DEVELOPMENTAL METHODOLOGY AND RESULT COMPARISON FOR THE DRY ASHING AND WET PRESSURE/DIGESTION PRETREATMENT TECHNIQUES IN THE DETERMINATION BY ATOMIC ABSORPTION SPECTROPHOTOMETRY OF COPPER, NICKEL, ZINC, LEAD, CADMIUM AND VANADIUM IN WHOLE RAINBOW TROUT TISSUE

Joseph De Luca

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ABSTRACT

Joseph De Luca

DEVELOPMENTAL METHODOLOGY AND RESULT COMPARISON FOR THE DRY ASHING AND WET PRESSURE/DIGESTION PRETREATMENT TECHNIQUES IN THE DETERMINATION BY ATOMIC ABSORPTION SPECTROPHOTOMETRY OF COPPER, NICKEL, ZINC, LEAD, CADMIUM AND VANADIUM IN WHOLE RAINBOW TROUT TISSUE.

Complete methodologies are tested for carrying out the dry ashing and wet pressure/digestion techniques for the treatment of whole rainbow trout tissue prior to analysis to determine copper, nickel, zinc, lead, cadmium and vanadium by atomic absorption spectrophotometry.

The linear zones for concentration versus absorbance, for the estimation of each trace metal by the flame atomic absorption method, are reviewed. These were found to agree generally with the literature values. The dry/char/atomize temperature cycles for the "flameless" of graphite furnace atomic absorption technique are re-established for each metal, and the associated linear zones are reviewed. Although minor differences were noted relative to the charring and atomizing cycle characteristics, these agreed in general with the values indicated in the literature. The linear limits for each trace metal also showed general agreement with the literature values.

The amount of each trace metal in whole rainbow trout tissue is determined by both pretreatment methodologies and the results are compared. The values for each trace metal, with the exception of nickel, showed statistical agreement relative to the two methodologies. The nickel value from each methodology showed differences outside the uncertainty parameters. This was attributed to the fact that the nickel content determined was too close to the detection limit for this element in the flame technique. The recovery characteristics for each metal, and for each methodology, are determined by a spiking technique, and the ability of this technique to yield reliable analytical results indicated. Again, with the exception of the nickel determination data, recoveries for each spiking technique were found to approximate 100 percent. There was an indication that the wet ashing technique yielded lower spike recoveries of about 95 percent, and this was attributed to losses based on the additional handling characteristics of this method.

Finally, the recovery characteristics for each trace metal, and for each methodology, as based on aliquoting procedures, are determined and the adaptability of each pretreatment technique to aliquoting established. The use of aliquoting as a means of reducing high solution concentrations was generally shown to be satisfactory, being about 100 percent recovery on spiked and aliquoted dry-ashed samples and about 95 percent on wet-ashed samples.

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DEDICATED

to

MY WIFE DONNA

and

MY PARENTS

LIST OF SYMBOLS

r	=	linear regression correlation coefficient
b	=	linear regression y - intercept
m	=	linear regression slope
S	=	standard deviation
F	=	ratio of the square of standard deviations
$F_{(0.99)}$	=	tabulated F value with 99 percent confidence limits
s	=	combined standard deviation of two separate populations
$\bar{x}_i$	=	mean for a given population (i)
x_i	=	single result for a given population (i)
t	=	Student's "t"
$t_{(0.99)}$	=	tabulated Student's "t" with 99 percent confidence limits
n_i	=	number of measurements for a given population (i)
d.f.	=	degrees of freedom
1% level	=	99 percent confidence limits
$\bar{x}$	=	difference in means from two populations
$\sum x^2$	=	sum of variances squared
$s_{\bar{x}}$	=	standard deviation of combined means from 2 separate populations
$s_{\bar{x}}^2$	=	variance of mean

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1. INTRODUCTION

1.1 General

The presence of metals in fish tissue as trace contaminants has caused considerable concern with respect to their possible effect on the ecological system and eventually on man. In addition to those metals known to be essential to aquatic life (1), a variety of others are consistently present. Many of these "non essential" metals have little or no biochemical function and are labeled environmental contaminants (2). However, high levels of the essential metals may also prove to be damaging if not fatal to aquatic life. The nature and amount of these trace metals as they move through the ecosystem greatly influences the proper functioning of "all" living systems (3).

The biologist utilizes the information attained from trace metal analysis to ascertain tolerance levels within fish species, which can then be used to help make determinations on the cause of "fish kills" (47). Fish showing high levels of any one or more of these contaminants may find its way into the food chain and induce irreparable disorder to the human organism. A knowledge of trace element levels in rainbow trout, being an important part of the fish food chain, will elucidate some of the limiting factors that are of great ecological significance.

The biological influence of most metals in this study have been reviewed over recent years, and new data with respect to the lower level effects of these elements have provided some rather surprising information. Cadmium is increasingly recognized as an important environmental pollutant with toxic effects both on human and animal life at relatively low concentration levels (4,5,6). Environmental concentrations of this metal are of serious concern since cadmium accumulates in the human body throughout life - from approximately 1 ug at birth to about 30 mg with approximately one third to be found in the kidneys (7). Based on animal studies, cadmium is preferentially retained in the liver as well as the kidneys (5) and it has been shown to be associated with arterial hypertension in man (8). Lead interferes with a number of body functions, most notably the central nervous system (48). Prolonged exposure to relatively low levels of lead has been shown to be a possible cause of brain damage (9). A recent study of the toxicity of $ZnSO_4$ to rainbow trout dealt with only acute toxicity measured by fish mortality (10). Another study examined the chronic toxicity of copper, cadmium and zinc mixtures at sublethal concentrations to the fathead minnow using mortality, physical characteristics and reproduction as bioassay methods (11).

The above studies increase our understanding of biological effects

for relatively concentrated heavy metal pollutants in aquatic systems. However, no evidence has been presented for the actual rate of accumulation or the levels of trace metals present in fish unexposed to any metal contaminant either through the laboratory-test breeding environment or the food. Moreover, only rarely in natural waters do the concentrations of toxic metals attain a level used in most acute and chronic bioassay studies (49). Thus, experimental evidence for heavy metal accumulation and distribution in organisms exposed to environmentally realistic levels of heavy metal pollutants in natural waters should make possible more reliable and general prediction for the long term effects of such pollutants.

When experimental data indicates metabolic effects on aquatic life as a possible result of heavy metal content contamination at levels much lower than previously expected, it becomes increasingly important that attempts be made to devise and adapt methods of analysis providing for very high sensitivity and ease of application. This study was partially carried out with the above thought in mind. It involves the exploration of the sensitivity, concentration range of linear response and the ease of application of both the dry - ashing and wet pressure/digestion pretreatment techniques followed by the determination of copper, nickel, zinc, lead, cadmium and vanadium by atomic absorption spectrophotometry (flame and graphite furnace methods).

