. So, the derivatization was conducted in 30, 50 or 70% potassium carbonate solutions. As summarized in Table III, the best results can be obtained by adding 1 ml of 50 or 70% potassium carbonate solution. If the hydrolysis and the PFB derivatization of oxine-copper occur quantitatively, the relative yield was 82.5% $\{[(2 \cdot \text{PFB-hydroxyquinoline/oxine-copper})/(\text{PFB-hydroxyquinoline/8-hy-}$

TABLE II

EFFECTS OF THE AMOUNT OF POTASSIUM CARBONATE, REACTION TEMPERATURE AND REACTION TIME ON THE PFB DERIVATIZATION OF OXINE-COPPER

Values are the means of duplicate experiments. Reaction conditions: oxine-copper, 100 μ g; 8-hydroxyquinoline, 100 μ g; temperature, 100°C; time, 3 h; 1% PFB-Br in acetone, 2 ml.

Amount of K_2CO_3 (mg)	Temperature ($^{\circ}C$)	Time (h)	Relative yield ^a (%) mean \pm S.D.
20	70	1	0.7
		3	0.9
		5	1.5
20	100	1	1.4
		3	1.8
		5	1.8
50	100	3	3.1
100	100	3	8.1
200	100	3	26.3
300	100	3	17.0

^a Ratio of peak heights: (oxine-copper/8-hydroxyquinoline) \cdot 100.

TABLE III

EFFECT OF POTASSIUM CARBONATE ON THE PFB DERIVATIZATION OF OXINE-COPPER

Values are the means of triplicate experiments. Reaction conditions: oxine-copper, 100 μg ; 8-hydroxyquinoline, 100 μg ; temperature, 100°C; time, 3 h; 1% PFB-Br in acetone, 2 ml.

Percentage of K_2CO_3	Volume of K_2CO_3 (ml)	Relative yield ^a (%) mean \pm S.D.
30	0.2	7.6 \pm 1.0
	0.5	34.5 \pm 4.6
	1.0	90.4 \pm 9.0
50	0.5	26.0 \pm 4.3
	1.0	116.4 \pm 3.6
	1.5	99.1 \pm 19.2
	2.0	89.8 \pm 4.8
70	0.5	23.1 \pm 2.9
	1.0	116.1 \pm 12.8

^a Ratio of peak heights: (oxine-copper/8-hydroxyquinoline) \cdot 100.

droxyquinoline] \cdot 100 = (650/351.9)/(325/145.15) \cdot 100; because 1 mol of oxine-copper theoretically produces 2 mol of 8-hydroxyquinoline. However, in this study, the yield was 116%. This is considered to be due to the low yield of 8-hydroxyquinoline used as a control compound.

From these results, the reaction conditions for the PFB derivatization of oxine-copper in alkaline media were selected as follows: reaction temperature, 100°C; reaction time, 3 h; volume of 50% potassium carbonate, 1 ml.

Mass spectrum of the PFB derivative of oxine-copper

The electron impact (EI) mass spectra of the PFB derivatives of oxine-copper and 8-hydroxyquinoline were identical, as shown in Fig. 2. The molecular ion appeared at m/z 325 and major fragment ions were found at m/z 181 [$\text{C}_6\text{F}_5\text{CH}_2$]⁺, 158 [$\text{M} - \text{C}_6\text{F}_5$]⁺, 144 [$\text{M} - \text{C}_6\text{F}_5\text{CH}_2$]⁺, 129 [C_6F_3]⁺ and 116 [$144 - \text{CO}$]⁺.

Limit of detection

Under the given GC conditions, the minimum detectable amount of oxine-copper was 0.25 ng. Therefore, the limit of detection was 0.005 ppm when a 2- μg portion of 2 ml of the final acetone extract obtained from 50 g of orange was injected into the gas chromatograph.

This value was significantly better than those ($>$ 0.02 ppm) from the Extrelut and steam distillation methods.

Results from orange

The recovery experiments for the PFB derivatization of oxine-copper were carried out at fortification levels of 0.2, 0.4, 0.5 and 2 ppm. Untreated orange was treated with standard acetone solution of oxine-copper prior to the addition of the extraction solvent. The results are shown in Fig. 3 and Table I. The chromatogram shows no interfering peak from the sample, and oxine-copper was quantitatively recovered.

