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163. Shunji Ishikura and Keiko Yokota : Analyses of Organic Mercury I. Sensitive and Differential Determination of Phenylmercury Acetate and Inorganic Mercury.

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A number of organic mercury compounds are available as disinfectant, spermidide, fungicide, diuretic for medical or agricultural purpose. Thus, the necessity of establishment of a reliable analytical method for the determination of the organic mercurials has prompted researches in this line.

The methods reported so far, however, consist essentially of the determination of inorganic mercury resulted from the oxidative decomposition of the organic materials. Such methods are hardly capable of differential determination of organic mercurial from the inorganic. Moreover, a portion of mercury present in the material is liable to be lost during oxidative decomposition.

Presently available analytical methods claiming specificity for the organic mercurials in the presence of inorganic mercury compounds are based in principle upon colorimetry by use of dithizone.

Miller, *et al.*[1~3] and Kanazawa[4] reported that phenylmercury compounds can be determined in the presence of mercury ions using diphenylthiocarbazone (dithizone). According their method, an aqueous sample solution is extracted with dithizone chloroform solution, and the chloroform layer is washed with acetate buffer. After washing, unreacted dithizone in chloroform is determined by a photoelectric colorimeter. However, dithizone is proved to combine with many kinds of metals, so the errors caused by the other metals and stability of dithizone itself should be put in considerations in this case.

The present paper describes a method for the separation of organic and inorganic mercuric dithizonates from each other and for the determination of those. Many adsorbents, eluants and chromatographic techniques were tested for deciding the method described below. Phenylmercury dithizonate, mercury dithizonate and unreacted dithizone can be easily separated by chromatography using an alumina column. By this procedure the specific and simultaneous determination of phenylmercury acetate and mercuric ions is effectively conducted.

Experimental

Reagents and Apparatus

Reagent 1——5*N* HCl. Conc. HCl is diluted to 5*N*.

Reagent 2——15.4 mg. dithizone was dissolved in 100 ml. CCl_4. This solution is kept refrigerated ($6\times10^{-4}M$). Diluted with CCl_4 to $2\times10^{-4}M$ before use.

Reapent 3——CCl_4-$CHCl_3$ (95:5 by volume).

Reagent 4——CCl_4-$CHCl_3$ (1:1).

Reagent 5——CCl_4-$CHCl_3$-AcOH (10:10:1). It was prepared freshly before use.

Standard Mercury——Stock solution. 500 mg. of purified metalic Hg was dissolved in 15 ml. of conc. HNO_3 and diluted to 500 ml. with H_2O, and kept in refrigerator. This solution contains 1000 μg. Hg/ml.

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1) V. L. Miller, D. Polley : Anal. Chem., **23**, 1286 (1951).
2) *Idem* : *Ibid.*, **24**, 1622 (1952).
3) *Idem* : *Ibid.*, **26**, 1333 (1954).
4) J. Kanazawa, R. Sato : Bunsekikagaku, 8, 440 (1959).

Standard Mercury——Working solution. 1 ml. of the stock standard was diluted to 100 ml. with H_2O. This solution contains 10 μg. Hg/ml.

Standard Organic Mercury——Stock solution, 419.7 mg. of recrystalized phenylmercury acetate (from EtOH) was dissolved in 10 ml. of glac. AcOH and diluted to 500 ml. with H_2O, and kept in refrigerator. This solution contains 500 μg./ml.

Standard Organic Mercury——Working solution. 2 ml. of the stock standard solution was diluted to 100 ml. with H_2O. This solution contains 10 μg./ml.

Aluminum oxide——Standardized for chromatographic adsorption analysis acc. to Brockmann (Merck Co.) was activated by heating in a 270~330° oven for 2 hr. and after cooling, H_2O was added to Al_2O_3 at the ratio of 6% (w/w), and shaken vigorously for 3 min. Then it was kept tightly closed for 24 hr. or more before use.

Spectrophotometer——Hitachi Photo-Electric Spectrophotometer EPU-2A type.

Procedure

Preparation of column——Conventional 5 mm. in diameter and 300 mm. in length glass tube was used. 1.5 g. of Al_2O_3 was added to 10 ml. of CCl_4 with stirring and the slurry was applied into the tube.

