# Inhibitors of Lead Analysis in Atomic Absorption Spectrometry

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## INTRODUCTION

As methods of determining trace lead contained in foods, dithizone colorimetry, polarography and atomic absorption spectrophotometry are generally carried out on test solutions prepared by the wet or dry ashing of samples. Of these methods, atomic absorption spectrophotometry has come to establish its place as a major tool for lead analysis in the past few years.

In the atomic absorption spectrophotometric method, when the direct injection of a test solution is possible, lead determination is favorably simple, but the coexistence of various negative or positive ions is known to hamper lead determination. This tendency is more pronounced in the presence of negative ions. In order to eliminate these effects, for instance, strontium, lanthanum, etc., are added. Moreover, lead is extracted with MIBK and other organic solvent in the presence of a chelate reagent, such as DDTC, APDC and organic amine, to remove inhibitory ions and concentrate test solutions. Atomic absorption spectrophotometry employs two methods of atomizing elements : flame atomizer and flameless atomizer. The latter has been recently used more frequently because of to its better sensitivity.

As fundamental experiments on the adaptability of the method of directly injecting a hydrochloric acid solution of a sample and determining the lead content in the sample by a flameless atomic absorption spectrophotometric analyzer, this study selected potassium, calcium and magnesium ions as ions contained in relatively great quantities in animal and vegetable foods and investigated by a model approach the effects of coexistence of these ions on the determined lead contents. In addition, a few other examinations were made.

## EXPERIMENTAL

1. Instruments

1-1) Perkin Elmer atomic absorption spectrophotometer Type 303 with heated graphite atomizer HGA72 and deuterium background correction.

| | |
|---|---|
| Wavelength | 283.3 nm |
| Step 1 | Drying at 100℃ for 30 sec |
| Step 2 | Ashing at 550℃ for 30 sec |
| Step 3 | Atomization at 2,040℃ for 5 sec |
| Step 4 | Heating-out at 2,600℃ |
| Purge gas | $N_2$ |

1-2) All glassware was washed with dilute nitric acid [$HNO_3$(1 + 1)] before use.

2. Reagents

JIS guaranteed reagents or reagents manufactured by Merck were used and N-HCl was used to dilute solutions.

2-1) Lead Standard Stock Solution

0.1599g of lead nitrate [$Pb(NO_3)_2$] was weighed and dissolved in 10 ml of $HNO_3$ (1 → 100). Water was added to prepare 100 ml of lead standard stock solution. 1 ml = 1 mg Pb.

2-2) Potassium Standard Stock Solution

19.067g of potassium chloride (KCl) was dissolved in N-HCl to prepare 100 ml of potassium standard stock solution. 1 ml = 100 mg K.

2-3) Calcium Standard Stock Solution

24.9726g of calcium carbonate ($CaCO_3$) was dissolved in approx. 85 ml of 20% HCl. An excess of hydrochloric acid was removed by heating on a hot plate and the resultant product was dissolved in N-HCl to obtain 200 ml of calcium standard stock solution. 1 ml = 50 mg Ca.

2-4) Magnesium Standard Stock Solution

16.5828g of magnesium oxide (MgO) was dissolved in 135 ml of 20% HCl and treated in the same way as the calcium standard stock solution to prepare 200 ml of magnesium standard stock solution. 1 ml = 50 mg Mg.

2-5) Ammonium Citrate Solution

45g of ammonium citrate dibasic [$(NH_4)_2HC_6H_5O_7$] was dissolved in 100 ml of water and two to three droplets of phenol red T.S. were added. Ammonia water was added until the solution exhibited a red color. To remove lead, this solution was extracted with 20-ml portions of the dithizone-chloroform solution described later until the dithizone solution maintained its peculiar green color. Furthermore, dithizone remaining in the solution was extracted with chloroform.

2-6) 20% Hydroxylamine Solution

Hydroxylamine hydrochloride solution was made alkaline by ammonia water beforehand. DDTC was added and the resultant solution was extracted with chloroform. This extraction was repeated until the water layer did not exhibit a yellow color when the chloroform layer was shaken with 1% $CuSO_4$. HCl was then added to the solution to prepare a 20% hydroxylamine solution.

2-7) Dithizone Chloroform Solution

30 mg of dithizone was dissolved in chloroform to prepare 1,000 ml of dithizone chloroform solution. The obtained solution was washed twice with half volume of N-HCl before use.

