Journal of Chromatography, 604 (1992) 247–253
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 225

New analytical methods for quantitation of four fungicides by gas and high-performance liquid chromatography

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(First received September 6th, 1990; revised manuscript received March 21st, 1992)

ABSTRACT

New analytical methods were developed for the quantitation of para-nitrophenol, 2,2′-methylenebis(4-chlorophenol), salicylanilide and copper 8-quinolinolate, by high-performance liquid and gas chromatography. The first three were extracted for 4–8 h from leather, cotton and felt samples, respectively, by acetonitrile in a Soxhlet, and then quantitated by high-performance liquid chromatography using a C_{18} reversed-phase column and a UV detector. Copper 8-quinolinolate was extracted by dilute sulfuric acid and the acid extract was treated with a chelating agent to release 8-hydroxyquinoline. 8-Hydroxyquinoline was in turn extracted and quantitated by gas chromatography with a flame ionization detector.

INTRODUCTION

Treatments with fungicides are usually part of a functional finish for materials, which may include treatments for water repellency, coloring, and flame retardation. Present specifications for the quantitation of the fungicides *p*-nitrophenol (pNP), 2,2′-methylenebis(4-chlorophenol) (dichlorophene, G-4), salicylanilide and copper 8-quinolinolate (Cu-8) are based on colorimetric techniques, which are not precise, specific, sensitive or reproducible [1–5]. Therefore new analytical methods, high-performance liquid chromatography (HPLC) with UV detection, and gas chromatography (GC) with flame ionization detection (FID), were evaluated for the precise and sensitive quantitation of these fungicides in the presence of other functional finishes. Fig. 1 gives the chemical structures of the four fungicides studied.

Earlier studies for the determination of Cu-8 have shown that the yellow color extracted from the

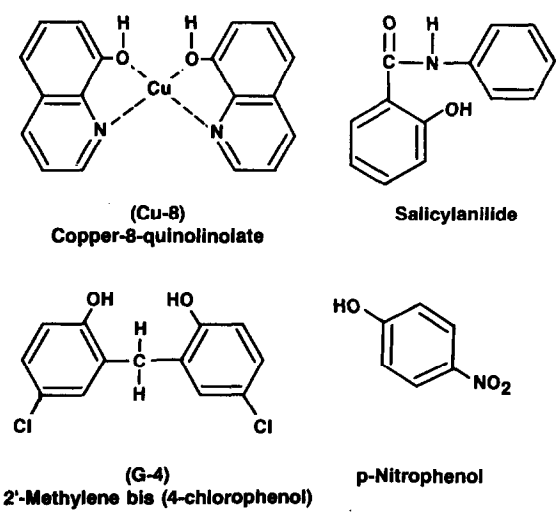

Fig. 1. Chemical structures of fungicides.

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test material interfered with the analytical method. To prevent interference in the quantitation of Cu-8, column chromatographic removal of the dye was recommended [6,7]. There was significant interference with a number of phenolic compounds including *o*-phenylphenol, salicylanilide, 4,4′-isopropylidenebis(2-chlorophenol), and 2,4-dichlorophenol by the present colorimetric method used for quantitation of G-4 [8]. Some of these interferences with the colorimetric procedures used in the quantitation could result in incorrect values, which would result in incorrect recommendations for the optimum treatment of material with these fungicides. The new analytical methods developed and reported here are reliable, accurate, sensitive and free from interferences from other substances generally present in the material being evaluated.

EXPERIMENTAL

Materials

Materials tested

Leather treated with *p*-nitrophenol; wool felt treated with salicylanilide; and cotton duck and tent lining with flame retardant [tetrakis(hydroxymethyl)phosphonium hydroxide–ammonia (THPOH–NH_3)] and treated with G-4 or Cu-8 were used for the fungicide quantitation.

Fungicides

The following fungicides were purchased from commercial sources: Cu-8 (Monsanto, St Louis, MO, USA); *p*-nitrophenol (J. T. Baker, Phillipsburg, NJ, USA); G-4 (Givaudan, Clifton, NJ, USA); and salicylanilide (Aldrich, Milwaukee, WI, USA).

