SHIMADZU APPLICATION NEWS

LA122-E006B

● SPECTROPHOTOMETRIC ANALYSIS

No.A342B

Speciation of Arsenic in Blood by Cold Trap Vapor Reduction Atomic Absorption Method

The toxicity of arsenic differs depending on its chemical form. Inorganic arsenic has stronger toxicity than methylated arsenic species such as monomethyl, dimethyl and trimethyl arsenics. When ingested into human body, inorganic arsenic is metabolized and changes its chemical form into methylated arsenic, and to dimethylated arsenic. The arsenic toxicity decreases through this process of metabolism. The level of arsenic exposure in a person can be evaluated by analyzing these metabolites.

One method for speciating arsenic is “the cold trap - vapor reduction - atomic absorption spectroscopy”. This Application News introduces an example of speciating arsenic in human blood using this method. It has been generally thought that speciating arsenic in human blood is difficult because the arsenic concentrations in blood are usually extremely low, and interfering substances largely affect the analysis. However, by combining “the cold trap - vapor reduction - atomic absorption spectroscopy” and a simple pretreatment procedure, arsenic in blood can be easily analyzed.

■ System Configuration and Applicable Samples

The system consists of the following four units:
1. Unit where the sample is mixed with the reaction solution and reducing agent to produce arsine gas
2. Unit where the generated arsine gas is dehumidified and trapped at an extremely low temperature
3. Unit where the arsine gas is atomized and analyzed using atomic absorption spectroscopy
4. Unit where data is processed

Environmental Samples

This system is applicable for a variety of environmental samples, including fresh water from rivers, ground, or hot springs, seawater, and soils. When analyzing fresh or salt water, trivalent and pentavalent species of inorganic arsenic can be individually measured without pretreatment. When analyzing solid samples such as soils, thermal alkaline decomposition is required.

Food Products

This system is applicable for the majority of commonly distributed food products. When analyzing liquid samples such as soft drinks or soy source, trivalent and pentavalent species of inorganic arsenic can be individually measured without pretreatment. When analyzing solid samples, thermal alkaline decomposition is required.

Atmosphere or Work Environments

Dust containing arsenic is collected onto a membrane filter and thermally decomposed in alkaline for measurement.

Biological Samples

This system can be applied to a wide range of biological samples, including human, test animal, or cell samples. When analyzing arsenic in urine from humans or other mammals, generally, thermal alkaline decomposition is not necessary. However, the urine of humans and other mammals usually contains arsenobetaine, a type of trimethylated arsenic, which cannot be analyzed using this system. Thermal alkaline decomposition is required for analyzing arsenobetaine. In the special case where the urine contains trimethylarsine oxide, another type of trimethylated arsenic, thermal alkaline decomposition is not required.

For biological samples such as blood, hair or tissue, thermal alkaline decomposition is normally necessary. Arsenic in samples those don’t decompose by this method is difficult to speciate. In such a case, acid decomposition is performed and the total arsenic concentration is obtained.

■ Pretreatment for Arsenic Speciation

Generally, 0.2 to 2g of samples are used for pretreatment, though the appropriate amount varies depending on the quantity of arsenic contained. The recommended pretreatment weights for biological samples are about 1mL for human urine or blood, 0.2 to 0.5g for hair, and 1g for biological tissue. Those for food products, normally about 1 to 2 wet g are necessary. For seafood product samples, whose arsenic concentration levels are 10 to 1000times higher than for terrestrial plants and animals and also vary over a wide range, a special care is required for pretreatment.

This system can not measure the trimethylated arsenic arsenobetaine. Therefore, if arsenobetaine is contained in the sample, thermal alkaline decomposition must first be performed to convert it to trimethylarsine oxide. A typical procedure for thermal alkaline decomposition pretreatment is shown below.

Place a specified quantity of the sample in a 10mL heat resistant plastic container and add 2 - 4mL of 2N sodium hydroxide solution. Heat it at 100˚C for 2 - 3hours using a heating device such as a block heater. Insoluble matter may be observed after thermal decomposition. However, centrifugal separation or filtering is not necessary, because arsenic will elute into the solution. It is preferable to analyze the sample immediately after pretreatment. However, it can be stored by freezing at -30 to -180˚C. Slowly thaw the frozen sample and dilute it with distilled water for analysis.

In this example, the sample solution was prepared by adding 1mL of 2N sodium hydroxide solution to 0.5mL of human blood. The mixture was thermally decomposed, let it cool, and then diluted to 2mL with distilled water.

■ Measurement Example

A reaction solution of 1% hydrochloric acid and a reducing agent of 10% sodium borohydride were used. Fig. 1 shows the profile for a standard arsenic mixture (1 ppb for each species). The injected sample volume was 1.8mL, so the peaks correspond to 1.8ng of arsenic. Fig. 2 shows the profile for the sample, and Table 1 the measurement results. The measurement results are multiplied by the dilution factor.