3. Preparation of Test Solutions

3-1) Lead Standard Solutions

The lead standard solution was diluted to a 10-ppm lead solution. 0, 0.5, 1.0, 1.5 and

2.0 ml portions were weighed from the solution and diluted to 100 ml. The resultant dilute solutions contained 0, 0.05, 0.1, 0.15 and 0.20 ppm of lead, respectively.

3-2) Potassium Coexistent Lead Standard Solutions

A total of 24 types of N-HCl solutions were prepared which contained 0, 0.05 or 0.15 ppm of lead and 0, 50, 100, 250, 500, 1,000, 2,500 or 5,000 ppm of potassium.

3-3) Calcium Coexistent Lead Standard Solutions

Solutions were prepared which contained 0, 0.05 or 0.15 ppm of lead and 0, 50, 100, 250, 500, 1,000, 2,500 or 5,000 ppm of calcium.

3-4) Magnesium Coexistent Lead Standard Solutions

In the same procedure as described for the potassium coexistent lead standard solutions in Section 3-2), solutions were prepared which contained 0, 50, 100, 250, 500 or 1,000 ppm of magnesium.

3-5) Canned Apricots in Syrup

After a can containing apricots in syrup was opened, pits were removed and the contents including the syrup were homogenized with a mixer. 20-g portions of the contents were transferred in beakers. Lead was incorporated in the samples in the quantities of 0, 2 μg (= 0.1 ppm) and 4 μg (= 0.2 ppm) using the lead standard solutions. The sample was ashed by the dry method and left to cool. 5 ml of HCl (1 + 1) was added to the ash and the solution was evaporated to dryness on a water bath. The residue was dissolved in N-HCl to prepare 50 ml of solution. 5 ml of the resultant solution was used for lead determination.

3-6) Bottled Mandarin Orange Juice

The contents were sufficiently homogenized and the lead standard solutions were added to 20-g portions of the contents. Each sample was ashed and treated in the same way as for the canned apricots in syrup mentioned above to prepare 50 ml of solution. 10 ml of the obtained solution was used for lead determination.

4. Measuring Methods and Results

Eppendorff micropipettes were used to inject test solutions in the graphite tube of an atomic absorption spectrophotometric analyzer.

4-1) Direct Method

The test solutions 1) through 4) were determined for lead content.

(1) Effect of Acid Type and Concentration

The effects of acid type and concentration on the absorbance of lead were examined for hydrochloric acid, nitric acid and sulfuric acid. When these acids bearing 0.1 ppm of lead and having an acid concentration of 0.01, 0.05, 0.1, 0.5, 1.0 or 2 N were determined for lead, HCl was found to be most stable, great in absorbance and not subject to a difference in concentration. For this reason, N-HCl was used in the following experiments.

(2) Working Curve

One example of working curve is illustrated in Fig. 1 and examined results of reproducibility are given in Table 1. A typical waveform is shown in Chart 1.

