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Arsenic Determination in Autopsy Material Using Atomic Absorption Spectroscopy

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Summary. In a case of fatal arsenic intoxication samples of blood, liver and bile were analysed using atomic absorption spectroscopy. Different flames were used. The liver sample was ashed in a low temperature asher. The method is simple and requires a minimum of sample handling.

Zusammenfassung. In einem Fall von tödlicher Arsen-Intoxikation wurden Proben von Blut, Leber und Galle mit Hilfe von einem Atom-Absorptions-Spektrophotometer analysiert. Die Leberprobe wurde unter niedrigen Temperaturbedingungen verascht. Die Methode ist einfach und erfordert ein Minimum an Vorbehandlung.

Key words: Arsenic, atomic absorption — Atomic absorption, arsenic — Autopsy material, arsenic determination.

Arsenic is one of the most famous compounds in forensic toxicology. Fatal intoxications with arsenic trioxide are now rare, but the determination of arsenic is still an important analysis in forensic chemical laboratories.

Different techniques have been used in arsenic determinations. The most commonly methods used are neutron activation-analysis, spectrophotometry and atomic absorption spectroscopy. Neutron activation is the most sensitive method, but it requires access to a reactor with specialized equipment. Spectrophotometric arsenic determination requires a lot of sample preparation work. After ashing the biological material to form arsenic oxide, these oxides are reduced with zinc in hydrochloric acid solution evolving arsine.

The evolved arsine forms a coloured complex with silverdiethyldithiocarbamat in pyridine or chloroform (George *et al.*, 1973; Kopp, 1973). Both wet and dry ashing procedure can be used.

Atomic absorption is a simple and rapid method for arsenic determination but its sensitivity is relative poor due to the occurrence of the resonnance lines of arsenic in the far UV region. Most of the radiation from the light source is absorbed by the air and the flame gases absorb strongly at these wavelengths. This results

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in a weak signal. Fluctuation in the flame also gives rise to variation in the readings. In spite of all this, atomic absorption has been applied for arsenic analyses in different samples (Hill, 1969; Spielholtz *et al.*, 1971; Williams, 1972). Different atomic absorption techniques have been used (flameless (Weltz, 1973), arsine reduction (Fernandez, 1973)) to determine trace amounts, but ordinary flame technique was used in this work.

Low temperature ashing is an excellent technique for the combustion of biological samples. The temperature is normally as low as 100—200°C depending on the high frequency current, but the exact temperature in each experiment is difficult to determine. Tissues are ashed in an atmosphere of oxygen excited by the radiofrequency current under low pressure (Sanui, 1970; Schwab, 1968). Under proper conditions the temperature is so low that the loss of arsenic is negligible.

Material and Methods

Case History. A 52-year-old woman was left in bed by her husband at 10 a.m. She had been mentally depressed for 1 year. When the husband came home 2 hrs later, his wife was still in bed. She was very ill and vomited. She told her husband that she had taken some poison which had been prescribed for animals 11 years ago. A doctor was sent for and she was admitted to hospital at 2.30 p.m. and she died there about 11 p.m. the same evening. In the meantime her husband had thrown away all that was left of the poison. It was, however, suspected that it contained arsenic trioxide.

Sample Preparation. Samples of blood, bile and liver were collected at autopsy and delivered for toxiological examination. Blood and bile were diluted four times with water and centrifuged. The supernatants from these mixtures were analysed directly by atomic absorption using the argon/hydrogen flame. Biological reference samples containing known amounts of arsenic were made from blood. These blood samples were both analysed directly after dilution with water and after ashing with the low temperature asher. Both methods gave the same result. The liver sample was dried to constant weigth at 70°C and finely crushed in a mortar. 0.5 g of the powder was placed in the low temperature asher overnight. The residue was dissolved in 5.0 ml of 1 N nitric acid and examined by atomic absorption with air/acetylene flame and argon/hydrogen flame. The method with argon/hydrogen flame could not be used.

Equipment. The atomic absorption measurements were made with a Perkin Elmer model 303 atomic absorption spectrophotometer equipped with a readout and a Hitachi-Perkin-Elmer recorder. No electrodeless lamp was available, and an Intensitron hollow cathode lamp was used. The wavelength 1973.0 Å was chosen. All measurements were made with a deuterium background corrector to compensate for light scattering interferences.

The flame type is extremely critical for arsenic determination. Different gas mixture were tried. Argon/hydrogen gave a stable flame and a good sensitivity, but the low temperature of this flame causes interferences. Air/acetylene on the other hand is relatively free of interferences, but gives poorer sensitivity. Both these flames were used in the further work.

A low temperature asher Type 500 A from the firm Upsala Elektronik (Uppsala, Sweden) was used for the ashing of dried liver sample. The oxygen flow was 25 ml/min, the working pressure 1.5 mm Hg and the high frequency current was regulated to 100 μA. The ashing was performed overnight. The sample was spread out in an uniform layer in the ashing boat. Dried blood samples containing known amounts of arsenic trioxied was used to control the loss during the ashing step.

Standard Solution. A primary standard solution of 1000 μg/ml As was made from arsenic trioxide. 0.6602 g As_2O_3 was dissolved in hydrochloric acid and diluted to 500 ml with destilled water.

Secondary standard solutions of 1, 2, 5, 10, 20 and 50 μg/ml As were made by diluting the primary standard solution with water.