Other chemicals

The following chemicals were purchased from sources indicated: 2-hydroxyquinoline, 5-hydroxyquinoline, 8-hydroxyquinoline, 2,4-quinolinediol, isoquinoline, 5,7-dibromo-8-hydroxyquinoline, 2-hydroxypyridine, 8-hydroxyquinaldine, 8-hydroxyquinoline N-oxide, salicylaldehyde, salicylic acid, salicylamide, phenylsalicylate, phenol, nitrobenzene, *m*-nitrotoluene, *o*-nitrotoluene and *p*-nitrotoluene (Aldrich); 2,2′-methylenebis(3,4,6-trichlorophenol) (Givaudan) and *p*-nitrophenol (J. T. Baker). These chemicals were selected based on the functional groups, precursors, and breakdown products of the fungicides for which new analytical methods were to be developed, and to determine the potential interference of these compounds.

Solvents

Acetonitrile and methyl alcohol (HPLC grade, with UV cut-off at 190 nm and 205 nm, respectively, Caledon, Ontario, Canada) were used for extractions, preparation of standard solutions, and HPLC analysis. Cu-8 was solubilized in pyridine (Aldrich) and used in recovery studies. Methylene chloride (HPLC grade, 233 nm cut-off, Burdick & Jackson, Baxter Travenol, Muskegon, MI, USA) was used for the extraction and the preparation of standard solution of 8-hydroxyquinoline.

High-performance liquid chromatography

An HPLC System (Waters Div., Millipore, Bedford, MA, USA) with Model 721 system controller, Model 730 data module, Model 6000A solvent pump, Model 710B WISP and Model 441 UV detector was used for the quantitation of G-4, pNP and salicylanilide.

Gas chromatography

Hewlett-Packard gas chromatography (Model 5880A; Hewlett-Packard, Avondale, PA, USA) with flame ionization detector and a glass column (305 cm × 4 mm I.D.) with packing of 1.5% OV-17 with 1.95% QF-1 on Gas-Chrom Q, 100–120 mesh (lot SP 981; Applied Science Laboratories, State College, PA, USA) was used to quantitate Cu-8.

Methods

Treatment of material

Ca. 0.1–0.5 g of material (leather, felt, cotton duck and tent lining) containing fungicide was cut up and weighed. Control samples [leather, felt, cotton duck, tent lining, etc., without any fungicide, but with other functional finishes (flame retardant, water repellent, etc., present in the test materials)] were also extracted to determine the potential interference of these functional finishes in the analytical methods being developed. These control samples were spiked with a known amount of fungicide, and used for recovery studies. The treatment level in re-

covery samples represents the actual spiking concentrations. These samples were extracted and fungicides quantitated as described below.

Preparation of fungicide solutions

p-Nitrophenol, G-4 and salicylanilide were solubilized in methanol and used to spike control samples for recovery studies. These fungicides, dissolved in 86.7% (v/v) acetonitrile (ACN) in Milli-Q water, were used as the HPLC standards. A solution of Cu-8 in pyridine was used in the recovery studies because of the solubility of the fungicide in this solvent of the many solvents tested.

Method of extraction

Two procedures were developed for the extraction of fungicides from the test materials. The first procedure was for the extraction of pNP, G-4, and salicylanilide by acetonitrile solution and the second one was for the extraction of Cu-8 by H_2SO_4 solution.

Extraction of pNP, G-4 and salicylanilide. Soxhlet extraction was used for the extraction of *p*-nitrophenol, G-4 and salicylanilide. This extraction setup consisted of a heating mantle with a rheostat, 250 ml flat bottom flask for the solvent, 100 ml Soxhlet extractor to hold the sample in a thimble and a water-cooled condenser. Fungicides were extracted with 86.7% (v/v) ACN in Milli-Q water. The boiling point of this azeotrophic mixture is 76.5°C. Time required for the complete extraction of the above fungicides from test samples was from 4–8 h. Afterwards the extract was concentrated to less than 25 ml volume using the same solvent. An aliquot of the extract was quantitated using HPLC as described below.

