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IDENTIFICATION AND DETERMINATION OF ORGANOMERCURIAL FUNGICIDE RESIDUES BY THIN-LAYER AND GAS CHROMATOGRAPHY

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SUMMARY

A study has been made of the thin-layer and gas–liquid chromatographic characteristics of the dithizonates of a number of organomercurial fungicides in common use. A method is given for the extraction of these fungicides from apples, potatoes and tomatoes and their identification and determination, as their dithizonates, by thin-layer and gas chromatography.

INTRODUCTION

Organomercury compounds have been in use as fungicides in agriculture and horticulture on a fairly large scale for a number of years. In certain countries they are also used by the wood pulp industry to prevent slime formation. The main agricultural use in the U.K. is as a dressing for seeds of cereal crops but seeds of beet and mangolds and seed potatoes are often similarly protected by the compounds. Organomercurial sprays are applied to apple and pear trees to prevent scab and similar sprays are used extensively on tomatoes, especially glass-house crops. Currently there is some interest in the mercury residue problem but concern has also been expressed from time to time about the contamination of our general environment[1–3].

Studies on the translocation of these compounds and the residues occurring in foodstuffs have been recently reviewed[1]. Most of the mercury residue work carried out so far has consisted of the determination of mercury as Hg by the traditional methods such as acid digestion of the sample followed by estimation of the resultant inorganic mercury as the dithizonate[4]. These methods, however, give no indication of the chemical nature of the mercury compound present in the sample or even whether it is in an organic or inorganic form. The nature of the mercury compound present is actually of some importance as the toxicities of individual mercury compounds differ considerably. Methylmercury compounds, for instance, are far more toxic than their phenylmercury analogues. However, very little work has been done on identification and determination of individual organomercurials. Some has been carried out in Sweden, a country which has experienced serious mercury pollution problems associated with the use of the compounds in agriculture and its large wood pulp

industries. WESTÖÖ[5,6] has investigated thin-layer and gas–liquid chromatographic methods for estimating methylmercury compounds in fish, meat, liver and eggs and showed incidentally that gas chromatographic separation of some organomercurials, as their dithizonates, was possible. This present study examines the TLC and GLC systems required to separate and identify most of the organomercury compounds in common use in agriculture and horticulture, including the alkyl-, alkoxyalkyl- and arylmercury compounds. The application of these techniques to the identification and determination of these compounds in potatoes, apples and tomatoes is also described.

THIN-LAYER CHROMATOGRAPHY

The following types of organomercury compounds were examined:

Me–Hg–X	MeO–Et–Hg–X	Phenyl–Hg–X
Et–Hg–X	EtO–Et–Hg–X	Tolyl–Hg–X

The nature of X affects the properties of the compound. In general, when X is an anion such as sulphate, nitrate or acetate, the compound tends to be ionic and water soluble. When X is a halogen or dicyandiamide the compound tends to be non-polar and soluble in organic solvents. As all these variations of X are likely to be encountered in practice, it is difficult to devise a single TLC system suitable for separating all these compounds. If, for example, silica gel plates are used with an organic solvent as the mobile phase, then the chlorides and diphenylmercury move up the plate and the more salt-like compounds remain at the origin line. Visualisation can be achieved satisfactorily with a spray of 0.05% dithizone in chloroform, but efficient TLC separation of the intact organomercurials was judged unlikely to be successful for all the compounds likely to be encountered.

As the actual identity of the X moiety is not so important from a residue point of view, and as its identity can usually be established by simple chemical tests, the chromatography of the dithizonates was investigated. All the mercury compounds examined readily yielded characteristic stable intensely yellow to red complexes with dithizone on simply shaking the organomercurial, in solid form or in solution, with a chloroform solution of dithizone until a slightly green colour indicated an excess of reagent. Inorganic mercury compounds which may be present give the usual mercury di(dithizonate): diphenylmercury is at least partially converted to phenylmercury dithizonate. A number of solvent systems were tried with silica gel and alumina absorbents and the R_F values for the most satisfactory combinations are given in Table I. This shows that by appropriate selection, all the dithizonates can be clearly separated and identified. The dithizonates of methyl- and ethylmercury compounds are, perhaps, the least well separated of the spots but any doubt as to the identity of these two compounds can be easily resolved by the gas chromatographic procedures detailed below. Visualisation is not a problem; as little as 2 μg of these compounds are self-indicating as yellow or red spots.

Nearly all the commercially available samples of organomercury compounds used in this study were found to contain varying quantities of inorganic mercury and other organomercury compounds including diphenylmercury. TLC was found very useful for isolating pure specimens of the organomercury compounds as standards.