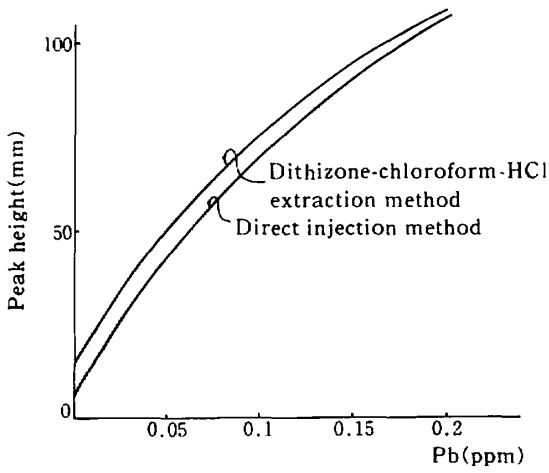


Fig.1 Working Curves

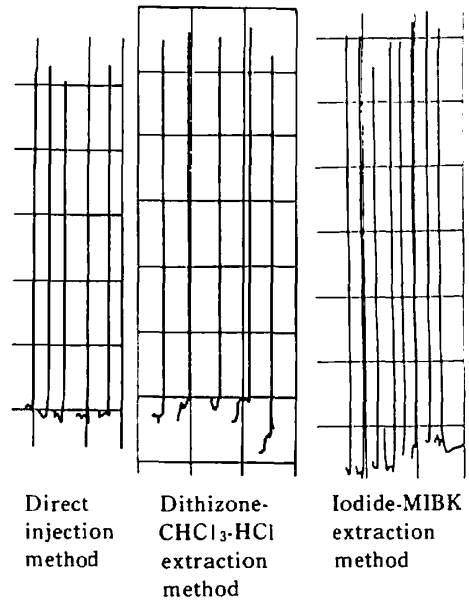


Chart 1 Waveforms of 0.1 ppm Lead Solutions

Table 1 Comparison in Reproducibility of Determined Lead Content
(Expressed in peak height in mm at injection of 10 $\mu\ell$ of 0.1 ppm Pb solution)

| Determination No. | Direct injection method | Iodide-MIBK extraction method | Dithizone-$CHCl_3$-HCl extraction method |
|---|---|---|---|
| 1 | 65.4 | 85.5 | 73.4 |
| 2 | 67.5 | 78.0 | 71.5 |
| 3 | 66.0 | 83.0 | 71.6 |
| 4 | 68.0 | 80.3 | 72.5 |
| 5 | 67.0 | 83.8 | 73.7 |
| Average | 66.8 | 82.1 | 72.5 |
| Standard deviation | 0.956 | 2.657 | 0.901 |
| Coefficient of variation | 0.016 | 0.036 | 0.014 |

(3) Effect of Coexistent Positive Ions

The effects on the peak height of lead of coexistent potassium and calcium ions

are presented in Figs. 2 and 3, respectively. Calcium was far greater than potassium in effect and found to exhibit the phenomenon of waveform distortion during determination. Magnesium was greater than the former two in effect and drew two-step waveforms as shown in Chart 2, rendering lead determination impossible.