The amount of each trace metal in dried whole rainbow trout tissue unexposed to experimentally high trace metals in the water and/or food is determined by both pretreatment methodologies and the results compared. The recovery characteristics for each metal, and for each methodology, are determined by a spiking technique, and the ability of this technique to yield reliable analytical results indicated.

Finally, the recovery characteristics for each trace metal, and for each methodology, as based on aliquoting procedures, are determined and the adaptability of each pretreatment methodology to aliquoting established.

1.2 Trace Metal Detection and Determination in Fish Tissue

The importance of trace metal detection in biological materials (including fish tissue) has grown enormously over the past decade. It has become clear that even trace and ultratrace quantities of certain heavy metals can be detrimental. Numerous sophisticated techniques and instruments have been developed in order to analytically detect and measure these metals.

When choosing an analytical technique to determine trace metals in tissue samples it is essential to consider the advantages and disadvantages. Considering the purpose, scope and requirements of the

present project, the following is a list of essential properties the "ideal" method must possess:-

(a) high elemental selectivity
(b) high sensitivity
(c) high accuracy
(d) capable of multielement determination per sample
(e) unaffected by changes in matrix, relatively free from interferences
(f) capable to accept minute samples
(g) relatively inexpensive
(h) provide rapid analysis
(i) simple to operate
(j) efficient

Many analytical techniques have been successful in past years, but as requirements increase new methods are developed or old methods are adapted to suit the present requirements. Following are some of the widely used methods for the determination of trace metals in fish tissue.

Colorimetry

Colorimetric analysis is based on the principle of complexing the required metal with an agent such as dithizone at a specified pH followed by the development of a coloured solution. The actual measurement can take place by colour comparison in nessler tubes or spectrophotometrically.

Numerous methods have been developed for the determination of trace metals in tissue by colorimetry (50 - 54). Fogg et al. (12)

made use of xantenes, triphenylmethane and other basic dye cations as reagents for the determination of ion-associated complexes of metals such as chromium, zinc and antimony. These can then be measured as coloured complexes. Sandell (13) developed a procedure for the determination of copper and zinc in biological materials by extraction with dithizone (16). The extraction procedure was followed by a colorimetric determination of these metal complexes. The absorptiometric determination of small amounts of copper in organic materials by extraction with ammonium diethyldithiocarbamate in carbon tetrachloride was investigated by the Analytical Methods Committee (15). Lead and cadmium can also be determined in tissue (14) by extraction after pH adjustment with dithizone/carbon tetrachloride mixture. The final analysis of the metal dithizonate is finally determined by nessler tubes or spectrophotometrically.

Some of the advantages of this method are its ease of operation and maintenance as well as the low cost of the instruments involved. Although colorimetric analysis has been and still is used for metal determination it has some serious disadvantages. The technique requires extensive sample preparation and manipulation. The rather large number of interferences (12, 55 - 57) which plague the colorimetric method limit its use as a general method and have led to several erroneous literature reports (58).

Neutron activation analysis

Neutron activation analysis has been an old and well established technique with regards to trace analysis in biological materials (19 - 22). Many variations of the method exist, but essentially it relies on bombardment of the sample and subsequent detection of the radioisotopes usually by a gamma ray spectrometer.

Livingston and Smith (92) adapted the technique to the determination of vanadium as V^{52} in biological materials. Recently a more up to date method with a sensitivity as high as 30 ppb was developed by Blotcky et al. (59) for the determination of vanadium in marine biological specimens. Hahn et al. (24) developed a rapid and simple continuous radiochemical separation technique for copper and zinc in biological materials. A different adaptation was carried out by Lieberman (25) where cadmium was first extracted as a dithizone complex from biological material and then determined by neutron activation analysis. Determination of trace metals in fish products has been carried out by Lunde (60,62) and Luca (61) very successfully.

The extremely high sensitivity, accuracy and precision achievable for most elements and the fact that there is hardly any risk of contamination of the indicated samples by elements present in the chemicals

used for treatment makes this method especially attractive. It is an excellent technique for simultaneous multielement determinations. The main disadvantage of the technique is the need for preconcentrating the sample which is tedious and time consuming. A second disadvantage is the cost of a fission reactor on site at ones disposal for activation and the highly skilled personnel necessary to operate and maintain the equipment. Another disadvantage is the lengthy period of time for the activation and counting.

Atomic fluorescence

Fluorescence methods (26,63,64) are of immense advantage in the analysis of minute tissue samples. A logical approach by Wallach and Steck (27) was the introduction of metal chelating groups into fluorescent dyes with the expectation of forming derivatives which would change fluorescent intensity and/or wavelength upon formation of metal complexes. Dye et al. (28) has developed a fluorometric technique for the detection and measurement of low concentration of trace metals using 3,3' - Diaminobenzidine as the fluorescent component. Later, Watkinson (29) made use of 2,3 - Diaminonaphthalene for the same purpose.

Not enough work has been carried out in fluorescence determination of trace metals in fish tissue to make it an attractive method.

The manipulation of the sample, the uncertain formation of a derivative and finally the complex itself may prove to be a lengthy procedure prior to the actual determination.

Gas-liquid chromatography

Little work has been carried out by gas-liquid chromatography in terms of determination of trace metals. The adaptation of gas-liquid chromatography for the determination of metals in biological materials has however been successfully attempted by various analysts (30,31,32). The metals are extracted with trifluoroacetylacetonate [Cr (tfa)$_3$] and quantitatively measured by gas-liquid chromatography with an electron capture detector. Attempts for the determination of aluminum, iron and copper as volatile metal chelates in biological materials has shown some promise (34,35,36).

Gas-liquid chromatographic analysis of metals has shown promise but is not considered a popular technique because of the lack of general analytical procedures and extensive sample manipulation.

Polarography

Polarography is particulary suited for trace analysis. In recent years, research for the adaptation of this electrochemical technique to trace metal detection has grown considerably (65,66). This is mainly due to the new and improved equipment available to the analyst.

Gajan and Larry (17) developed a method for the determination of lead in fish tissue by pulse polarography. Then a collaborative study (18) was carried out with excellent results.

Polarography is ideal for trace metal determination due to its high sensitivity. However it has some serious disadvantages. The range of metals that can be detected is limited, there is a lack of standard methods, the chance that a sequestered metal can go undetected and the high degree of skill that is required by the operator do not make this a popular technique for trace metal analysis.

