PROBLEMS INVOLVING THE USE OF ASPIRATION FLAME ATOMIC ABSORPTION SPECTROSCOPY IN THE ESTIMATION OF METALS IN PLASMA, SERUM OR WHOLE BLOOD

Pages with reference to book, From 17 To 18

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Much work is being currently done in the detection, analysis and estimation of metals in body fluids and special efforts are going into investigations concerning relationships between abnormally raised or lowered levels and disease states and also their actual role in biological processes. The investigative process presents certain difficulties which should point out to anyone using aspiration flame atomic absorption spectroscopy. Trace metal analysis of biological samples was not well established until the development of atomic absorption spectroscopy. With this new technique, methods for the analysis of trace metals began to appear, but numerous problems in the sampling procedure affected accuracy. Because of viscosity problems several methods involved the dilution of plasma or serum with water or mineral acids but the dilution had to be small in order to achieve adequate sensitivity. However, the high protein content of serum still resulted in aspiration anomalies and also burner clogging. To overcome this problem protein precipitation methods were used but found to be inaccurate as part of the metal precipitated with the protein. Together with volume exclusion errors losses of upto 20% have been reported[1]. Any differences between the viscosity of the sample and the standard solution causes substantial measuring errors. The viscosity of the standard solution should, therefore, match the viscosity of sample. For this reason standard solutions have been prepared in glycerol solutions, for example, to match the viscosity of plasma[2]. This improved the accuracy of the result. Laboratory air quality also influences the precision and accuracy of elemental analysis. Most elements cannot be adequately and accurately determined in an uncontrolled laboratory environment. The effect of such variability on the precision and accuracy of trace element analysis has been reported[3]. In our studies on metals we used whole blood specimens and not serum, for logistical reasons. Digestion of the blood is carried out using concentrated nitric acid. Proteins are completely hydrolyzed which eliminates the need for, their removal. A whole blood standard is similarly treated. This minimises viscosity differences. Initially the blood standard is calibrated by the standard addition of at least 3 concentrations of the metal under investigation. Calibration is time consuming as 25 — 45 curves need to be prepared involving at least 12 absorption readings per curve for each metal. An aqueous control may also be incorporated into a run of samples whose mean value ±2 standard deviations for that metal has been pre-determined 25 — 30 times against the whole blood standard. We found that by calibrating the instrument with an aqueous standard the level of Cu in whole blood was 77.9% of the true value. That for Zn was 85.5%, Pb 188.4% and Mg 884%. It should also be realised that absorption Vs concentration for a metal may be linear in blood whereas in water it may not be, or otherwise. Although for copper it is linear in both blood and water, for zinc it is linear in only blood and for magnesium it is linear in neither. The digestion procedure is lengthy but is simple and provides an accurate, precise and sensitive method for measuring metals in whole blood.

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