Table 1. The concentration of arsenic (μg/ml or μg/g) in blood, liver and bile in a fatal intoxication. The water content in the liver sample was 68.9% and the arsenic concentration was calculated on wet weight basis

	Blood	Liver	Bile
Concentration	16 μg/ml	99 μg/g	40 μg/ml

Results and Discussion

When using atomic absorption for the determination of arsenic in biological samples the choice of flame is very critical. In the present study it was found that blood and bile could be diluted four times and analysed directly using an argon/hydrogen flame. This flame gave stabile results, and a detection limit of 1 μg/ml As was obtained.

The dry ashed liver sample could not be analysed with the argon/hydrogen flame. The arsenic was not reduced completely in this low temperature flame and the results were only about 50% of the total amount. The air/acetylene flame on the other hand was found suitable and a quantitative recovery was obtained.

The use of the low temperature asher is also critical for the arsenic determination. A too high effect on the instrument gives too high temperature and this will result in loss of arsenic. Therefore a high frequency current of 100 μA was used, this requiring an ashing time of about 15—20 hrs. In the experiment mentioned above, where dried blood with known amount of arsenic was analysed, no loss was seen during the ashing step.

The results of the arsenic determinations are presented in Table 1. When arsenic poisoning is suspected, it is important to determine the distribution of arsenic in the body. In acute poisoning cases a high arsenic content is found in blood, liver and other inner organs and usually also in the stomach content. In subacute and chronic intoxications a high arsenic content may be found in liver and other inner organs while blood may have a near normal concentration. Death may, however, occur at a time when also the arsenic content in the inner organs are far below what is found in acute intoxications (Grusz-Harday, 1966). In these cases it is important that samples of hair and nails are analysed. The accumulation of arsenic in hair is well known and enhanced concentrations are found long time after ingestion (Wyttenbach *et al.*, 1967; Barrowcliff, 1971; Pearson, 1971). In the present case no hair or nail samples was submitted for analysis. Most living organisms contain small amounts of arsenic. It is, however, not known if arsenic plays an essential role in the human body (Schroeder *et al.*, 1966). The normal level of arsenic may differ in different parts of the world (Heydorn, 1970), but the concentrations in the human body seldom exceed 10 μg/100 g (Bäumler, 1968). Urine may reach a higher concentration.

The results presented confirm that the present case was an acute intoxication. The prescribed method for the determination of arsenic in autopsy material is relatively simple and requires a minimum of sample handling.

Note Added in Proof. After this study was finished, an electrodeless discharge lamp was tried. For the blood determinations this lamp gave a sensitivity of about 0.5 μg/ml.

References

Barrowcliff, D.: The stoneleigh abbey poisoning case. Med.-leg. J. (Camb.) **39**, 79—90 (1971)

Bäumler, J., Im Obersteg, J., Shafer, R.: Determination of the level of arsenic in human bodies (population of Basle). Dtsch. Z. ges. gerichtl. Med. **64**, 56—61 (1968)

Fernandez, F. J.: Atomic absorption determination of gaseous hydrides utilizing sodium borohydride reduction. Atomic Abs. Newsl. **12**, 93—97 (1973)

George, G. M., Frahm, L. J., McDonnell, F.: Dry ashing method for the determination of total arsenic in animal tissues: Collaborative study. J. Ass. Off. Analyt. Chem. **56**, 793—797 (1973)

Grusz-Harday, E.: Arsengehalt des menschlichen Organismus bei Vergifteten in klinischen und tödlichen Fällen. Arch. Toxikol. **22**, 164—175 (1966)

Heydorn, K.: Environmental variation of arsenic levels in human blood determined by neutron activation analysis. Clin. chim. Acta **28**, 349—357 (1970)

Hill, U. T.: Determination of arsenic in steels, iron ores, and spelters by atomic absorption. ASTM Special Technical Publication **443**, 83—89 (1969)

Kopp, J. P.: 1-ephedrine in chloroform as a solvent for silver diethyldithiocarbamate in the determination of arsenic. Analyt. Chem. **45**, 1786—1787 (1973)

Pearson, E. F., Pounds, C. A.: A case involving the administration of known amounts of arsenic and its analysis in hair. J. forens. Sci. Soc. **11**, 229—234 (1971)

Sanui, H.: Activated oxygen ashing of biological specimens for the microdetermination of Na, K, Mg, and Ca by atomic absorption spectrophotometry. Analyt. Biochem. **42**, 21—28 (1971)

Schroeder, H. A., Balassa, J. J.: Abnormal trace metals in man: Arsenic. J. chron. Dis. **19**, 85—106 (1966)

Schwab, R.: Kalte Trockenveraschung in der analytischen Chemie. Z. Anal. Chem. **243**, 208—209 (1968)

Spielholtz, G. I., Toraballa, G. C., Steinberg, R. J.: Determination of arsenic in coal and insecticides by atomic absorption spectroscopy. Mikrochim. Acta 918—923 (1971)

Weltz, B.: Personal communication (1973)

Williams, A. I.: The use of atomic-absorption spectrophotometry for the determination of copper, chromium and arsenic in preserved wood. Analyst **97**, 104—110 (1972)

Wyttenbach, A., Barthe, P., Martin, E. P.: The content of arsenic in the hair in a case of acute lethal arsenic poisoning. J. forens. Sci. Soc. **7**, 194—197 (1967)

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