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A Rapid Method for Analysis of Tissues for Heavy Metals Utilizing Atomic Absorption Spectrophotometry

Abstract

A rapid analytical technique for analyzing heavy metals in tissue samples is described. The technique utilizes Corning glass ignition tubes which are used as a container for storing, ashing, and digestion of samples. This process reduces the amount of glassware needed in the analytical procedure, reduces exposure of samples to contamination by excessive glassware use, and provides the analyst with a clean, economical, and efficient system for measuring heavy metals in animal tissues.

Introduction

Numerous studies in our laboratory dealing with heavy metal contamination in the environment (Scanlon 1979, Scanlon *et al.* 1979, Scanlon *et al.* 1980, Kendall and Scanlon 1979, Kendall 1980) have necessitated development of a rapid analytical technique for heavy metal measurements (i.e., lead (Pb), cadmium (Cd), nickel (Ni), zinc (Zn), and copper (Cu)) in animal tissues. Although large numbers of samples are processed, individual handling of each sample is of utmost importance so as to avoid contamination and maintain identity. For this reason, a tissue processing technique was developed utilizing Corning glass ignition tubes for ashing of samples prior to analysis by atomic absorption spectrophotometry. The present report describes our technique for drying, ashing, digestion, and analysis of tissue samples for heavy metals by atomic absorption spectrophotometry.

Methods and Materials

Preparation of laboratory glassware was an important consideration in the heavy metal measurements. Before use, glassware was soaked (> 24 hr) and washed in 5 percent Lakesal Laboratory Glassware Cleaner (Peck's Products, St. Louis, Mo.) and rinsed in distilled-deionized H₂O. Glassware was then leached in 20 percent HNO₃ (> 24 h) and rinsed in distilled-deionized H₂O before usage. Glassware was then transferred to a 5 percent HNO₃ solution (> 24 h), rinsed in distilled-deionized water, and stored in a clean cabinet. All clean glassware was placed in a 5 percent HNO₃ bath at least 24 h prior to use and rinsed 9-10 times with deionized water.

All tissue samples were dissected free using acid-washed stainless steel surgical instruments. Plastic gloves were worn throughout this procedure. Tissue samples were

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then placed in acid-washed sample vials, covered with parafilm, frozen, and later lyophilized at -50°C for 24 h. After lyophilization, all samples were placed in a desiccation chamber to prevent rehydration and possible contamination. Tissue samples were then added to tared 16 x 125 mm ignition tubes (glass etched with numbering system) and weighed to the nearest 0.10 mg. Samples were then ashed overnight in a muffle furnace (500°C) and the ash brought into solution in distilled-deionized H_2O (Barnstead Demineralizer)/ HNO_3 (reagent grade, Fisher Scientific)/ HCl (reagent grade, Fisher Scientific). An automatic repipette system (20 ml) was used to maintain precision in acid delivery. Samples were allowed to digest, and the ignition tubes were capped with parafilm and centrifuged for 5 min. at 761 g. Appropriate dilutions were made, and heavy metal concentrations were measured in tissues using an Instrumentation Laboratories Model 351 atomic absorption spectrophotometer (Wilmington, Mass.). Automatic background correction was accomplished with a deuterium continuum light source; unknown samples were attained from a standard curve.

Water pollution quality control samples for trace metal analyses were obtained from the Environmental Monitoring and Support Laboratory, Environmental Protection Agency, Cincinnati, Oh. These samples were used to verify and standardize analytical procedures in addition to bovine liver standards obtained from the National Bureau of Standards, Washington, D.C.

Standards were made up in 100 ml acid-washed pyrex volumetric flasks. New standards were routinely checked against old standards. Standard additions were used to check for matrix interferences. Spiked samples were run to obtain percent recovery. Blank tubes were routinely run through the procedure to check for contamination. Deionized water and acids used were analyzed by flameless atomic absorption spectrophotometry to check for metallic contamination. Lead concentrations were calculated in terms of μg lead/g dry weight of tissue.

Results and Discussion

Using this technique, one has the capability of analyzing upwards of 300 different tissue samples per week for various heavy metals. A slow point in the process is the weighing out of samples in the ignition tubes by a Mettler balance. We suggest use of a 5 decimal place analytical top-loading balance to facilitate getting samples weighed out. This technique should speed up the process considerably.

In the past, the process has often been slowed by the use of porcelain crucibles for ashing, requiring excessive space in the furnace. However, use of ignition tubes circumvents this problem, as many ignition tubes can be stacked horizontally (approximately 10 degree tilt) in the furnace. Care must be exercised, however, in placing the ignition tubes in a cool furnace and bringing the temperature up slowly (room temperature to 500°C in about 5 hr) to avoid having samples boil out of tubes which may occur if the temperature is increased too rapidly.

Samples can be stored in the tubes before ashing if parafilm is used for sealing the tube. Sample storage can also be facilitated by use of test tube racks where a large number of samples can be stored in a small area.

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