Atomic emission spectrometry

Atomic emission is based on the principle that a metal atom in a vapor that has been excited can decay to a lower energy state by irradiating light.

The multielement capabilities of this technique coupled with its selectivity as an analytical tool for trace element determination has been recognized by various investigators (36,37,68). Bedrosian et al. (67) has developed a sensitive rapid and comprehensive spectrographic procedure for the direct determination of a large number of elements in biological materials without appreciable alteration of the samples. Only a few spectroscopists (38,39,40) have been able to successfully analyze unaltered biological material.

Perhaps the greatest advantage of of this technique is its ability to perform simultaneous multielement determinations and the need of no pretreatment required for the sample. The technique is also relatively free from interferences. The major disadvantages are the cost and the size of the instrument. Another disadvantage is the sensitivity which is somewhat lower than that of various other techniques such as atomic absorption spectrophotometry and polarography, and is not sensitive enough to cover the analytical range required for biological tissues.

Ultraviolet Photometry

A rather different application of ultraviolet photometry has been described by Thomas et al. (41) for the detection and measurement of total mercury in fish tissue. After the expelling of elemental mercury from the combustion of whole tissue, into a carrier stream and after removal of interfering gases, the mercury content is measured by a scanning ultraviolet photometer. The measurement of trace metals by ultraviolet determination originates from work by Pappas and Rosenberg (42).

Precipitation ion exchange

Very little work has been carried out in terms of precipitation ion exchange for the determination of trace metals in tissue or other biological materials. Experiments in this field by Tera et al. (43) have

shown some promise. The matrix and traces were sorbed on a cation exchange column, the matrix was precipitated and finally the traces were eluted. This technique centers on the preconcentration and separation of trace metals from large amounts of matrix.

X-ray fluorescence

X-ray fluorescence has not been an attractive technique for the determination of trace metals in biological materials. Basically it involves the bombardment with high energy photons or particles which excite the inner electronic levels of the analyte metal atom. A certain fraction of the excited atoms decays with the emission of x-rays.

One of the few reported experiments was that of Alexander (44) where x-ray fluorescence was adapted to the determination of zinc, copper and iron in biological tissue ash. The technique proved to have a high enough sensitivity for the stated metals and was relatively free of interferences due to changes in matrix.

Some of the advantages of XRF are its ability to make simultaneous multielement determinations and the general simplicity of the mechanical design. For solid samples, no pretreatment preparation is required. The most serious disadvantage in using XRF is the expensive equipment,

the relatively lower sensitivity and the high degree of skill to operate and maintain the instrument.

Spark source mass spectrometry

The applicability of spark source mass spectrometry to the quantitative determination of trace metals in biological materials has been evaluated by Evans and Morrison (45) on work previously accomplished by Sasaki and Watanabe (46). The method is capable of providing information simultaneously on 50 or more trace elements in the concentration range from 100 ppm to a few ppb with good reproducibility. Spark source mass spectrometry (45) for the analysis of solids employs a vacuum spark in which a high voltage radio frequency discharge is produced between two closely spaced electrodes made from the material being analyzed. The electrodes are sparked resulting in vapourization and ionization of the sample constituents. After acceleration of the ions the magnetic field separates the beam according to mass-to-charge ratio, providing mass analysis at the same time. The ions are recorded electrically or photographically.

Inspite of the high sensitivity capable of being achieved, certain disadvantages do not make this method a very popular tool. Because of the time required for the measurement (2-6 hours) and the complexity

of the technique, quantitative mass spectrometry remains primarily a research tool.

Atomic Absorption Spectroscopy

Atomic absorption spectroscopy is based on the principle that metal atoms absorb radiation at frequencies which are characteristic of a particular metal, the amount of light absorbed being a function of its concentration (Figure 2.1).

Atomic absorption has become established as an important tool and is practically indispensable in trace-metal analysis. It is considered by many analysts (69-78) to be the most widely used technique for the detection of trace metals in biological materials.

Much work has been done on the detection and measurement of trace metals in fish tissue by atomic absorption. With the demands for lower limits of detection, atomic absorption has clearly led the way into ultratrace determinations. The determination of copper was carried out by various analysts (79,80,85,87) in a wide variety of fish tissues, from cod and bullhead to shrimp tissue. Julshamn and Braekkan (81) successfully established a good percent recovery of cobalt in three types of fish tissues (sand eel, saithe and ballan wrasse) with no major problems due to interferences. Atomic absorption is a popular procedure

for the determination of mercury in tissue (83,84). Other tests were carried out on cadmium (79,82,85,86), zinc (79,80,85), lead (17,18), with excellent results.

Gajan and Larry (17,18) compared atomic absorption determination of lead in fish tissue to its closest competitor, polarography. The indication was that more sample mainpulation was required for polarographic analysis than for atomic absorption. Also, atomic absorption appeared to be the simpler-to-operate of the two methods.

Current atomic absorption instruments available are relatively easy to operate, and are readily automated. The option for background correction (Sub-section 2.1) removes most interference problems. A recent study by Childs and Gaffke (89,90) on the possible interference in the measurement of lead and cadmium from elements found in fish muscle gave surprising results. It was found that the measurement of Pb and Cd is not only sensitive, accurate and precise, but with modern equipment, interferences can be easily eliminated. Instruments are moderately priced when compared to such instruments as emission spectrometers or x-ray fluorescent equipment.

Atomic absorption is particularly suited for routine, repetitive quantitative analyses of a wide range of metals (88). With current instrumentation, the method however is not suitable for qualita-

tive or simultaneous multielement analyses. The sensitivity of this technique is usually comparable to if not better than any of the previously discussed methods. Concentrations in parts per billion are in some cases easily achieved. The accuracy and precision of the method are excellent. The equipment is easy to operate and to maintain. In conclusion, atomic absorption spectrophotometry is a very powerful if not the most powerful tool for trace metal analysis in biological materials.

1.3 Dry Ashing Method of Pretreatment Prior to Trace Metal Determination

Trace metals analysis in fish tissue or organic materials in general has given rise to numerous publications concerning analytical procedures. Atomic absorption is perhaps the most suited method for the determination of metals and its use is widespread. The destruction of organic matter, however, is a prerequisite for metal ion determination to prevent interference to the analysis and also to release any metal ions that may be complexed with such tissues. The problems with the atomic absorption precedure are mainly related to the destruction of organic matter.