Extraction of copper 8-quinolinolate. Cu-8 is a chelated compound of 8-hydroxyquinoline with copper and the analytical method developed and reported is for the quantitation of 8-hydroxyquinoline. The extraction procedure used involved extraction of Cu-8 from the test material, treatment of extracted Cu-8 with a strong chelating agent to remove copper from Cu-8 and the extraction of 8-hydroxyquinoline released. Cu-8 treated fabric was extracted three times with hot, 0.5 *M* H_2SO_4 solution (8.0 ml each time) and the extracts were pooled and brought up to 25 ml with 0.5 *M* H_2SO_4. A 1-ml volume of this acid extract was treated with 1.0 ml of tetrasodium ethylenediaminetetraacetate (Na_4-EDTA, 1.0 *M*) and mixed on a Vortex mixer for 1 min. This treatment of Cu-8 solution with EDTA was to remove copper present in Cu-8 and release 8-hydroxyquinoline. 8-Hydroxyquinoline that was released (from Cu-8) was extracted with 0.2 ml methylene chloride by vortexing the mixture for 1 min. The supernatant containing copper salt was aspirated off and 8-hydroxyquinoline present in methylene chloride was quantitated by GC as described below.

Recovery studies

Recovery studies were carried out by spiking control samples [0.2–1.0 g cut piece of the test material (leather, felt, or cotton duck with no prior fungicide treatment, but with other functional finishes)] with a known amount of the fungicide solution. The amount of fungicide used to spike the control samples varied from fungicide to fungicide and depended upon the amount of the commercial fungicide treatment normally required for protection. These spiked samples were extracted and the amount of the fungicide in the extract was quantitated by the methods developed. The fungicides (except Cu-8) dissolved in methyl alcohol were applied onto the test materials and the solvent was removed by air drying. Cu-8 dissolved in pyridine was applied to test materials as above, and pyridine was removed by air drying. Since not enough cotton duck without G-4 pretreatment was available, the cotton duck commercially treated with this fungicide plus other functional finishes was also used for the G-4 recovery studies.

HPLC operating conditions

Reversed-phase column (C_{18}, 10 μm, Waters, Millipore) with either acetonitrile or methanol solution and a UV detector were used for the HPLC analysis. The wavelength selected for the HPLC detector was based on the absorption maximum determined by UV–VIS scanning in a spectrophotometer (Model Lambda 3; Perkin-Elmer, Oak Brook, IL, USA).

GC operating conditions

Nitrogen, with a flow-rate of 30 ml/min, was the carrier gas for GC. The flow-rates of air and hydrogen to the flame ionization detector were 400 and 40

ml/min, respectively. The injector temperature was 170°C, and the column temperature was programmed from 170–250°C at a rate of 10°C per min for 8 min. A 10-μl volume of the sample was injected into the column and the data were collected for 4 min.

Standard curves

The standard curves of the four fungicides were prepared from standard solutions by HPLC and GC methods described above. The range, linearity and correlation coefficient of the standard curves of fungicides were determined by 2–4 independent analyses.

RESULTS AND DISCUSSION

p-Nitrophenol

Leather samples commercially treated with pNP were extracted by Soxhlet for 4–8 h with 86.7% (v/v) ACN in water as described earlier. The retention time of the fungicide eluted from the C_{18} (10 μm) column was 10.7 min, using 25% (v/v) ACN in Milli-Q water as the eluent. Other details of the extraction procedure and the HPLC method used for the analysis of pNP are given in Table I. The following compounds were evaluated for potential interference in the quantification of pNP: phenol, nitrobenzene, *o*-nitrotoluene, *m*-nitrotoluene and *p*-nitrotoluene. These compounds eluted at times different from the retention time of pNP. There was also no interference from other substances extracted from untreated leather by the acetonitrile solution. The standard curve for pNP was linear from 2–12 mg/l (correlation coefficient = 0.9982, intercept = $-0.0278 \cdot 10^6$ and slope = $0.0792 \cdot 10^6$). Fig. 2A gives the chromatogram obtained with a typical HPLC run. The average amount of moisture in the leather samples tested was 9.3%. Mean value for the *p*-nitrophenol (after the moisture correction) in the leather samples tested varied from 0.26–0.27% (g/100 g) and standard deviation varied from 0.002–0.007. With extraction times from 4–8 h, there was no significant difference in the levels of pNP quantitated by HPLC.