Standard Curve——0.1, 0.5, 1, 2 and 3 ml. of the working standard solution was pipeted into 100 ml. separatory funnels respectively, made 50 ml. with H_2O in all flasks, and followed by additions of 5 ml. of reagent 1 and 5 ml. of reagent 2, then shaken each funnel vigorously for 1 min. After separation, the CCl_4 layer was transfered into a test tube, and 3 ml. of each solution was loaded onto each column. Successive elution with 10 ml. of CCl_4, 5 ml. of reagent 3, and then 5 ml. of reagent 4 gave a yellow eluate of phenylmercury dithizonate first, and then orange eluate of mercury dithizonate separately, and unreacted dithizone and oxidized dithizone were adsorbed on the upper part of the column.

Samples——An aliquat containing 0~30 μg. of Hg was pipeted into a 100 ml. separatory funnel and treated as in the standard curve.

Spectrophotometry——Each fraction was collected into a 10 ml. graduated flask containing 1 ml. of reagent 5, and then the volume was adjusted 10 ml. with reagent 4. The optical density of the sample was determined at 480 mμ for phenylmercury dithizonate, and at 490 mμ for mercury dithizonate against the blank effluent by spectrophotometer.

Calculations——From standard absorbance, read the number of micrograms corresponding to the absorbance of the sample.

Resuts and Discussion

Calibration——Known amounts of mercury or phenylmercury acetate to be tested were subjected to the procedure described above and the results are recorded. The both curves follow Beer's law at range of 0~30 μg. of mercury with practical error of below 5% (Fig. 1).

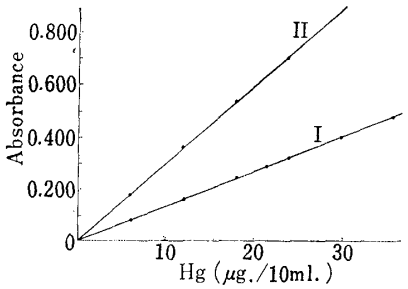
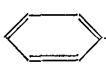


Fig. 1. Calibration Curve for Organic and Inorganic Mercury Dithizonate

I :  -Hg-Dz at 480 mμ

II : inorganic-Hg-Dz at 490 mμ

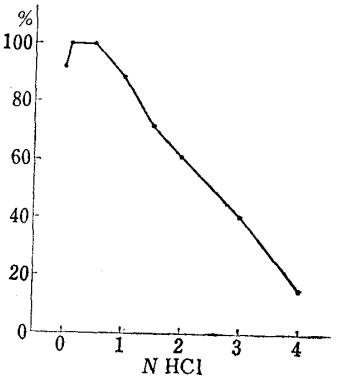


Fig. 2. Effect of Hydrochloric Acid Concentration on Recovery of Phenylmercury Acetate

Effect of Hydrochloric Acid Concentration on Extraction——To determine the most suitable concentration of hydrochloric acid for the extraction, a series of experiments was conducted with several hydrochloric acid concentration. The results obtained is shown in Fig. 2.

Elution Diagrams of Alumina Chromatography of Dithizonates——Many experiments were made by twelve different pure or binary eluants, from alumina ranging in activity from calcined material to that which contained 9% water, to determine the best chromatographic condition for the elution of dithizonates.

Calcinated or 3% water added alumina absorbed dithizonates too strong, and on 9% water added alumina, phenylmercury dithizonate was not adsorbed. And it was found that 6% water added alumina was most suitable for these experiments.

Inorganic mercury dithizonate could be eluted first with carbon tetrachloride while the organic dithizonate required long time and a large amount of the solvent to be eluted out. Addition of chloroform to the eluant made organic dithizonate readily elute and gave a sharp peak as shown in the elution diagram (Fig. 3).

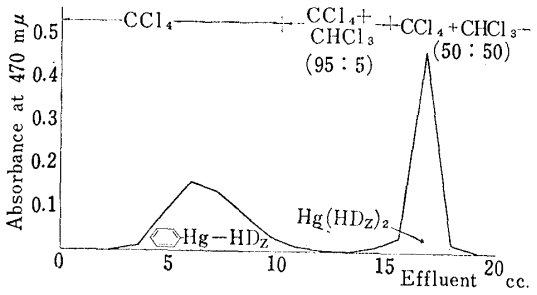


Fig. 3. Elution Diagram of Dithizonates on Alumina Column

Stabilization of Dithizonates——Absorption spectra of dithizone, mercury dithizonate and phenylmercury dithizonate dissolved in the mixture of carbon tetrachloride, chloroform and glacial acetic acid (100:100:1) were determined by Hitachi Recording Spectrophotometer EPS-2, and are shown in Fig. 4. Eluated dithizonates, especially phenylmercury dithizonate was rather unstable to ordinary laboratory light,[5] and the effluent became green in a short time. But it became stable by addition of purified glacial acetic acid to the effluent (1% v/v).[6]

Absorption spectra of eluated dithizonates were determined after 5, 10, 20, 40, and 60 minutes at room temperature without addition of glacial acetic acid, and after 24 hours with preliminary addition of glacial acetic acid. The results obtained are shown in Fig. 5.

