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## DETECTION OF MERCURY AND ALKYL MERCURY COMPOUNDS BY REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY

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## SUMMARY

A reversed-phase thin-layer chromatographic method for separation and detection of the dithizonates of inorganic mercury compounds and a series of alkylmercury compounds ( $C_1$ - $C_{18}$ ) was investigated. The specificity of this method was that all the compounds tested were distinctly separated and detected with high sensitivity. The influence of some of the other inorganic metals and excess dithizone was also examined. The method is applicable to the separation and identification of mercury compounds in foods and sewages.

## INTRODUCTION

The contamination of foods by mercury compounds from our environment has been observed. There is no doubt that the mercury compounds in shellfish and fish which caused the Minamata disease and a kind of nervous disease in the Agano River area in Japan was a methylmercury compound<sup>1</sup>. Since then, mercury compounds have toxicologically been evaluated; the organic mercury compounds have longer biological retention times in several tissues and have a greater specific effect on nervous systems than do inorganic mercury compounds. In the field of health sciences, therefore, a simple and rapid method for the analysis of mercury compounds in foods and sewages is needed.

Several authors have presented methods for the detection and determination of organic mercury compounds<sup>2,3</sup>. The compounds are generally separated by extraction from the acidified samples with a suitable solvent such as benzene, concentrated by shaking the extract with a small portion of a cysteine solution, and subsequently extracted with benzene. With or without concentration, the compounds are detected and determined by thin-layer chromatography (TLC) and gas chromatography (GC). Especially in the separation of inorganic and organic mercury compounds, ISHIKURA AND YOKOTA<sup>4</sup> reported that these dithizonates were satisfactorily separated by chromatography on an alumina column. WESTÖÖ<sup>5</sup> reported that using a combined TLC and GC method, the mercury compound found in fishes was identified as a methylmercury compound and quantitatively determined. TANABE<sup>6</sup> investigated

the metabolic fate of mercury compounds in plant tissues applying a similar method. However, using these methods it was impossible to separate a series of alkylmercury compounds which may be found in waste water.

In the present paper, methods are given for the detection of trace amounts of a series of alkylmercuric dithizonates by reversed-phase thin-layer chromatography using corn starch or Avicel SF coated with liquid paraffin.

## EXPERIMENTAL

### Materials and methods

**Adsorbents.** Corn starch (obtained from Wako Pure Chemical Ind., Ltd.) and Avicel SF (obtained from FMC American Viscose Division) were used as the adsorbents.

**Reagents.** Liquid paraffin, chloroform, benzene, ethanol, methyl cellosolve and dithizone were of analytical reagent grade. Mercuric chloride was obtained from Tokyo Kasei Kogyo Co., Ltd. All the alkylmercuric chlorides listed in Table I were synthesized and purified according to the method reported by SLOTTA AND JACOBI<sup>7</sup>.

TABLE I

CHEMICAL FORMULAS AND MOLECULAR WEIGHTS OF MERCURY AND ALKYL MERCURY COMPOUNDS

Compound	Chemical formula	Molecular weight	
		X = Dithizone	X = Chloride
Mercury	Hg-X <sub>2</sub>	711.23	271.50
Methylmercury	CH <sub>3</sub> -Hg-X	470.96	251.08
Ethylmercury	CH <sub>3</sub> -CH <sub>2</sub> -Hg-X	484.98	265.11
n-Propylmercury	CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>2</sub> -Hg-X	499.01	279.14
n-Butylmercury	CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>3</sub> -Hg-X	513.04	293.17
n-Amylmercury	CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>4</sub> -Hg-X	527.07	307.20
n-Octylmercury	CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>7</sub> -Hg-X	566.12	349.28
Stearylmercury	CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>17</sub> -Hg-X	696.31	489.55

Dithizonates of all the compounds were prepared as follows. 10 mg of mercuric chloride or methylmercuric chloride were dissolved in 20 ml of water and 10 mg of the other compounds were dissolved in 20 ml of benzene. After a portion of a benzene solution of dithizone (0.4%, w/v) had been added to each solution with stirring until a green coloration took place, the benzene solution was shaken with 10 ml of 1 N sulfuric acid. It was then shaken with 30 ml of dilute aq. ammonia and subsequently washed twice with 20 ml of water to remove excess dithizone. The solution was then evaporated to dryness under reduced pressure.

By dissolving 10 mg of the dithizonates in 20 ml of benzene, test solutions were prepared. Aliquots ( $\mu$ l) of these solutions were used for TLC.

**Apparatus.** The thin-layer applicator and accessories were obtained from Yasawa Seisaku Co., Ltd. Glass plates were 20 cm  $\times$  5 cm. The chromatographic chamber was 9 cm in diameter and 27 cm in height.