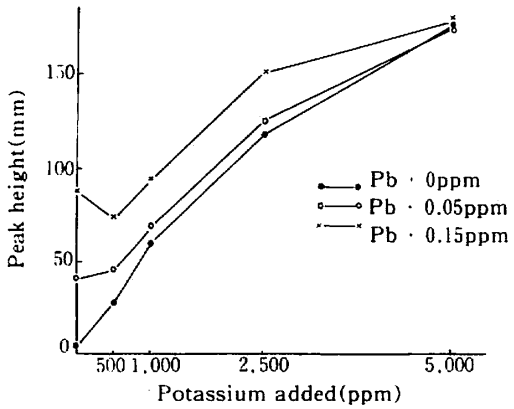


Fig.2 Effects of Potassium Coexistence in Direct Injection Method

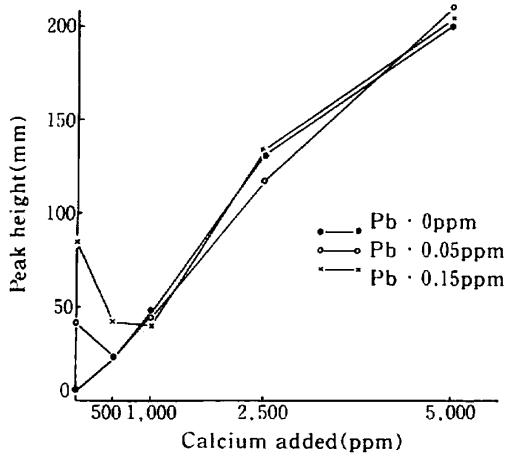


Fig.3 Effects of Calcium Coexistence in Direct Injection Method

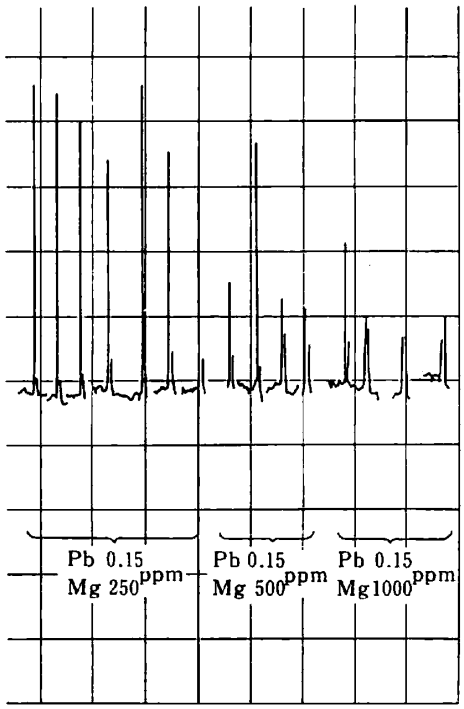


Chart 2 Waveforms in Presence of Magnesium

4-2) Iodide-MIBK Extraction Method

A certain amount of the test solution was weighed into a separatory funnel. N-HCl was added to obtain 40 ml of solution. 20 ml of 85% $H_3PO_4$ was added and mixed. 7 ml of a saturated potassium iodide solution was added and shaken. After 5 min, 10 ml of MIBK was added for extraction. The MIBK layer was batched off, dehydrated and injected. Table 1 compares peak heights obtained under this method and the other two when five repetitive determinations made on solutions containing 0.1 ppm of lead. The difficulty of maintaining the injection amount constant, when compared with injection in the water system, was shown to greatly vary peak heights, as illustrated in Chart 1, and offer poor reproducibility.

4-3) Dithizone-Chloroform-HCl Extraction Method

10 ml of the test solution was transferred into a 100-ml separatory funnel and 5 ml of ammonium citrate solution, 2 ml of 20% $NH_2OH$ solution and 2 to 3 droplets of phenol red T.S. were added and ammonia water was added for alkaline. The test solution was repeatedly extracted with 10, 5 and 5 ml of the dithizone-chloroform solution. The chloroform layer was collected into a stoppered test tube and 10 ml of N-HCl was added. The extracted water layer was injected.

(1) Working Curve

A typical working curve is shown in Fig.1 and examined reproducibility results are presented in Table 1. An example of waveform is diagrammed in Chart 1.

(2) Effect of Coexistent Positive Ions

The coexistence of potassium, calcium and magnesium ions was found not to interfere with lead determination at all.

(3) Measurement of Recovery

Table 2 shows measurements of additive recovery made on the test solutions (5) and (6).

Table 2 Measurements of Lead Recovery

| Sample | Lead detected in sample (ppm) | Lead added (ppm) | Lead detected (ppm) | Recovery (%) | Tin (ppm) |
|---|---|---|---|---|---|
| Canned apricot in syrup n = 4 | 0.15 | 0.10 | 0.25 | 100 | 155 |
| | | 0.20 | 0.37 | 110 | |
| Mandarin orange juice n = 3 | 0.05 | 0.10 | 0.14 | 90 | 0.1 |
| | | 0.20 | 0.23 | 90 | |

## DISCUSSION

As the method of determining the amount of lead contained in canned foods, we have been traditionally using mainly the dry ashing polarographic method because of the ease of treating many samples. This method was, however, very difficult to determine lead quantities less than 0.1 ppm and had the problem of poor reliability of determined values. Since the need has been increasing recently for grasping background values, we attempted several investigations to apply the flameless atomic absorption spectrophotometric method which insures high sensitivity among atomic absorption spectrophotometric methods and enables the determination of trace amounts.

When test solutions derived from the ashing of sample foods to measure background values are examined, the acid type and concentration employed pose problems. Our study of this point showed that N-HCl is suitable and assures good reproducibility.

Foods contain numerous ions. In general, potassium and calcium ions are found in greater quantities. Considering the amount of ions contained in ordinary foods, we individually added these ions to test solutions and studied the influence of these ions on the determination of lead by a model approach. The direct injection method showed that the coexistence of any ions affects the determination of lead. We, therefore, attempted the iodide-MIBK extraction method which is capable of eliminating the influence of inhibitory ions and "concentrating" and relatively easy to employ. In the MIBK extraction method, MIBK deposits to the opening of a carbon rod or deposits and remains in trace quantity at the outside end of a micropipette, thereby making it considerably difficult to inject a constant amount. As is evident from Table 1, the standard deviation under the MIBK extraction method is greater than when a water system test solution is injected, making determined values less reliable. For these reasons, we tried the method in which the sample was extracted with the dithizone-chloroform solution long used for lead determination, transferred into a water system by reverse extraction with N-HCl and made an injection test solution in order to assure correct measurements despite complicated operations. Because of the nature of atomic absorption spectrophotometry, KCN, a maskant in general dithizone methods, was not used. As shown in Fig. 1, the working curve obtained by this method assumes the same shape as is obtainable by the direct injection method but exhibits a peak height approximately 7 mm higher when compared at various concentrations. This is because N-HCl was used in a quantity twice as great as in the direct injection method. There was recognized no great difference in reproducibility between this method and the direct injection method. The influence of coexistent ions recognized in the direct injection method was completely eliminable. To ascertain this capability, peak heights when 0, 0.05 and 0.15 ppm lead solutions were treated were assumed to be unity. Ratios were obtained of such peak heights to the peak heights measured when solutions bearing lead at the same concentrations and containing potassium, calcium or magnesium were treated and