p-Nitrophenol recovery studies were carried out with 0.5 g control leather samples (without any fungicide treatment) after spiking with 1.5 mg of the fungicide. These recovery samples were extracted for 4, 6, and 8 h and the fungicide in the extract was quantitated as described above. Recovery studies indicate that the amount of pNP recovered was from 102–111% (mean value 106 ± 3.9% S.D.) and the extraction of pNP was complete from the fungicide-spiked leather samples after 4 h of the Soxhlet extraction.

2,2'-Methylenebis(4-chlorophenol)

Cotton fabrics commercially treated with G-4 and flame retardant (THPOH–NH_3) treatment were used for the HPLC analysis. The Soxhlet extraction with acetonitrile was carried out for 4, 6 and 8 h, to determine the extraction efficiency. G-4 extracted was quantitated by HPLC using a C_{18} (10

TABLE I

SUMMARY OF ANALYTICAL METHODS FOR FUNGICIDES

Reference details in Experimental.

	pNP	G-4	Salicylanilide	Cu-8
Extraction solvent	86.7% ACN	86.7% ACN	86.7% ACN	0.5% *M* H_2SO_4
Extraction time (h)	4–8	4–8	4–8	1.5
Chromatography	HPLC	HPLC	HPLC	GC
Column packing	C_{18}, 10 μm	C_{18}, 10 μm	C_{18}, 10 μm	1.7% OV-17 with 1.95% QF-1
Eluent/carrier gas	25% ACN	50% ACN	75% Methanol	Nitrogen
Flow-rate (ml/min)	1.0	1.0	1.0	30
Retention time (min)	10.7	9.0	3.25	2.8
Detector	UV	UV	UV	FID
Wavelength (nm)	340	214	229	–

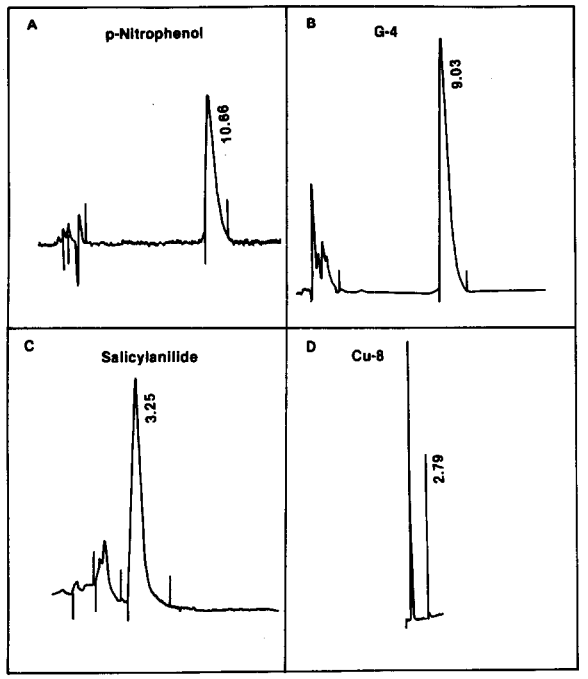

Fig. 2. Chromatograms of fungicides with retention times in min.