**Preparation of chromatoplates.** (a) Corn starch-liquid paraffin: According to the general method, glass plates were evenly coated with a homogeneous slurry composed of 30 g of corn starch, 3.0 ml of liquid paraffin and 40 ml of chloroform by using

an applicator giving a thin layer approximately  $300\ \mu$  thick. The plates were dried in air at room temperature and stored overnight before use in a desiccator containing calcium chloride.

(b) Avicel SF-liquid paraffin: The chromatoplates were prepared in the same manner as (a) with a homogeneous slurry composed of 30 g of Avicel SF, 6 ml of liquid paraffin and 60 ml of chloroform. The plates were stored overnight before use in a desiccator containing calcium chloride as were the corn starch-liquid paraffin plates.

*Solvent systems for TLC.* The solvent systems used in the experiments were prepared as follows. To ethanol and methyl cellosolve, respectively, water was added at ratios from 0 to 30%.

*Application of samples and development of chromatoplates.* 0.2–0.5  $\mu$ l of the test solutions of dithizonate of each mercury compound were spotted with a micropipette on the starting line 2 cm from the lower edge. The plate was then placed inside a chamber containing the solvent system to a depth of about 1 cm and the sample was developed by the ascending method until the solvent front had travelled a distance of 10 cm from the starting line. The spots were observed under visual light.

## RESULTS AND DISCUSSION

### *Reproducibility of $R_F$ values of the dithizonates of mercury compounds*

It is well known that the nature of the adsorbent, the thickness and activity of the plates and the solvent saturation in the chromatographic chamber are factors that control the reproducibility of  $R_F$  values in TLC. In the separation of the dithizonates of mercury and alkylmercury compounds tested, the most important factor that controls the reproducibility of  $R_F$  values, except those listed above, was the preparation of the layers. The content of liquid paraffin in the layers of corn starch, and Avicel SF, and the amount of moisture which adhered to the layers while chloroform was vaporizing from the layers, after the glass plates had been coated with both slurries, were found critical. In order to resolve the latter problem, the layers prepared were dried overnight in a desiccator containing calcium chloride before use.

### *The content of liquid paraffin in corn starch- and Avicel SF-liquid paraffin layers*

In order to find the content of liquid paraffin in corn starch- and Avicel SF-liquid paraffin layers most suitable for separation of the dithizonates of these mercury compounds, each layer containing liquid paraffin at a ratio of 1.5, 3.0, 4.5 and 6.0 ml, respectively, for 30 g of each adsorbent was prepared according to the sequence and steps described above. The test solutions were applied to the layers which were then developed with a solvent system composed of ethanol and water.

The patterns of the distribution of the spots of the mercuric dithizonates on the chromatograms of both layers were similar when developed with the same solvent system.  $R_F$  values of the compounds decreased as the length of the carbon chain increased; and when increasing the content of liquid paraffin in the layers,  $R_F$  values of the compounds decreased relatively with good distribution of the spots. From the results of this experiment, the content of liquid paraffin in corn starch- and Avicel SF-liquid paraffin layers was adjusted to that described in EXPERIMENTAL.

### Solvent systems

In order to obtain solvent systems which would be able to separate all the mercuric and alkylmercuric dithizonates tested on corn starch- and Avicel SF-liquid paraffin layers, the compounds were applied to each layer and developed with either ethanol-water or methyl cellosolve-water, the water content of which was 0, 10, 20, 25 and 30%, respectively.

As shown in Fig. 1a and 1b, the distribution of spots of the mercury compounds on corn starch-liquid paraffin layers was better using methyl cellosolve-water systems than using ethanol-water systems. It was found from Fig. 1b and 1c that there was