determined for lead. The peak height ratios thus calculated are indicated by broken lines in Figs. 4, 5 and 6. The values are all unity, showing that there are no effects of these ions.

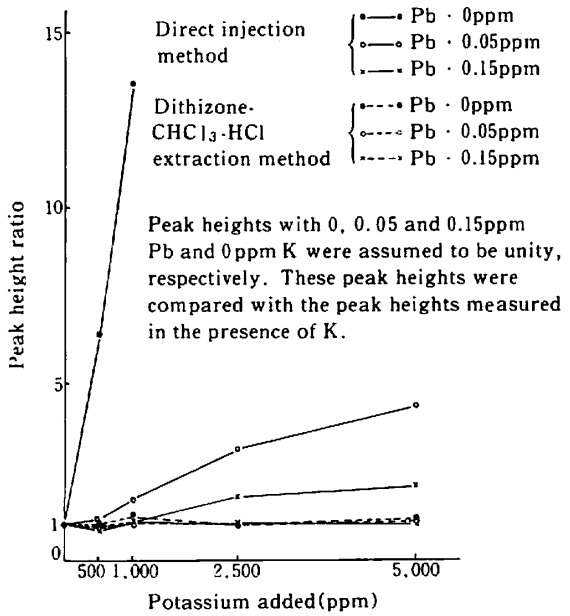


Fig.4 Comparison in Effect of Potassium Between Dithizone-$CHCl_3$-HCl Extraction Method and Direct Injection Method

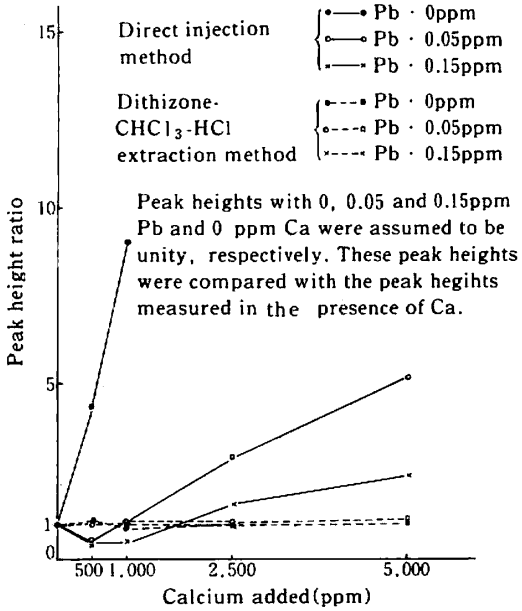


Fig.5 Comparison in Effect of Calcium Coexistence Between Dithizone-$CHCl_3$-HCl Extraction Method and Direct Injection Method

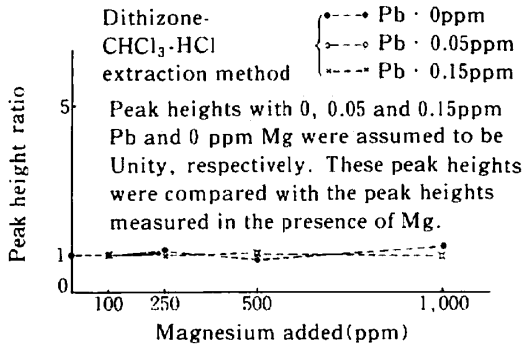


Fig.6 Effect of Magnesium Coexistence in Dithizone-$CHCl_3$-HCl Extraction Method

Since the above model experiments provided good results, we made additive recovery experiments on actual foods packed in cans and bottles. The results are shown in Table 2. The recovery ranges from 90 to 110%, a range among which lead can be determined accurately enough for practical purposes.