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Determination of Copper-oxinate in Plant Tissues by High Performance Liquid Chromatography using Fluorimetric Detector

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A high performance liquid chromatography method for the determination of copper-oxinate in plant tissues was developed. The method involves isolation of the residue by extraction with acetone, decomposition of extracted residue to 8-hydroxyquinoline by masking copper (II) ion with potassium cyanide followed by high performance liquid chromatography with a fluorimetric detector. Non-fluorescent 8-hydroxyquinoline separated from copper-oxinate was converted to a highly fluorescent chelate and eluted from the column by the eluent containing Al (III) ion. The lowest limit of detection and recoveries from plant tissues sample were about 0.01 ppm and 86.1—103.1%, respectively.

Keywords——copper-oxinate; fluorescence high performance liquid chromatography; residual analysis; fungicide; fluorimetry; bis(8-hydroxyquinolinato)copper (II)

Copper-oxinate[bis(8-hydroxyquinolinato)copper (II)] is widely used as a systemic fungicide that has preventive and curative properties and also a mite ovicide effect. Gas chromatography has been generally used for the residual analysis of pesticides in plant tissues and soil. Many metal chelate compounds have been also determined by gas chromatography,[2] but copper-oxinate has not been assayed by gas chromatography. The quantitative analysis of 8-hydroxyquinoline(ligand of copper-oxinate) can be performed by bromometric titration[3] and spectrophotometric method using *p*-nitrobenzene diazonium fluoroborate as a color reagent after extraction.[4] The bromometry can not be applied to the residual analysis because of its low sensitivity. The spectrophotometric method suffers interference from various colored substances extracted from plant tissues and its sensitivity is too low to determine small amount of the residual (0.1—0.01 ppm). Recently, developement of a more sensitive method has been required.

Fluorometry is known to be about 100 times more sensitive than colorimery. The authors reported previously the fluorescence high performance liquid chromatographic analysis for pesticide residue.[5] 8-Hydroxyquinoline has been used as a fluorescent reagent for the microdetermination of various metal ions such as Al (III), Zn (II), and Ga (II) *etc.*,[6–8] and the fluorimetric determination of small quantities of alminum in plant tissues using 8-hydroxyquinoline was also reported.[9] In this paper, the residual analysis of copper-oxinate is examined by a combination of fluorescent chelate formation and high performance liquid chromatography.

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Experimental

Apparatus——A Hitachi Model 634 high speed liquid chromatograph, a Hitachi Model 204 fluorescence spectrophotometer equipped with a Hg lamp and a LDC ultraviolet (UV) monitor (254 nm) were used.

Materials——Analytical standard of copper oxinate (99.9%) was obtained from Sankyo Co. (Tokyo Japan). 8-Hydroxyquinoline and other chemicals of reagent grade were used without purification.

Chromatographic Conditions——Column packing; Hitachi gel No. 3010 (particle size, 20—30 μm) was packed in stainless steel column (2.1×500 mm). Column temperature; room temperature (27°), mobile phase; 1 w/v % aluminum nitrate [$Al(NO_3)_3 \cdot 9H_2O$] in methanol, carrier flow rate; 1.0—1.2 ml/min, inlet pressure; 40—45 kg/cm², UV monitor scale; 0.16 absorbance unit full scale, fluorimeter sensitivity; 1×8, chart speed; 2.5 mm/min. The effluent was monitored at an excitation wavelength of 365 nm and an emission wavelength of 505 nm with the fluorimeter, and at 254 nm with the UV monitor.

Standard Solution——Standard solutions of copper-oxinate were prepared by dissolving the analytical standard in methanol at seven different concentrations between 20 and 200 μg/ml.

Isolation of Residues from Plant Tissues and Assay Procedure——Weigh 50 g of chopped sample in a Waaring blender, add 100 ml of acetone and 20 ml of 1 M phosphoric acid solution, and blend at high speed for 5—10 min. Filter the blended mixture through a filter paper into a 300 ml round-bottomed evaporation flask. Repeat the extraction with two additional 100 ml portions of acetone. Evaporate the combined acetone extracts under reduced pressure below 40° until acetone is completely removed, and adjust the residual solution to pH 9—10 with 0.5 N NaOH solution or saturated Na_2CO_3 solution. Add 1 ml of 4% potassium cyanide solution to it, and extract with two 20 ml portions of $CHCl_3$. After phase separation, transfer the $CHCl_3$ layer into a round-bottomed flask and evaporate it to dryness under reduced pressure. Dissolve the dried residue by addition of 1 ml of methanol, and inject an appropriate amount of the resultant solution into the liquid chromatograph. Determine the amount of copper-oxinate by comparing the peak height with the working curve prepared by the following procedure.