Dry ashing techniques have been considered by several authors (73,80,81,91). The procedure simply involves the ashing of the sample

in a furnace. However, by using normal dry ashing procedures, losses of the more volatile elements are experienced (102,103) and to avoid this, some analysts have used sulfated ash (104) and ashing aids (103,105). Anderson (79) successfully determined zinc, chromium, copper, lead and cadmium in fish tissue by a simple ashing procedure at 500 °C in a furnace for sixteen hours, followed by dissolving the ash with 5 percent nitric acid and direct metal determination by atomic absorption.

The advantages of dry oxidation methods are the lack of added reagents and the simplicity of the method. The disadvantages are loss by volatilization on the more volatile metals and retention of the desired metals on the surface of the vessel used at high temperatures. To eliminate volatilization, Gorsuch (102) pointed out that it is essential to maintain the lowest possible temperatures consistent with completion of oxidation.

Gajan and Larry (17,18) preferred the conventional dry ashing technique for lead in fish tissue over the wet digestion method for various reasons. The Lead Panel of the Analytical Methods Committee in its report (101) on the determination of lead in foodstuff found no evidence of loss by volatilization if the ashing temperature does not exceed 500 °C. Dry ashing also needs little attention and is suitable for breaking down tissue material. The Analytical Methods Committee

of the Society for Analytical Chemistry recommends dry ashing techniques for the determination of zinc and magnesium in biological materials. Therefore, the analysis of 12 species of fresh and salt water fish was carried out with excellent results.

Weighing the advantages and disadvantages, the conventional dry ashing pretreatment technique was chosen as one of the pretreatment methods in this project.

1.4 Wet Ashing Method of Pretreatment Prior to Trace Metal Determination

Concentration measurements by atomic absorption may be made very rapidly, but the preparation of samples can often be time consuming. No single procedure for wet digestion will effectively handle a wide range of biological materials. Various methods have been reported (73,78,79,80,96,97,98,100) for the determination of heavy metals in fish tissue by atomic absorption after the digestion of the sample by different procedures of wet ashing methods.

Leonard (87) used a mixture of concentrated nitric and perchloric acids (5:1 by volume) for oxidation in the determination of copper in fish tissue. Excellent recoveries of 95-100 percent were obtained. However, digestion over a hot plate proved to be a lengthy process. Copper was also determined by Smeyers-Verbeke (78) and lead and cadmium by Friend et al. (91) by atomic absorption. To the tissue samples, concentrated H_2SO_4 was added and heated to sulphur trioxide fumes. Following was the addition of 50 percent H_2O_2 dropwise until

a clear solution was obtained. The Analytical Methods Committee(23) recommends 50 percent H_2O_2 to be used as the oxidation agent in wet digestion procedures. It provides a rapid but smooth oxidation with no fumes and water being the only side product. However, extreme care in handling and storage plus the need for safety equipment is required. Egaas and Julshamn (83) made use of concentrated HNO_3 and H_2SO_4 (1:1 by volume) acid mixtures with V_2O_5 utilized as a catalyst. The procedure required the use of a digestion vessel with a condenser. When the mixture reached the boiling stage, H_2O_2 (30 percent) was added dropwise (2-3 drops). The complete oxidation lasted 20 to 30 minutes. There were no significant differences when results were compared to the more conventional wet digestion methods.

Another variation of the wet digestion procedure was proposed and carried out by Jackson (94) for zinc and copper determination using soluene-100, a quaternary ammonium hydroxide tissue solubilizer. This technique was improved upon by Barlow and Khera (95) for the determination of lead in fish tissue mainly through the use of soluene-350 which is an improvement over the previous solubilizing agent. The advantages of this method of pretreatment are the very little handling required, the rapid breakdown of tissue, the loss of metals is reduced and the improvement in sensitivity by atomic absorption spectrophotometry. Luyten et al. (97) found, however, that while soluene digestion yielded excellent results for cer-

tain types of tissues, it was a poor solubilizer for a variety of others.

Paus (99) made use of an aluminum bomb with an inner teflon vessel for the decomposition of biological materials. Concentrated sulphuric and nitric acids were added to the sample in the bomb which was sealed and heated to 100 °C. This technique has been successfull in the determination of zinc, cadmium and copper in fish tissue. The only major disadvantage is the high cost of the decomposition equipment.

Recently, a technique by Adrian (93) based on work by Paus (99) was introduced. This method utilized not only acid digestion but also the use of pressure to oxidize fish tissue and make it suitable for determination by atomic absorption. Four different combinations of concentrated perchloric, nitric and sulphuric acids were added to the sample in a nalgene bottle with a polypropylene screw cap. The samples were first allowed to digest overnight and then under heat. The results were encouraging, but definitively more work had to be carried out. Luyten et al. (97) was very successfull in the determination of copper and zinc in fish tissue samples by the pressure/digestion technique and atomic absorption detection. A mixture of $HNO_3/HClO_4$ (1:1 by volume) was utilized. Sperling (82) instead, used concentrated H_2SO_4 and HNO_3 acids for the determination of cadmium in marine organisms by heating the mixture overnight at 90 °C followed by direct atomic absorption measurement of cadmium.

Other reports for the determination of cadmium in fish tissue (86) and zinc, copper, lead and cadmium (85) in a variety of fish tissues using variations of the pressure/digestion method yielded excellent results.

The pressure/digestion approach appears to be as precise as the conventional wet acid digestion methods for the analysis of fish tissues. It has the advantage of being fast, safe and simple.

A good digestion method should make use of a minimum amount of glassware to reduce handling errors and possible contamination, produce small final digest volumes containing the metal of interest and yield quantitative and reproducible recoveries of the metal sought. Conventional wet acid digestion techniques however, have some drawbacks. These methods produce high blanks from possible contamination caused usually by large amounts of reagents used and sample manipulation. They also require in general more man hours. The use of H_2SO_4/H_2O_2 digestion mixtures was not adopted because of the poor recoveries reported by Adrian (93).

The method of choice (Sub-section 2.2.3 - c) is a combination of the pressure/digestion method of Adrian (93) using concentrated nitric and perchloric acids and the conventional wet digestion of the resultant mixture (after digestion overnight) over a hot plate with concentrated nitric acid.

1.5 Purpose of the Investigation

The trace heavy metals chosen for determination throughout the course of the investigation were selected for the following reasons:-

(a) They represent trace heavy metals which are still under continuing investigation as to their single and multiple toxicity to marine life (47).

(b) These trace metals represent a range of volatilities under various dry ashing temperature conditions to be investigated. For example, copper, nickel and vanadium provide oxides under dry ashing with very low degrees of volatility. On the other hand, zinc, lead and cadmium can be expected to display, under the same conditions, higher degrees of volatility. The choice of the entire group of trace metals involved provides for the development of a dry ashing procedure able to yield reliable recovery data for both low and relatively high volatility metal oxides.