μm) column and eluted with 50% (v/v) ACN in Milli-Q water. Other details of the HPLC method are given in Table I. The amount of G-4 in fabric samples (extracted for 4–8 h) varied from 0.98–1.2% with a mean value of 1.08 ± 0.11% S.D. The results indicate that there was no significant difference between 4 and 8 h of extraction. 4-Chloro-3-methylphenol and 2,2'-methylenebis(3,4,6-trichlorophenol) did not interfere with the HPLC analysis of G-4. Fig. 2B shows a representative chromatogram with a G-4 by the HPLC method. The standard curve indicates that the response of the LC detector was linear up to 100 mg/l of G-4 (correlation coefficient 0.9998, intercept $-0.017 \cdot 10^6$ and slope $0.093 \cdot 10^6$).

The G-4 recovery studies were carried out with 627–773 mg control samples (commercially treated with THPOH–NH_3 but without G-4 pretreatment), spiked with 3.0 mg of the fungicide in methanol, and extracted for 6 h. The G-4 (commercially) treated fabric (141–213 mg) was additionally spiked with 0.5 mg of G-4 per sample and Soxhlet extracted for 4–8 h as described above. The amount of G-4 present in the sample (unspiked) was subtracted from the spiked sample to determine the percentage of recovery from the spiked sample. The data indicate that the G-4 extraction was complete in 6 h and the mean value of the recovery was 101 ± 15.2% S.D.

Salicylanilide

Felt samples were Soxhlet extracted for 6 and 8 h, and the extract was analyzed by HPLC using a C_{18} (10 μm) column with 75% (v/v) methanol in Milli-Q water as the eluent. Other details of the extraction and HPLC parameters are given in Table I. Fig. 2C is a typical HPLC chromatogram with salicylanilide with a retention time of 3.25 min. The standard curve was linear from 10–500 mg/l of salicylanilide (correlation coefficient 0.9685, intercept $0.702 \cdot 10^6$ and slope $0.064 \cdot 10^6$). The HPLC data indicate that there was no difference in the amount of salicylanilide quantitated in felt samples extracted for 6 or 8 h, and salicylanilide in felt samples varied from 1.01–1.05% (w/w), with a mean value of 1.03 ± 0.014% S.D. Salicylaldehyde, salicylic acid, salicylamide and phenylsalicylate did not interfere with the quantitation of G-4 by this HPLC method. The amount of salicylanilide-like substances was 0.03% or less in those felt samples untreated by salicylanilide but treated with other functional finishes.

Recovery studies were carried out with salicylanilide-spiked felt samples. Untreated felt samples (*ca.* 200 mg in weight each) were spiked with 4 mg of salicylanilide and extracted for 6 and 8 h with 86.7% (v/v) ACN in Milli-Q water as described above. The salicylanilide extracted was quantitated by HPLC method described earlier. This study indicates that 94% (w/w) of the salicylanilide was recovered from felt samples after 6 h of extraction, and the percentage of recovery ranged from 90–95% (mean value 92 ± 2.0% S.D.).

Copper 8-quinolinolate

Since Cu-8 is a chelated compound of 8-hydroxyquinoline with copper, repeated attempts to quantitate this chelated compound by HPLC and GC were not successful. HPLC methods using normal- and reversed-phase column packing, with a number of eluents from polar to non-polar solvents were tried for the separation of Cu-8. The column packings evaluated included Bondapak, Phenyl, CN,

and NH_2 (Waters, Millipore) and ion-exchange columns. In many instances, the Cu-8 broke down slowly and irreversibly bound with the column packing, and thus changed the retention time and shape of the fungicide peak from run to run. Separation of this fungicide by GC was equally unsuccessful with and without derivatization of Cu-8 (including in-column derivatization) using a number of GC column packings. Since 8-hydroxyquinoline is the major (82%) and expensive ingredient of Cu-8, it was decided to quantitate 8-hydroxyquinoline, to measure the amount of Cu-8 present in samples.