Take 0.5 ml each of standard solutions containing copper-oxinate (20—200 μg/ml), adjust to pH 9—10 with 0.5 N NaOH solution and mix with 1 ml of 4% potassium cyanide solution. Extract each mixture with $CHCl_3$ (3 ml) for 1 min. Transfer the organic layer into a test tube and evaporate to dryness under reduced pressure. Dissolve each resideu by addition of 1 ml of methanol and inject each 5 μl portion of the resultant solutions into the liquid chromatograph.

Results and Discussion

The following considerations were made in order to obtain the optimal conditions for the determination of copper-oxinate residue in plant tissues.

Selection of Mobile Phase

Among the solvent systems tested such as methanol, methanol-water, methanol containing various concentrations of aluminum nitrate, *etc.*, 1 w/v% almunum nitrate nonahydrate-methanol was found most suitable, because it gave good separation and stable fluorescence

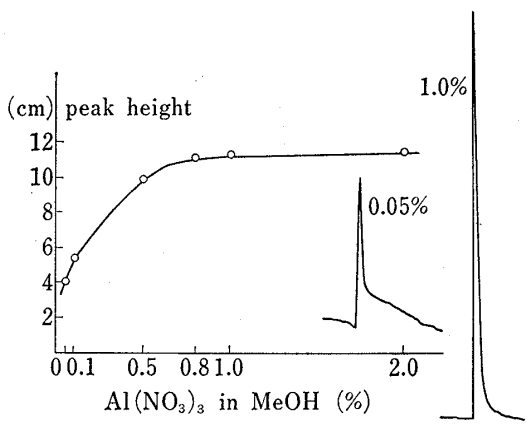

Fig. 1. Effect of the Concentration of Aluminum Nitrate as a Fluorescent Reagent

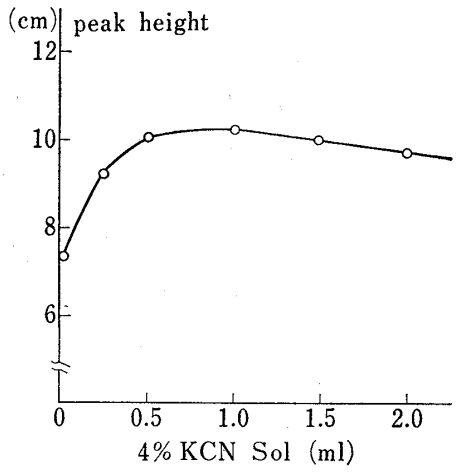

Fig. 2. Effect of the Concentration of KCN Solution as a Masking Reagent

of 8-hydroxyquinoline. Non-fluorescent 8-hydroxyquinoline was converted to highly fluorescent aluminum chelate in this mobile phase separated on the column, and detected by a fluorimeter. Nitrates of zinc, magnesium, and calcium were also tested as fluorescent reagent to give lower fluorescence intensities than that obtained by aluminum nitrate. The concentration of aluminum nitrate in effluent ranging from 0.06% to 2.0% was examined in order to attain the highest intensity of fluorescence. As shown in Fig. 1, the peak height (fluorescence intensity) for a given amount of 8-hydroxyquinoline increased with increasing concentration of aluminum nitrate up to 0.8% and took plateau between 0.8 and 2.0%. Such an increase in aluminium nitrate concentration improved the peak shape to become sharp and symmetric as well, permitting a quick and accurate peak height measurements for quantitative calculation.

Effect of the Concentration of Potassium Cyanide Solution as a Masking Reagent for Copper Ion

Masking and extraction procedures were studied as possible means of eliminating copper ion. The experimental results shown in Fig. 2 indicate that a constant peak height was obtained by addition of 0.5 ml of 4% potassium cyanide solution. Therefore, 1 ml of 4% potassium cyanide solution was added to eliminate the interference of copper ion.

Working Curve and Sensitivity

The working curve was prepared according to the peak height method. The relationship between amount of copper-oxinate and peak height was linear in the range from 50 to 500 ng.