Primarily, the purpose of the investigation was to test specific methodologies for the dry ashing and wet ashing pressure/digestion techniques of pretreatment of rainbow trout tissue prior to analysis for the trace heavy metals indicated. These methodologies were to be applied in the analysis of the fish tissue involved. This analysis was intended to, first, obtain by each pretreatment technique analytical values for each metal. The second objective was to compare the data from each technique in order to determine agreement and where possible, to obtain a final value for each trace metal content representing input from both techniques.

Associated objectives related to the pretreatment methodologies were, first, to investigate the extent to which the analytical re-

sults from each methodology represented complete recovery of each metal as it existed in the original tissue material. This portion of the investigation was based on the use of spiking additions of each trace metal to the original tissue and the determination of the extent to which the spiking addition was recovered. The second associated objective was to ascertain whether or not, for each pretreatment method, analytical values derived from aliquoted samples agreed with those from non-aliquoted samples. Such agreement would indicate that, where the original fish tissue contained an amount of any trace metal involved such that, in the normal routine of approach, it would yield a concentration in the test-solution high enough to exceed the concentration versus absorbance linear limits for the atomic absorption approach, the aliquoting procedure could be used to yield linear limit concentrations and reliable analytical results.

2. EXPERIMENTAL PROCEDURES - RESULTS AND DISCUSSIONS

2.1 Equipment Used

Atomic absorption spectrophotometry, carried out by the modified flame photometer, is one of the simplest of modern instrumental techniques adapted to the determination of trace metal analysis. The concept of atomic absorption is shown on Figure 2.1. Throughout the investigation, the following equipment was used relative to the final determination of trace metals involved.

The main instrument, for both flame and graphite furnace approaches is the Perkin-Elmer model 503 atomic absorption spectrophotometer. Figure 2.2 shows a general flow diagram of its function. This unit utilizes a double beam optical system (101). The radiation sources used in conjunction are hollow cathode lamps. The single element lamps were preferred over multielement lamps because of the possibility of interferences from other metals not being determined.

Flame atomic absorption, a relatively less sensitive approach when compared to graphite furnace determinations, utilizes burners, mixing chambers and atomizers which are listed in Appendix E. The flame technique is very simple, more so than the graphite furnace method. The aspiration of the sample in liquid form by the nebulizer into the mixing chamber is followed by the detection of the atoms