TABLE I

R_F VALUES × 100 OBTAINED BY TLC OF DITHIZONATES OF ORGANOMERCURY COMPOUNDS

Systems: (1) silica gel, hexane–acetone (9:1);
(2) silica gel, hexane–acetone (19:1)
(3) silica gel, hexane–acetone (93:7);
(4) silica gel, light petroleum–acetone (9:1);
(5) alumina, hexane–acetone (19:1);
(6) alumina, light petroleum–acetone (19:1);
Layer thickness: 250 μ.

Dithizonate	*System*					
	1	*2*	*3*	*4*	*5*	*6*
Methylmercury	64	48	57	77	89	86
Ethylmercury	64	51	62	78	91	87
Methoxyethylmercury	32	16	25	44	58	49
Ethoxyethylmercury	44	23	34	55	71	67
Phenylmercury	48	34	46	62	72	69
Tolylmercury	52	40	53	69	79	76
Mercury di-dithizonate	19	9	17	28	19	15

The same solvent systems were employed but chromatoplates 500 μm thick were used so that much larger amounts of the dithizonates could be applied. Appropriate areas of absorbent from the developed plate were then scraped off and the pure organomercury dithizonate eluted with diethyl ether.

GAS–LIQUID CHROMATOGRAPHY

WESTÖÖ[5] in his work on methylmercury compounds in fish used 10% Carbowax columns with electron-capture detection to show that various alkylmercury compounds, including their dithizonates, could be separated by GLC. TERAMOTO *et al.*[7], also working with methylmercury compounds, used a 25% diethylene glycol succinate column. These and a number of other stationary phases on various supports, with electron-capture detection, have been investigated. Again it was found much more convenient to use the dithizonates of the compounds under study. In general, the more polar phases such as Carbowax 20M and ethylene glycol adipate, on Chromosorb G, were found to give good separations but had a distinct tendency to produce tailing peaks on the chromatograms. By far the most satisfactory column consisted of 2% of polyethylene glycol succinate on Chromosorb G. Typical retention times for this column are given in Table II. The dithizonates of the various alkyl- and alkoxyalkylmercury compounds have fairly short retention times but are clearly separated from one another. Sensitivity is good and the system can easily detect 0.05 ng of these compounds. By contrast the arylmercury dithizonates had relatively long retention times with peaks that were correspondingly broader at the base. The peaks corresponding to phenylmercury dithizonate and tolylmercury dithizonate were also slightly asymmetrical; this type of peak appears to be an inherent characteristic of the arylmercury dithizonates, for which no obvious reason could be found. It is very marked on some types of column. Stationary phases such as Apiezon L, Silicone GE SE-52, Cyanosilicone GE XE-60, Carbowax 1500M and ethylene and diethylene

TABLE II

TYPICAL GLC RETENTION TIMES FOR ORGANOMERCURY DITHIZONATES

(I) 2% polyethyleneglycol succinate on Chromosorb G (acid-washed, DMCS-treated, 60–80 mesh) in glass columns 1.5 m long, 3 mm I.D.; carrier gas, nitrogen.

Dithizonate	*Column temperature (°C)*				
	140	*150*	*160*	*170*	*180*
Methylmercury	3.8	2.8	2.2	1.6	1.2
Ethylmercury	6.6	4.6	3.6	2.7	2.0
Ethoxyethylmercury	17.0	11.6	8.7	6.2	4.9
Methoxyethylmercury	17.4	12.0	8.7	6.2	4.9
Tolylmercury	—	—	—	29.0	19.5
Phenylmercury	—	—	—	42.0	27.0

(II) 1% polyethyleneglycol succinate on Chromosorb G (acid-washed, DMCS-treated, 60–80 mesh) in glass columns, 1.2 m long, 3 mm I.D.; carrier gas, nitrogen.

Dithizonate	*Column temperature (°C)*	
	170	*180*
Tolylmercury	6.4	3.2
Phenylmercury	10.0	5.0

glycol succinates, on Chromosorb W, G or Q as support, all showed this feature to some extent. Teflon, 40–60 mesh, was probably the best support but has certain intrinsic disadvantages. Direct "on column" injection tended to minimise this effect and was used throughout. Nevertheless, excellent reproducibility of these peaks for the arylmercury dithizonates was obtained on the polyethylene glycol succinate column referred to above and 1 ng of these compounds could be readily detected. A shorter column, containing only 1% of polyethylene glycol succinate, specifically for the arylmercury dithizonates, was also useful in that shorter retention times were obtained together with narrower peaks on the chromatogram. Typical retention times obtained by use of this column are also given in Table II. This system would readily detect 0.5 ng of these arylmercury compounds.