Table I. Recovery from Plant Tissues

Plant tissue	No. of determinations	Added (ppm)	Average recovery (%)	Range[a] (%)
Cucumber	4	4.0	100.0	8.5
	3	3.5	103.1	4.1
Strawberry	5	0.5	95.5	3.7
Apple	2	0.15	97.7	1.5
Tomato	3	0.15	86.1	8.5

a) Difference % between maximum and minimum values of recovery.

The smallest amount that could be determined was 20 ng of copper-oxinate per injection. Therefore, the detection limit of this procedure is 0.01 ppm when 50 g of sample (various plant tissues) is taken for the assay, the final volume of extracted sample is made up exactly to 1 ml, and 20 μl of it is chromatographed under the condition described above.

Recovery and Reproducibility

The recovery and reproducibility data for copper-oxinate are given in Table I. The known amounts of copper-oxinate were added to various plant tissue homogenates and determined by proposed procedure. The recoveries were between 86.1 and 103.1%. Figure 3 shows the liquid chromatograms of control and fortified cucumber extracts.

Although the limit of determination for copper-oxinate by UV detector was almost same as that with fluorimeter, the latter detector was preferable,

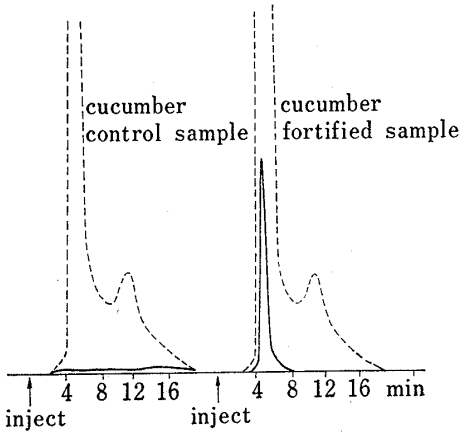

Fig. 3. Chromatograms of Cucumber Extracts

Operating conditions ; see in text.
Sample taken, 50 g; final volume, 1.0 ml; injected, volume, 20 μl; Broken line, absorbance at 254 nm (0.16 a. u. f. s); solid line, fluorescence intensity at excitation 365 nm and emission 505 nm (sensitivity; 1×8).

because the peak of 8-hydroxyquinoline overlapped with the strong UV-absorbing background peaks present in cucumber extract, whereas, as shown in Fig. 3, only the peak of 8-hydroxyquinoline appeared on the chromatogram obtained by the fluorimetric detector. The reliability of the present method was assessed by comparison with the results obtained by the conventional colorimetric method with *p*-nitrobenzene diazonium fluoroborate.[4] The results (Table II) obtained between both methods show a good agreement. The proposed method is suitable for the routine analysis of copper-oxinate residue in plant tissues, and is applicable to other pesticides and drugs that yield fluorescent complexes with metal ion.

Table II. Copper-oxinate Residues (ppm) in Fortified Samples after Spraying

Sample	Number of spraying	Amount of spraying	Days after the last spray	Number of determinations	Colorimetry[b]	HPLC
Tomato	0	—	—	2	0.05	0.01
	5	200 l[a]/10 a	3	2	0.26	0.23
Cucumber	0	—	—	2	0.05	0.01
	7	150 l[a]/10 a	3	2	0.14	0.16

a) 4% aqueous solution.
b) *p*-nitrobenzene diazonium fluoroborate colorimetric method.[4]

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Gas-Liquid Chromatographic Determination of Inositol in Multivitamin Preparation containing Large Amounts of Sugars

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A simple gas–liquid chromatographic method was developed for the determination of inositol in multivitamin preparation containing large amounts of sugars. Sample multivitamin preparation was trimethylsilylated and separated on a 2% OV-101 column. The quantitations were achieved by the peak height ratio method using methyl arachidate as an internal standard. Inositol in 13 synthetic mixtures containing large amount of various sugars (200 to 1000-fold amount) was determined successfully by the present method.

Keywords——inositol; sugars; multivitamin preparation; trimethylsilylation; gas–liquid chromatography

Niwa, *et al.*[2] established a spectrophotometric method for the determination of inositol in synthetic mixtures containing large amounts of sucrose using the color-reaction with hydroxamate following acetylation or the periodate oxidation. The reaction used, however, is not specific to inositol and complicated procedures are required for elimination of sucrose which interferes with the determination of inositol. As a result, their method is time-con-

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