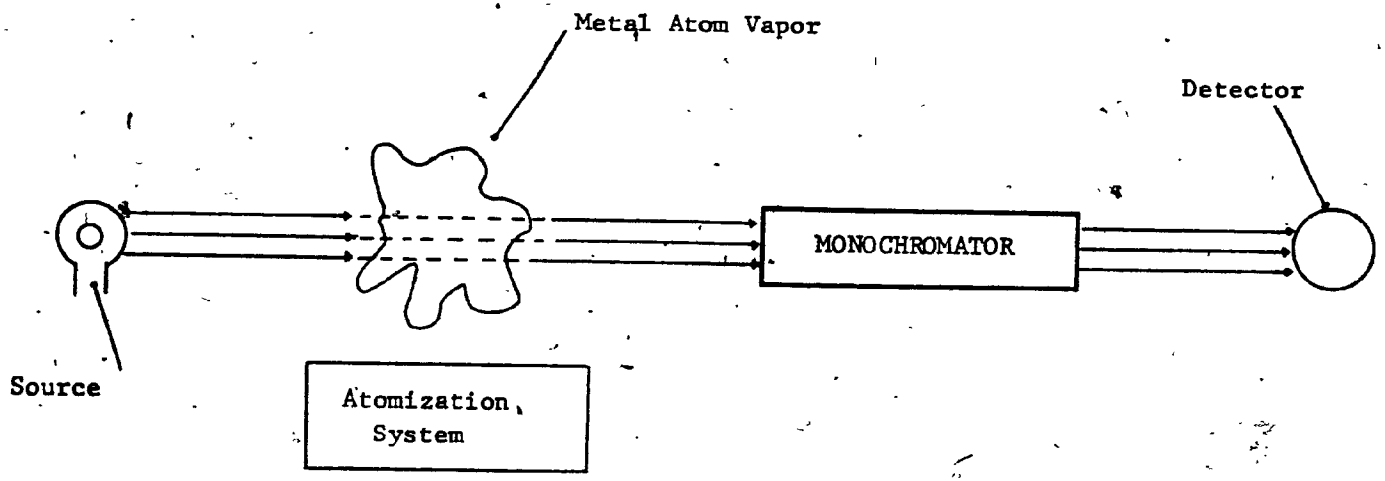

FIGURE 2.1 CONCEPT OF ATOMIC ABSORPTION

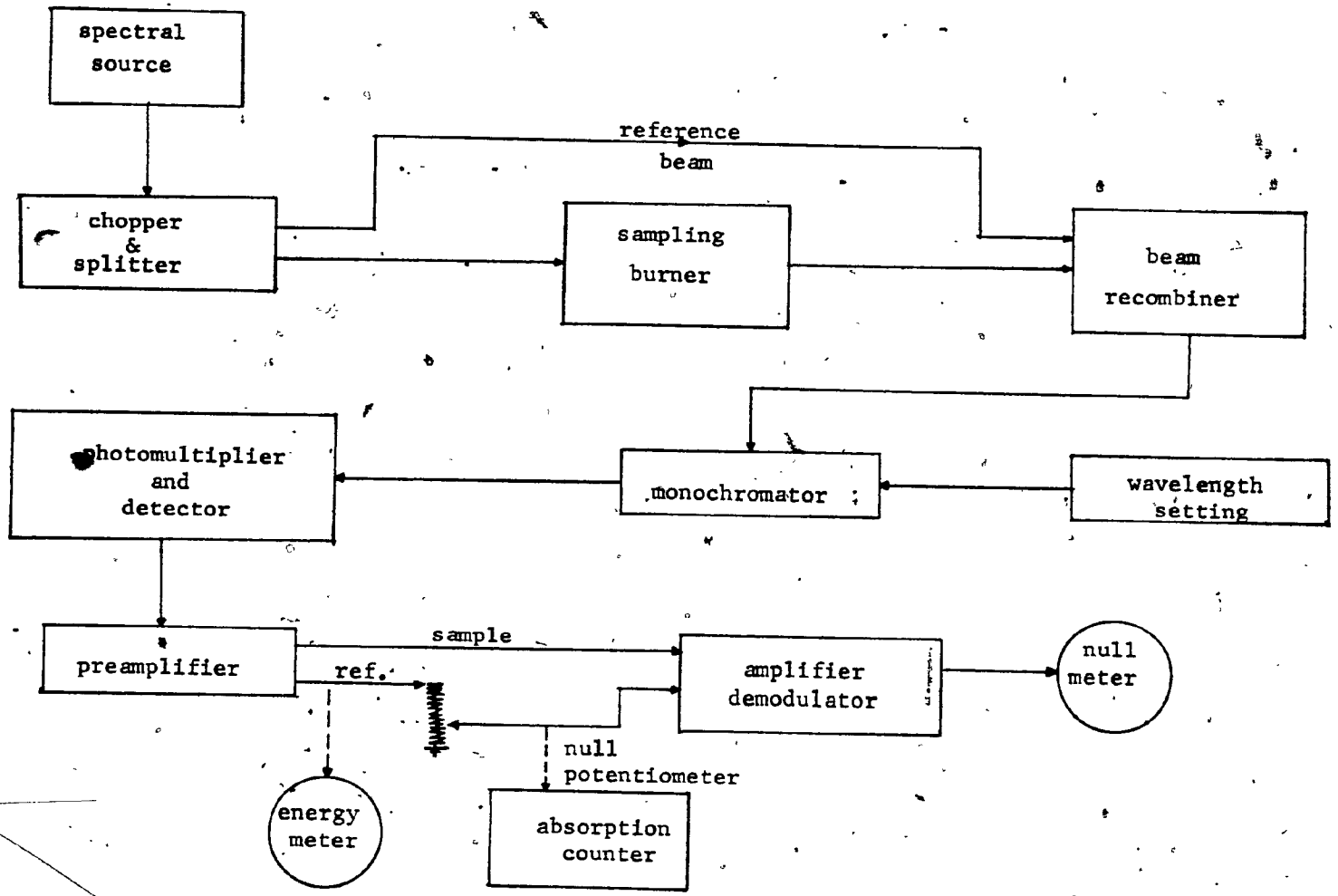

FIGURE 2.2 FLOW DIAGRAM OF THE COMPONENTS OF A PERKIN - ELMER SPECTROPHOTOMETER

as they pass through the flame.

For determinations where lower detection limits are required, the graphite furnace technique was employed. Two units were used, the Perkin-Elmer HGA 2000 and the improved version HGA 2100. Both atomizers function (109,106) in the same manner. A three stage heating program is used:-

(a) a low current/voltage to dry the sample
(b) an intermediate current/voltage to char and ash the sample
(c) and a high current/voltage to heat the atomizer to incandescence and atomize the sample.

The actual measurement of the metal ions of interest takes place at the atomization step. The only differences between the two models are:-

(a) the highest achievable temperature for the HGA 2000 is 2600 ^{o}C while 2800 ^{o}C for the HGA 2100.
(b) the use of an automatic purge step for the HGA 2100 which occurs after the integration of the peak takes place. This is to flush the graphite tube of any left over materials not completely atomized.
(c) the use of pyrolytically coated graphite tubes for the HGA 2100 whereas conventional graphite tubes were used for the HGA 2000 determinations.

With both atomizers, a Perkin-Elmer model 56 recorder was used on all determinations in order to record the peak result along with the digital peak integration result obtained through the spectrophotometer.

Part of the equipment used was a Perkin-Elmer Deuterium Arc Background Corrector (107). This unit is used in conjunction with

most atomic absorption spectrophotometers to remove unwanted absorption or light scattering. The continuum radiated by the D_2 arc replaces the ordinary reference beam in the double beam system. A variable attenuator serves to balance the reference beam energy of the D_2 beam and the sample beam energy of the hollow cathode lamp. Since the unwanted (background) absorption is of equal intensity in the two beams, its effect on the signal is cancelled. This leaves the sample beam absorption due to the metal of interest as a corrected signal. Some problems may arise at higher wavelengths (above 310 nm) where the intensity of the D_2 beam falls sharply making it impossible to balance with the hollow cathode energy beam. This problem can be solved in two ways, by reducing the sample beam energy (reducing the current on the hollow cathode lamp) or by changing the slit opening to a higher setting which increases the D_2 arc emission intensity in relation to the hollow cathode lamp intensity. The author believes that for metal determinations in tissue materials whether dry or wet ashed, the D_2 arc background corrector is a much needed requirement for the determination of trace metals by atomic absorption spectrophotometry.

Both the HGA 2000 and 2100 furnaces can be installed in the sample compartment of any Perkin-Elmer atomic absorption spectrophotometer. They both make use of a graphite tube placed so that the sample beam of the spectrophotometer passes through its middle.

Samples in solution are pipetted through a sample introduction hole at the center of the tube with the aid of an Eppendorf pipette.

As mentioned earlier, the HGA 2100 is capable of using pyrolytically coated graphite tubes. These tubes were originally introduced for the determination of refractory metals (108), to decrease the memory effect present in previous determinations with conventional tubes. Other advantages in the use of coated tubes (18,106,109) were the increased sensitivity for many metals and, particularly at higher temperatures, the extended tube life. The equipment needed for tube pyrolysis includes a Perkin-Elmer Pyro Coating gas control accessory (109). The coating gas was Linde P-10 consisting of 10 % methane and 90 % argon.

With the HGA 2100, elements can be determined with sensitivities and detection limits 50 to 1000 times that of the conventional burner-nebulizer systems. A given amount of metal in the graphite furnace gives a much higher absorbance signal than it would by flame for two reasons. In a burner, a large proportion of the sample flows down the drain, while the graphite furnace is much more efficient, all the sample is atomized. Also, residence time of the sample atoms in the graphite furnace is in the order of seconds while in the flame sample atoms leave the optical path in a fraction of a second.

For both the HGA 2000 and 2100 models the Energy Control Unit (power supply) temperature programming is automated. Separate

controls are provided to set the desired temperature for the drying, charring and atomizing steps. Timers are also provided for controlling the duration of each step. With the HGA 2100, it is also possible, using the Perkin-Elmer HGA Ramp Programmer, to increase the furnace temperature in each step gradually at a controlled rate (see Figure 2.3). This technique can yield significant performance advantages in many applications.

The Appendices A-1 to A-6 and B-1 to B-5 covering each process of determination and investigation provide additional details specific to the approach involved. Appendix E provides a complete listing of equipment, glassware and reagents used for the experimental approaches.