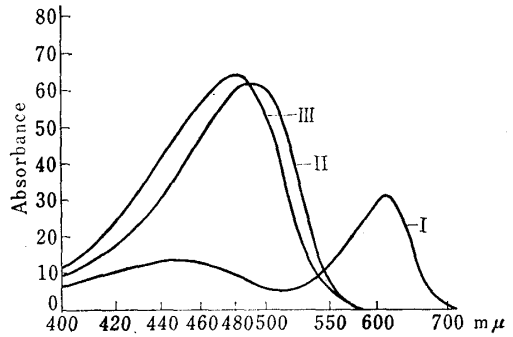


Fig. 4. Absorption Spectra of Dithizone (I), $Hg(HDz)_2$ (II) and $C_6H_5Hg\cdot HDz$ (III) in CCl_4+CHCl_3+AcOH (100:100:1)

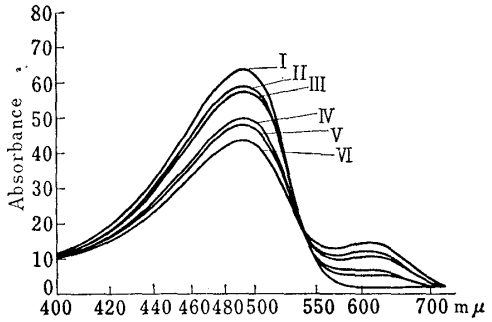


Fig. 5. Stability of $Hg(HDz)_2$ in CCl_4+CHCl_3

Absorption spectra of eluated dithizonates were determined after 5 (II), 10 (III), 20 (IV), 40 (V) and 60 (VI) minutes without addition of glacial acetic acid, and with preliminary addition of glacial acetic acid (I).

5) J. L. A. Webb : J. Am. Chem. Soc., **72**, 91.
6) T. Ashizawa : Bunsekikagaku, **10**, 443 (1961).

Effect of Other Metal Ions on Accuracy of Analytical Procedure——Various metal ions known to combine with dithizone in acidic medium were added to the test solution at the first step of procedure and carried through the extraction process. In the presence of 40 μg. of phenylmercury acetate, 200 μg. of copper, silver and tin did not interfere, but with zink a slight less recovery was observed. Table I gives the results of these experiments.

Table I. Effect of Other Metal Ions on Accuracy of Analytical Procedure

Inorganic ion (μg.)	Phenylmercury acetate (μg.)	Absorbance (490 mμ)	Recovery (%)
Cu^{++} 200	40	0.651, 0.647, 0.640	98.7
0	40	0.654, 0.653, 0.645	100
Ag^{+} 200	40	0.604, 0.600, 0.595	98.2
0	40	0.606, 0.610, 0.616	100
Sn^{++} 200	40	0.602, 0.604, 0.596	99.5
0	40	0.609, 0.605, 0.599	100
Zn^{++} 200	40	0.609, 0.590, 0.589	96.6
0	40	0.617, 0.623, 0.612	100
200	40	0.574, 0.572	97.0
0	40	0.590, 0.592	100

Other Oragnic Mercurials——It is revealed that the present method is applied successfully for the other organic mercurials than phenylmercury acetate. Details will be reported subsequently.

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Summary

A simple, sensitive analytic method for measuring to differentiate inorganic mercury and phenylmercury acetate has been developed. The method is found useful for analysis of mercurials used extensively as drug in medical and agricultural fields. An acidified sample solution was extracted with dithizone-carbon tetrachoride solution, the carbon tetrachloride layer was chromatographed on an alumina column, and the separated dithizonate solutions were measured by photoelectric colorimeter. By means of the method here reported, organic and inorganic mercury can be separately determined at levels to 0.02 p.p.m. with an error not exceeding 5%, using a minute amounts of materials and without subjecting it to the tedious procedure of preliminary oxidative degradation. This method is not disturbed by other metal ions. Stabilization of dithizonates is described.

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