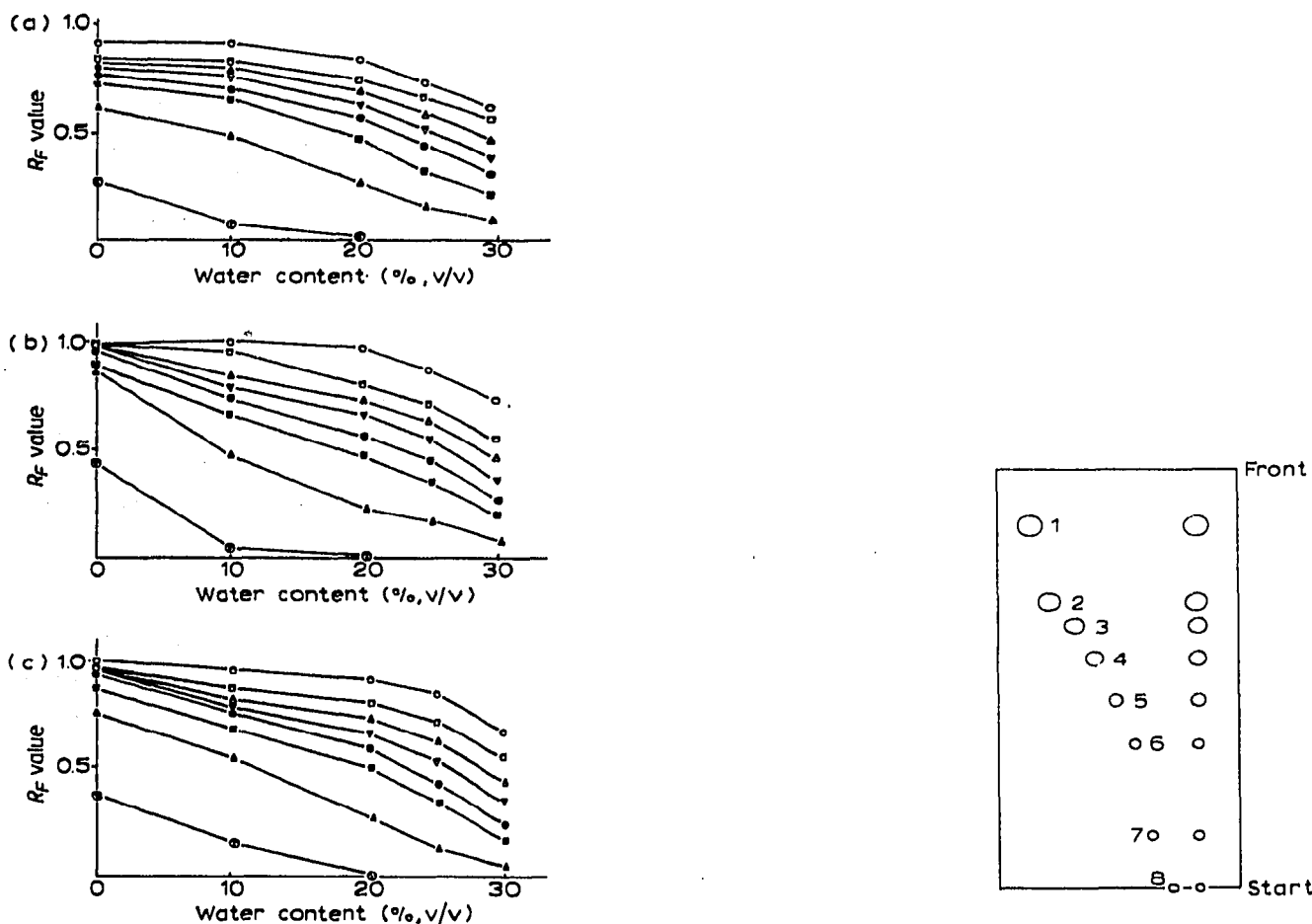


Fig. 1. Relationship between the distribution of mercuric and alkylmercuric dithizonates and the water content in the solvent systems. Mercuric dithizonate ( $\bigcirc$ — $\bigcirc$ ); ethylmercuric dithizonate ( $\square$ — $\square$ ); *n*-butylmercuric dithizonate ( $\triangle$ — $\triangle$ ); *n*-octylmercuric dithizonate ( $\nabla$ — $\nabla$ ); methylmercuric dithizonate ( $\bullet$ — $\bullet$ ); *n*-propylmercuric dithizonate ( $\blacksquare$ — $\blacksquare$ ); *n*-amylmercuric dithizonate ( $\blacktriangle$ — $\blacktriangle$ ); stearylmercuric dithizonate ( $\bigcirc$ — $\bigcirc$ ). (a) solvent system: ethanol-water; layer: corn starch-liquid paraffin; (b) solvent system: methyl cellosolve-water; layer: corn starch-liquid paraffin; (c) solvent system: methyl cellosolve-water; layer: Avicel SF-liquid paraffin.

Fig. 2. Chromatogram of mercuric and alkylmercuric dithizonates and the mixture of them on Avicel SF-liquid paraffin layer. Solvent system: methyl cellosolve-water (75:25). 1 = mercuric dithizonate; 2 = methylmercuric dithizonate; 3 = ethylmercuric dithizonate; 4 = *n*-propylmercuric dithizonate; 5 = *n*-butylmercuric dithizonate; 6 = *n*-amylmercuric dithizonate; 7 = *n*-octylmercuric dithizonate; 8 = stearylmercuric dithizonate.

some difference in the distribution of spots between both layers when using methyl cellosolve-water systems.

On corn starch-liquid paraffin layers, the dithizonates of inorganic mercury and alkylmercury compounds with a carbon chain from  $C_1$ - $C_8$  were distinctly separated with round spots when developed with the solvent systems having a water content of 15-25%, and it was assumed that the spots of dithizonates of alkylmercury compounds with a carbon chain from  $C_8$ - $C_{18}$  might be sufficiently separated from those of octyl- and stearylmercuric dithizonates when developed with a solvent system having a water content of 0-10%.