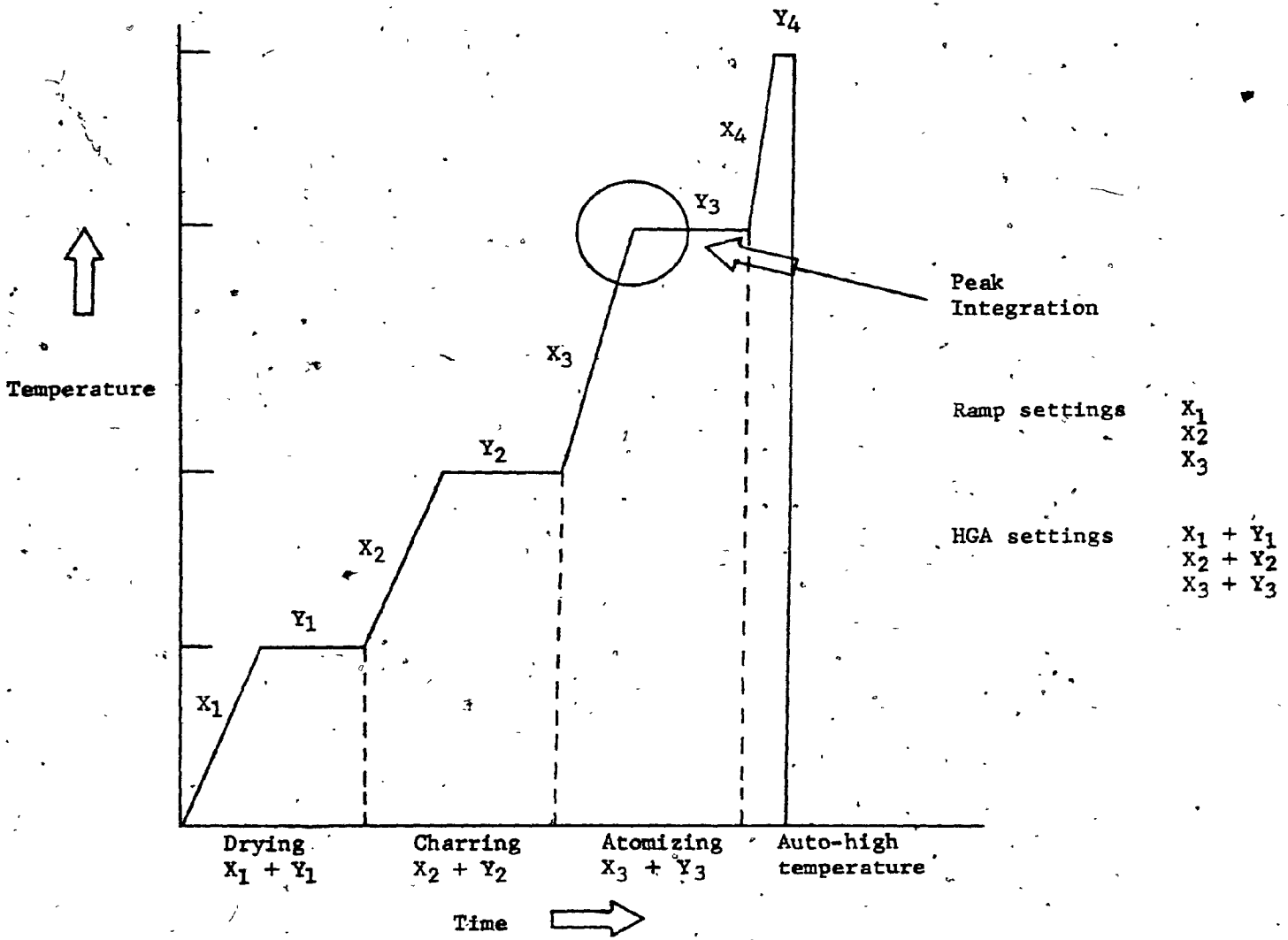

FIGURE 2.3 CONCEPT OF THE PERKIN - ELMER HGA RAMP PROGRAMMER

2.2 Generalized Experimental Approach

2.2.1 Flame atomic absorption approach

For each trace metal, tests were carried out to determine the linear range for the plot of concentration versus absorbance. In each case, standard solutions were prepared which included, within these initial series, concentrations outside the expected linearity ranges.

These solutions were tested under flame atomic absorption conditions, each solution being given three 3-second integration readings of absorbance. The three values obtained were averaged, and the standard deviation determined. The average values provided the final concentration versus absorbance raw data.

The linear range for concentration versus absorbance was determined in each case both graphically and by linear regression analysis. The regression analysis was carried out for all concentration/absorbance data, and then continued with successive deletion of data starting at the high concentration end of the series. With successive deletion of data, linearity was considered to be achieved when the intercept with the absorbance axis showed:-

(1) Agreement with zero within ± 0.002 absorbance units, where lamp stability provided absorbance data for the concentration series yielding an average for the standard deviations of lower than $\pm$ 0.001.

(2) Agreement with zero within $\pm$ 2x the average standard deviation for the concentration/absorbance data on absorbance where this provides values of $\pm$ 0.001 or higher (95 % factor).

An eyeball examination of the plot of concentration versus absorbance was used to verify the concentration limit for the linear zone as determined by regression analysis.

The limit of linearity data for concentration/absorbance were then used to obtain the linear equation, and this equation used, with the absorbance values for those concentrations within the linear range, to back-calculate the concentration data. Deviations of the calculated concentrations from the real values were then used to provide an uncertainty value for concentration data derived through the use of the linear equation.

Appendices A-1 to A-6 inclusive provide the data for these investigation series. Figures A-1 to A-6 show the respective concentration versus absorbance plots, while Table 1 shows the sensitivity, the linear zone concentration limit and the uncertainty for concentration data obtained from the linear equation representing the linear zone for concentration versus absorbance.

2.2.2 " Flameless " or graphite furnace atomic absorption approach

For each trace metal, with the exception of zinc, tests were carried out to determine the linear range for the plot of concentration versus absorbance. Zinc was not tested for under graphite furnace conditions, since its level in fish tissue is relatively high, eliminating any requirement for the high-sensitivity graphite furnace approach. For each of the metals investigated, standard

TABLE 1

FLAME ATOMIC ABSORPTION TECHNIQUE

SENSITIVITY, LINEAR RANGE AND CONCENTRATION UNCERTAINTY WITHIN LINEAR RANGE

Element	Sensitivity* (1% absorption) (ppm)	Tested linear range limit (ppm)	Uncertainty in concentration within linear range (ppm)
Copper	0.08^6	5.50	0.05
Nickel	0.1^5	5.00	0.04^6
Zinc	0.02^0	1.100	0.01
Lead	0.8^6	26.00	0.4
Cadmium	0.04^8	2.00	0.01^9
Vanadium	$2.^1$	150.0	0.7

* Using the arbitrary absorbance value of 0.0044 from Ramirez-Muñoz (115).

solutions were prepared which included concentrations outside the expected linear ranges.

