A SIMPLE METHOD FOR COLORIMETRIC DETERMINATION OF URINARY δ-AMINOLEVULINIC ACID IN WORKERS EXPOSED TO LEAD

Key words: urinary δ-aminolevulinic acid; simple method; colorimetry; lead worker; biological monitoring

Urinary excretion of δ-aminolevulinic acid (ALA) has been widely used as a measure of the biological effect of lead in workers occupationally exposed to lead. Generally, urinary ALA has been determined by colorimetric methods based on the color reaction of ALA-pyrole with Ehrlich’s reagent.1-4) By the difference of pretreatment, these conventional methods can be divided into two groups: the first group requires ion-exchange chromatography of urine1,2) and the other group involves liquid-liquid extraction instead of ion-exchange chromatography.3,4) In comparing the two groups, the former is more time-consuming and expensive than the latter.

In Japan, the method of Tomokuni and Ogata4) is widely used in large-scale routine analysis. However, when this method is applied to urine with higher density, urinary matrix interference is observed. A similar result is also obtained using the method of Wada et al.3) Recently, we modified the original method of Tomokuni and Ogata4) by diluting the urine specimen 3-fold with distilled water in advance to keep the matrix interference to a minimum.5)

In the present study, new findings were obtained which had not been described in our previous reports.4,5) These findings led to modification in the procedure. This is described here.

Materials and methods. All chemicals used were of the analytical grade. Acetate buffer (1 mol/l, pH 4.6); Dissolve 136g of sodium acetate trihydrate and 57ml of glacial acetic acid in distilled water and dilute to 1 l. Ehrlich’s reagent; Dissolve 2g of p-dimethylaminobenzaldehyde in 80 ml of glacial acetic acid, and add 10 ml of 60% perchloric acid and 10 ml of distilled water. ALA standard (5 mg/l); Prepare 100 mg/l stock solution of ALA by dissolving 12.8 mg of ALA-HCl and diluting to 100 ml with distilled water. Dilute the stock solution 20-fold with distilled water. The spot urine was diluted 3-fold with distilled water in advance to keep the matrix interference to a minimum.5)

In the present study, new findings were obtained which had not been described in our previous reports.4,5) These findings led to modification in the procedure. This is described here.

Materials and methods. All chemicals used were of the analytical grade. Acetate buffer (1 mol/l, pH 4.6); Dissolve 136 g of sodium acetate trihydrate and 57 ml of glacial acetic acid in distilled water and dilute to 1 l. Ehrlich’s reagent; Dissolve 2 g of p-dimethylaminobenzaldehyde in 80 ml of glacial acetic acid, and add 10 ml of 60% perchloric acid and 10 ml of distilled water. ALA standard (5 mg/l); Prepare 100 mg/l stock solution of ALA by dissolving 12.8 mg of ALA-HCl and diluting to 100 ml with distilled water. Dilute the stock solution 20-fold with distilled water. The spot urine was diluted 3-fold with distilled water, and 1 ml of this sample was pipetted into each of two 10-ml glass-stoppered tubes (sample and blank). Analysis was carried out according to the procedure shown in Fig. 1.

Results and discussion. In our recent report,6) we recommended the use of methyl acetoacetate as the condensation reagent because the formation of ALA-pyrole by methyl acetoacetate was about 7% higher than that by ethyl acetoacetate. However, in the present method, ethyl acetoacetate was used because of its fragrance.

In the original method,4) ALA-pyrole formed was extracted with 3 ml of ethyl acetate, and 2 ml of the extract was mixed with 2 ml of Ehrlich’s reagent (total 4 ml). In this reaction system, the 3-fold dilution of the extract (ethyl acetate layer)
was finally made. On the other hand, in the present method, ALA-pyrrole was extracted with 2 ml of ethyl acetate, and 1 ml of the extract was mixed with 2 ml of Ehrlich's reagent (total 3 ml). Therefore, 6-fold dilution of the extract was finally made. Judging from the dilutions, the color intensity (absorbance at 553 nm) in the present method is estimated to be half of that in the original method.4) However, the actual absorbance obtained by the present method was only 15% lower than that obtained by the original method.4) This finding indicates that the volume ratio of the extract and Ehrlich's reagent influences the color development.

As shown in Fig. 2, the relationship between aqueous ALA concentration and absorbance at 553 nm was linear up to 25 mg/l. The colored solution was stable for more than 40 min at room temperature.

Figure 3 shows the results concerning the stability of ALA-pyrrole. When the extract of ALA-pyrrole is allowed to stand in the light at room temperature, ALA-pyrrole seems to be gradually oxidized. Actually, when the extract of ALA-pyrrole was mixed with Ehrlich's reagent after it had been allowed to stand for 2 h at room temperature in the light, the color intensity decreased to 76% of the initial level for aqueous ALA and 56% for urinary ALA. In contrast, ALA-pyrrole was stable for at least 2 h when the extract was stored in the dark at room temperature. This finding has not been reported in our previous papers.4,5) From this result, the color development of ALA-pyrrole should be done within at least 1 h after the extraction.

In conclusion, the present method for determining urinary ALA is useful in mass screening for lead workers.

We are grateful to Mr. R. Kadoya and Mr. K. Furukawa, students of Saga Medical School, for their technical assistance.

References

Katsumaro TOMOKUNI and Masayoshi ICHIBA
Department of Community Health Science, Saga Medical School, Saga, 840-01 Japan

Received for publication, July 15, 1987
Reprint requests to K. Tomokuni, 通信先：友関あて