Cu-8 was extracted three times from cotton fabrics (*ca.* 1 g) with hot 0.5 *M* H_2SO_4 solution. The total treatment time was 1.5 h. The three extracts were pooled and diluted to 25 ml volume. An aliquot of the extract was treated with an equal volume of 1.0 *M* Na_4EDTA solution and shaken vigorously for 1.0 min to release copper-bound 8-hydroxyquinoline from Cu-8. 8-Hydroxyquinoline that was released was extracted with methylene chloride by vigorous shaking again for 1.0 min. Other details of the extraction procedure are given in Fig. 3. The supernatant was aspirated off, and an aliquot of the methylene chloride containing 8-hydroxyquinoline was injected directly into the GC column. The GC parameters used for the quantitation of 8-hydroxyquinoline are given above and in Table I.

Two standard curves were prepared for the quantitation of Cu-8 in cotton fabrics; the first standard curve was prepared using 8-hydroxyquinoline solution in methylene chloride; and the second one was prepared using 8-hydroxyquinoline in methylene chloride prepared from Cu-8 as described above (dissolving Cu-8 in 0.5 *M* H_2SO_4, followed by Na_4EDTA treatment, and extraction of 8-hydroxyquinoline by methylene chloride as illlustrated in Fig. 3). The values obtained with the 8-hydroxyquinoline standard curve were converted to Cu-8 by multiplying the 8-hydroxyquinoline values by 1.2195. This conversion factor was calculated from the theoretical amount of 8-hydroxyquinoline (82%) in Cu-8. The standard curve was linear from 50–500 mg/l for both 8-hydroxyquinoline and Cu-8 (correlation coefficient 0.9978, intercept $-1.107 \cdot 10^3$ and slope $0.107 \cdot 10^3$). Fig. 2D shows the chromatogram obtained with 8-hydroxyquinoline by

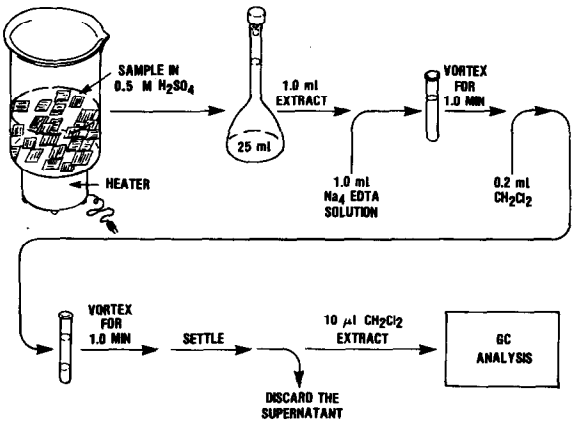

Fig. 3. Procedure for the extraction of copper 8-quinolinolate for the GC analysis.

GC, with a retention time of 2.79 min. Cu-8 values obtained with GC indicate that the amount of Cu-8 present in cotton fabric samples as calculated from 8-hydroxyquinoline and Cu-8 standard curves was comparable (0.64 ± 0.079 and 0.69 ± 0.079% S.D., respectively). GC studies with 2-hydroxyquinoline, 5-hydroxyquinoline, 2,4-quinolinediol, isoquinoline, 5,7-dibromo-8-hydroxyquinoline, 2-hydroxypyridine, 8-hydroxyquinaldine, and 8-hydroxyquinoline-N-oxide indicated that there was no interference from these compounds in the quantitation of 8-hydroxyquinoline (by GC).

Cu-8 recovery studies were carried out with cotton fabric samples (*ca.* 1.0 g each) spiked with 8.0 mg of the fungicide in pyridine and the solvent removed by air drying. Cu-8 was extracted and quantitated as described previously. The Cu-8 standard curve was used for the quantitation of 8-hydroxyquinoline in the Cu-8 spiked cotton fabric samples. The amount of Cu-8 extracted and quantitated by the GC method varied from 91–114% (with mean value of 101 ± 7.8% S.D.) of the spiked amount. The results indicate that the method will be able to quantitate accurately the amount of Cu-8 present in the test material [2,5,7].

The Cu-8 GC method developed and described here is reproducible, sensitive and could be automated. Cu-8 values calculated from 8-hydroxyquinoline data were consistent with those reported from copper determination (by atomic absorption) and colorimetric methods.

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