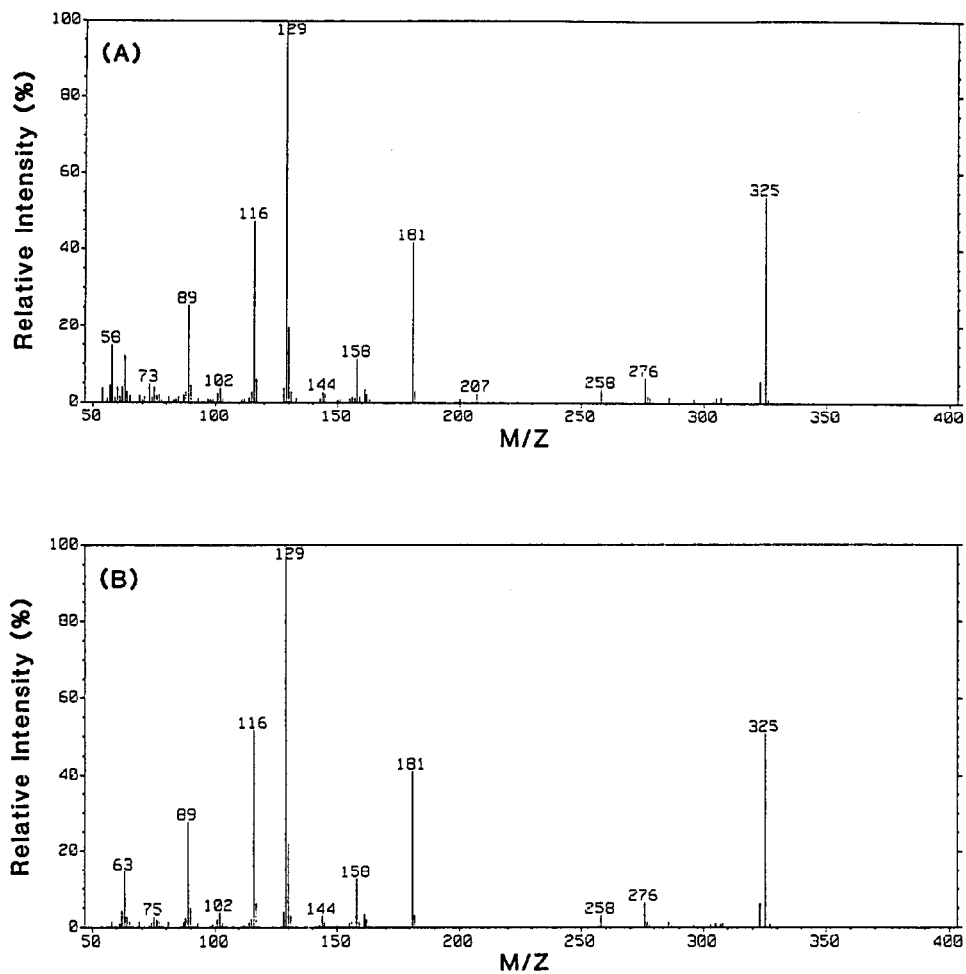


Fig. 2. Mass spectra of the PFB derivatives of oxine-copper (A) and 8-hydroxyquinoline (B).

TABLE IV

RESIDUES (ppm) OF OXINE-COPPER IN ORANGE FLESH (FIELD TRIALS)

The values in parentheses are mean \pm S.D. Active content of formulation: 80%. Concentrate in spray: 600 l per 10 acre. Type of formulation: wettable powder.

No. of applications	Days after last application	HPLC ^a		GLC ^a
		Extrelut	Steam distillation	Extrelut
5	30	0.27, 0.25, 0.25 (0.26 \pm 0.01)	0.26, 0.25, 0.28 (0.26 \pm 0.02)	0.27, 0.23, 0.29 (0.26 \pm 0.03)

^a Method of detection.

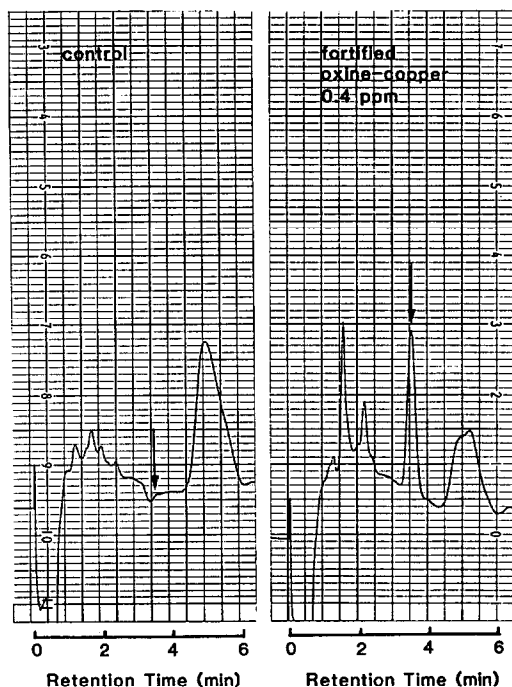


Fig. 3. Gas chromatograms of the recovery and control of oxine-copper from orange. Arrows indicate the retention times of the PFB derivative of oxine-copper. Conditions: final acetone solution, 2 ml (control); 4 ml (recovery). Injection volume: 2 μ l. Other conditions was given in the text.

These results indicate that the method is eligible for determination of trace amounts of oxine-copper.

The results obtained for actual samples are summarized in Table IV. The mean residue in oranges sprayed five times with 600 l per 10 acre was 0.27 ppm at 30 days after the last application, in accord with the analytical data obtained by steam distillation. Therefore, the clean-up with an Extrelut-20 column and the derivatization with PFB-Br will be very suitable for the determination of oxine-copper residues.

REFERENCES

- 1 W. Horiwitz (Editor), *Official Methods of Analysis of the Association of Official Analytical Chemists*, Association of Official Analytical Chemists, Washington, DC, 13th ed., 1980, p. 626.
- 2 W. T. Haskins and G. W. Luttermoser, *Anal. Chem.*, 23 (1951) 456.
- 3 S. Goto and S. Kato (Editors), *Analytical Methods of Pesticide Residues*, Soft Science, Tokyo, 1980, p. 198.
- 4 S. Ono and E. Yamazaki, *J. Pestic. Sci.*, 7 (1982) 531.
- 5 M. Maeda and A. Tsuji, *Chem. Pharm. Bull.*, 27 (1979) 797.