On Avicel SF-liquid paraffin layers, the dithizonates of mercury and alkylmercury compounds with a carbon chain from  $C_1$ - $C_6$  were separated with the above solvent systems having a water content of 25-30%, and the dithizonates of alkylmercury compounds with a carbon chain from  $C_8$ - $C_{18}$  with the solvent systems containing 0-20% water. In addition, the distribution and sharpness of spots on Avicel SF-liquid paraffin layers were superior to those on corn starch-liquid paraffin layers.

A chromatogram of the compounds on Avicel SF-liquid paraffin layers when using methyl cellosolve-water (75:25) is shown in Fig. 2.

The running time varied with the type of layer used and the water content in the solvent system. On corn starch-liquid paraffin layers 1-1.5 h and on Avicel SF-liquid paraffin layers 3 h were generally required at  $20 \pm 1^\circ$ .

TABLE II

## DETECTION LIMITS OF MERCURY AND ALKYL MERCURY COMPOUNDS

Solvent system: Methyl cellosolve-water (75:25). Layer: Avicel SF-liquid paraffin layer.

Compound	Dithizonate ( $\mu\text{g}$ )	Chloride ( $\mu\text{g}$ )
Mercury	0.01	0.004
Methylmercury	0.1	0.053
Ethylmercury	0.1	0.055
<i>n</i> -Propylmercury	0.1	0.056
<i>n</i> -Butylmercury	0.1	0.057
<i>n</i> -Amylmercury	0.05	0.029
<i>n</i> -Octylmercury	0.01	0.006
Stearylmercury	0.01	0.007

*Sensitivity test for the detection of mercury and alkylmercury compounds*

When spotting each 0.01-1.0  $\mu\text{g}$  of the mercuric and alkylmercuric dithizonates on Avicel SF-liquid paraffin layers and developing them with methyl cellosolve-water (75:25), each spot was detected under visual light. The detection limits of mercuric and alkylmercuric dithizonates and of their chlorides calculated from the corresponding dithizonates are shown in Table II. Detection was so sensitive that the compounds were found at the 0.005-0.057  $\mu\text{g}$  level when calculated as the chloride forms. The lower sensitivities of alkylmercury compounds having a shorter carbon chain in the molecules might be dependent upon any dispersion brought by their higher  $R_F$  values. In contrast to this assumption, mercuric dithizonates showed higher sensitivity than dithizonates of methyl, ethyl, propyl, *n*-butyl and amymercury compounds. This was, however, explained from the results that mercuric dithizonate was

observed as a red spot, which is most sensitive, while the other dithizonates were seen as orange-yellow spots.

#### *Influence of other metals and excess dithizone*

As mercury and alkylmercury compounds form the dithizonates with dithizone over an extensive pH range and even at a comparatively low pH, other metals except copper and some rare metals could be removed without formation of the dithizonates in the procedure described above. The metals, however, which may sometimes contaminate subsequent procedures and may be present in the layers, will react with small amounts of dithizone that may remain in the reaction mixture even after treatment with aq. ammonia or be formed by breakdown of the dithizonates during concentration of the reaction mixture and exposure under helio light. It is, therefore, necessary to know whether any dithizonates of the common metals and residual dithizone influence the detection of these mercuric dithizonates during TLC.

50 mg each of lead nitrate, copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), stannous chloride ( $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ ) and zinc sulfate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) were dissolved in 20 ml of water and neutralized with a small portion of diluted sodium hydroxide. To the solution, a portion of benzene solution of dithizone (0.4%, w/v) was added until the color of the mixture remained green. Treated with aq. ammonia and water, as in the preparation of mercuric dithizonates, the solution was taken to dryness under reduced pressure. After 10 mg each of the dried dithizonates and dithizone had been dissolved in 20 ml of benzene, aliquots of the solutions were spotted on Avicel SF-liquid paraffin layers and developed with methyl cellosolve-water (7:3).

On the chromatogram, all the compounds examined were found close to the solvent front. It is of interest that dithizone was observed as a pink spot. The spot might be of zinc dithizonate formed by the reaction between dithizone and trace amounts of the zinc compounds which had been present in the layer. Thus it was found that all the compounds examined did not have an influence on the detection of the mercury and alkylmercury compounds.

#### CONCLUSION

On either the corn starch- or Avicel SF-liquid paraffin layers, the inorganic mercury and a series of alkylmercury compounds were submitted to reversed-phase thin-layer chromatography. The distribution and sharpness of the spots of the compounds on the Avicel SF layers were superior to those on the corn starch layers, although the running time on the former was longer than on the latter layers. The best pattern of separation of all the compounds was obtained using development on the Avicel SF layers with methyl cellosolve-water (7:3). No interference of the other metals and the residual dithizone was observed.

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