Table 1 Measurement Results

	Equivalent concentrations in blood (ppb)
Inorganic arsenic	0.4
Monomethylated arsenic	1.0
Dimethylated arsenic	0.7

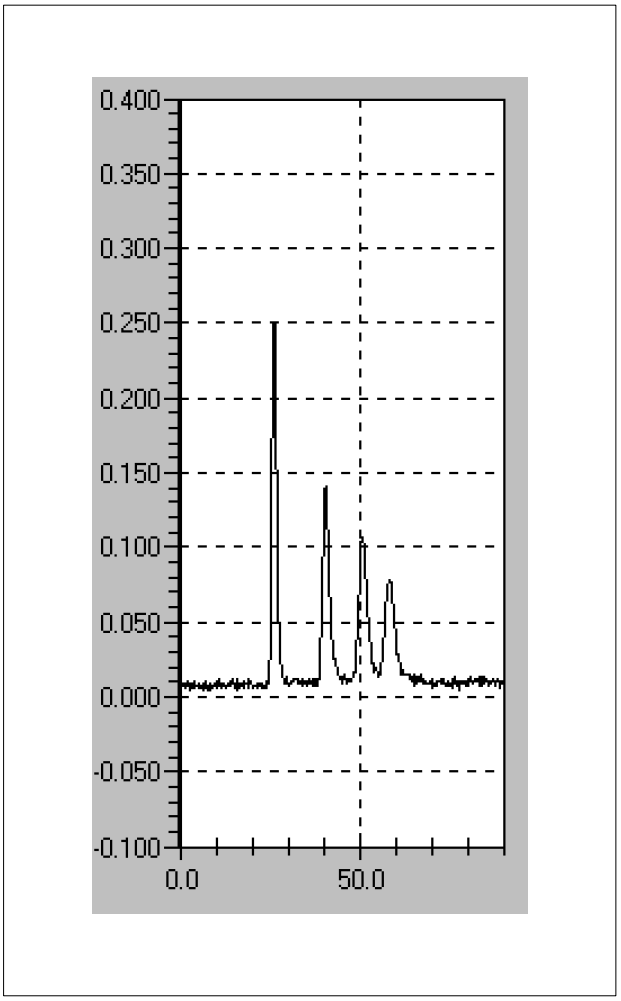

Fig.1 Profile of standard

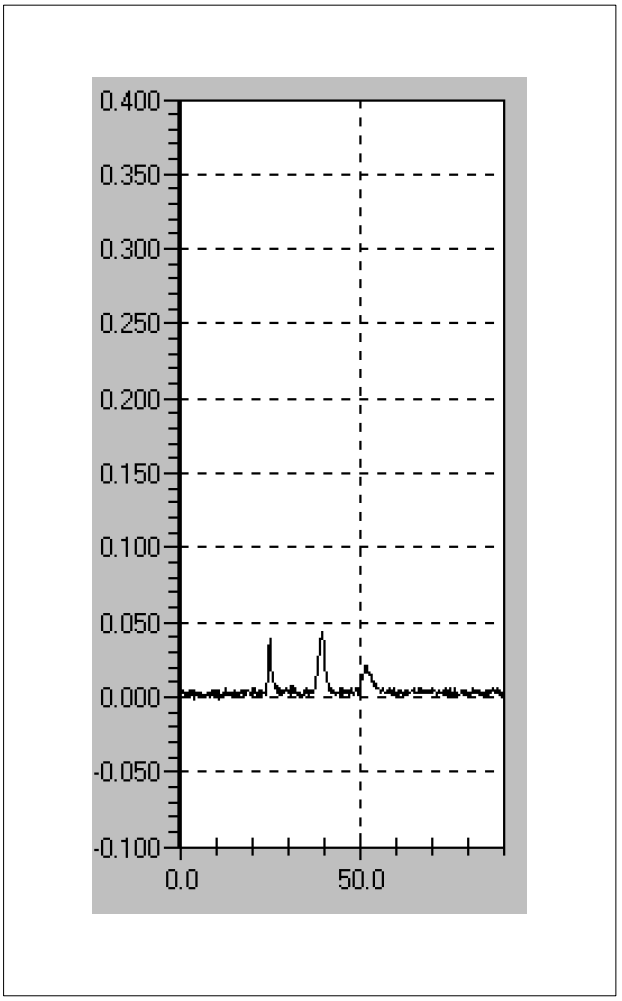

Fig.2 Profile of sample

■ Conclusion

Arsenic can be analyzed with high sensitivity by the furnace or hydride generation method. However, when analyzing samples such as blood containing extremely low concentrations of arsenic and high concentrations of complex interfering substances, the interfering substances significantly affect the results. The hydride generation method is also interfered by coexisting organic matter, and therefore, complex pretreatment using acid is required to eliminate organic matter. The system introduced here enables high-sensitivity arsenic speciation with simple pretreatment, so will take a important part in a wide range of applications.

*The published data was not acquired using an instrument registered by Japanese pharmaceutical affairs law.

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Printed in Japan 3100-06401-10A-IK