Mercury compounds are known to "poison" tritiated foil detectors. The tritium source is a very weak β-emitter and almost any coating deposited on the foil will reduce emission. This effect is even more marked when the coating has a high electron capturing potential, as is the case with mercury, and can result in emission falling to zero. In preliminary work, it was found that injections of large amounts of these mercury compounds at oven temperatures of 190° or higher led to rapid deterioration of detector response because of this effect. Sensitivity could be fairly easily restored by cleaning the foil gently with a mild abrasive polish[8] but this was clearly to be avoided if possible. Operation at temperatures below 150° reduced this effect to negligible proportions but it was far more satisfactory, in the interests of obtaining reasonable retention times, to maintain an oven temperature of 180° and restrict the mercury content of injections to 100 ng or less.

APPLICATION OF METHODS TO SAMPLES

The methods described by WESTÖÖ[5,6] were designed mainly for detecting residues of methylmercury compounds in fish. The organomercurial is converted to the chloride or bromide by treatment with hydrochloric or hydrobromic acids and then extracted with toluene. Clean-up of the extract is effected by conversion of the mercurial into a water-soluble form such as the hydroxide, sulphate or cysteine derivative followed by acidification and back extraction into benzene. Good recoveries were claimed for methylmercury compounds but these methods are not suitable for the detection of alkoxyalkyl compounds which are usually very unstable in even dilute acids. Further, the use of an aqueous system as a means of extraction appeared likely to give inadequate penetration into vegetable and fruit material and it is on these crops that organomercurial fungicides may be used in the U.K. and in countries from which we import these foodstuffs.

A method was sought, principally for potatoes, tomatoes and apples, by which all the organomercurials, including the alkoxyalkyl, could be extracted unchanged with good solvent penetration of the sample. The possibility of using an acetone solution of dithizone was examined and showed promise but clean-up of the initial extract proved difficult. This was overcome by conversion of the organomercury dithizonate to the water-soluble nitrate by extraction of the compound into 1% aqueous silver nitrate. The aqueous solution was then treated with potassium thiocyanate, filtered and the organomercury thiocyanate extracted with toluene. This procedure gave 60 to 70% recoveries for the alkyl and alkoxyalkyl compounds but poor recoveries for the arylmercurials.

An efficient method for the extraction and clean-up of all the organomercurials was finally developed using a slightly alkaline solution of cysteine hydrochloride in propan-2-ol. (The use of a slightly alkaline solution is essential if the alkoxyalkyl compounds are to be recovered unchanged.) The extract was then washed with diethyl ether or toluene and the organomercurials extracted with a diethyl ether solution of dithizone.

This method was applied to potatoes, tomatoes and apples and gave recoveries of 85 to 95% for samples spiked with 1.0, 0.1 and 0.01 p.p.m. of methyl-, ethyl- and ethoxyethylmercury as their chlorides, and 5 and 0.5 p.p.m. of phenyl- and tolylmercury acetates.

Method

The method described here was found suitable for potatoes, tomatoes and apples but could obviously be applied to other foodstuffs. In the case of apples and potatoes, the residues to be determined will in most cases be concentrated in the skin or outer layers. Samples of apples and potatoes are therefore coarsely peeled and the thick peel chopped to provide material for analysis. Mercury residues in tomatoes tend to be distributed more evenly in the fruit. Five grams of chopped peel of apples or potatoes, or 5 g of the macerated fruit in the case of tomatoes, are macerated with a mixture of 10 ml of propan-2-ol and 5 ml of alkaline cysteine hydrochloride solution (1% aqueous solution adjusted to pH 8.0 by the addition of 5 *N* ammonia solution). After allowing the liquor to settle, the clear layer is decanted and the extraction repeated twice more with further portions of extractant solutions. The combined

extracts are then centrifuged at 2500 r.p.m. for 5 min. The clear liquor is separated, diluted with 700 ml of 4% sodium sulphate solution and the solution washed with three 50-ml portions of diethyl ether. It was found that, at this stage, potatoes gave a gelatinous precipitate but this remained in the ether layer and could be discarded without apparently affecting appreciably the recovery of mercury compounds. The organomercurials are then extracted from the aqueous solution using three 25-ml portions of a 0.005 % solution of dithizone in diethyl ether. The combined extracts are then dried by passage through a short column of granular anhydrous sodium sulphate and concentrated to a suitable volume, usually 5 ml, in a Kuderna-Danish evaporator. The final solution can then be examined by TLC using silica gel as absorbent and a mixture of hexane and acetone, 93 : 7, as developing solvent (system 3 in Table I). If the results indicate that it is appropriate, then one of the other systems in Table I can also be tried. The final solution is also injected on to the first of the gas chromatographic columns described in Table II. The shorter column described in Table II should also be used if arylmercury compounds are present.

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