From among these standards, a specific solution was used at a set atomization temperature specific to the metal involved, and tests were carried out at varying charring temperatures in order to establish the maximum charring temperature and in turn the optimal operational charring temperature.

Figures B-1, B-4, B-7, B-10 and B-13 show the plots of absorbance versus charring temperature for the tests on copper, nickel, lead, cadmium and vanadium.

With the optimum charring temperatures established, specific standard solutions were now used at the optimum charring temperature to establish the optimum atomization temperature for each metal. Figures B-2, B-5, B-8, B-11 and B-14 show the plots of absorbance versus atomization temperature. In several instances, the optimum atomization temperature was beyond the capacity of either the HGA 2000 or HGA 2100 furnaces. In those cases, the maximum available temperature was used with consideration given to the degradation of the graphite tube.

Subsequent to these tests, the linear range for concentration versus absorbance was determined using, as the working conditions, the determined values for charring and atomizing temperatures.

Appendices B-1 to B-5 inclusive provide the data for these investigations. Table 2 shows the sensitivity, the linear zone concentration limits and the uncertainty for concentration data obtained from the linear equation representing the linear zone for concentration versus absorbance. It also shows the optimum charring and atomizing temperatures used.

2.2.3 Dry ashing and wet pressure/digestion pretreatments

(a) General

1. Whole fish, unexposed to experimentally-high trace metal concentrations in the water and/or food, were dried to constant weight at 65 ± 5°C.

2. Dried whole fish (approximately 50 grams dried weight per batch) was macerated using a 12-inch test tube as a pestle and a polyethylene beaker as a mortar. The fish skin tends to form small tissue balls during this process. These are crushed immediately, since delay in this action permits hardening and subsequent difficulty during the ashing procedure. The final tissue should be quite finely divided as the result of this operation.

3. The macerated tissue from various batches (a total of about 500 grams will be required for the experimental approach) is mixed together very thoroughly, and the entire mass of tissue is again dried to constant weight at 65 ± 5°C. The dried tissue is stored in a good dessicator.

(b) Dry ashing pretreatment

Based on the literature reviews noted in the introduction, the following very general procedure was adopted.

1. Specific weights of dried fish tissue were weighed accurately into 50 ml Pyrex beakers. The weights involved, which are given under the various analytical treatments, varied according to the expected range of trace metal content.

TABLE 2

FLAMELESS OR GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

SENSITIVITY, LINEAR RANGE AND CONCENTRATION UNCERTAINTY WITHIN LINEAR RANGE

Element	Optimum Charring temp. (°C)	Optimum Atomizing temp. (°C)	Sensitivity* (1 % abs.) (ppm)	Linear range (ppm)	Uncertainty in concentration in linear range (ppm)
Copper	750	2500	0.002^{7}	0.220	0.002
Nickel	800	2500	0.01^{7}	1.20	0.009
Lead	600	2200	0.0006	0.0500	0.0005
Cadmium	250	2100	0.00004	0.00350	0.00006
Vanadium	1400	2800	0.014	1.000**	0.01^{3}

* Using the arbitrary absorbance value of 0.0044 from Ramirez-Muñoz (115).

** This value represents the limit of the tested linear range, linearity may extend past this point.

2. A blank was carried through the preparatory ashing acid extraction procedure.

3. All samples, including the blank, were treated to the following ashing process:-

30 minutes at:-	100°C
30 minutes at:-	150°C
Increase temperature 50°C every 30 minutes until 500°C is attained	
Ash overnight (circa 14 hrs) at	500°C

Heavy smoke can be anticipated at between 250° - 300°C.

4. Remove the samples from the muffle furnace and allow to cool to room temperature. Add 10 ml of concentrated HNO_3. Heat without spattering to close to the boiling point to digest the ash and to oxidize as well as possible any carbon residue. Add additional small amounts of concentrated HNO_3 as required to maintain the original volume of 10 ml.

5. Evaporate carefully until the beaker contents are <u>just dry</u>. <u>Do not overheat or bake</u>.

6. A specific volume of concentrated HCl was pipetted into the beaker, and was swirled carefully to mix. The volume required is given under the various analytical treatments.

7. A specific volume of water was pipetted into the beaker and mixed well. The volume required is given under the various analytical treatments.

(c) <u>Wet ashing (pressure/digestion) pretreatment</u>

Based on the literature review, the following very procedure was adapted.

1. Specific weights of dried fish tissue were weighed accurately into Kimax screwcap 12-cm test tubes. The weights involved, which are given under the various analytical treatments, varied according to the expected range of trace metal content.

2. A blank was carried through the preparatory acid extraction digestion procedure.

3. All samples, including the blank, were treated to the following wet ashing pressure/digestion process:-

   i) Four (4) ml of a $HClO_4/HNO_3$ mixture (1:1) were pipetted into the test tube containing the weighed fish tissue.

   ii) The test tube was then tightly closed and allowed to stand overnight (circa 14 hrs.).

   iii) The contents of the test tube were then washed with the help of a 50 percent solution of HNO_3 in glass-distilled water into a 50 ml beaker.

   iv) The mixture was then evaporated on a hot plate to dense $HClO_4$ fumes.

   v) Concentrated HNO_3 was continuously added until a clear solution was attained with the $HClO_4$ fumes.

   vi) The beaker contents were then carefully evaporated until just dry. Do not overheat or bake.

4. The beaker walls were then washed by pipetting a specific volume of concentrated HCl and swirling to mix. The volume required is given under the various analytical procedures.

5. A specific volume of water, as indicated in the various analytical treatments, was then added, either by pipetting or by making up to the mark in a volumetric flask, and mixed well.

2.2.4 Analysis of fish tissue

Copper

Appendix C-1 provides the experimental data for the analysis of dried whole rainbow trout tissue for copper using the dry ashing pretreatment approach. The 21 values obtained during this experimental work yielded an average and standard deviation of:-

$$\text{Copper} = 3.8^{9} \pm 0.2^{9} \text{ ug Cu/g dried whole tissue}$$

Appendix D-1 provides the data obtained during the analysis of dried whole rainbow trout tissue for copper employing the wet ashing pressure/digestion method. The 19 values obtained yielded an average and standard deviation of:-

$$\text{Copper} = 4.1^{4} \pm 0.2^{4} \text{ ug Cu/g dried whole tissue}$$

The F-test was applied to determine whether or not the two series showed any significant difference in the standard deviations.

$$F = \frac{(S_1)^2}{(S_2)^2} = \frac{(0.29)^2}{(0.24)^2} = 1.46$$

$$F_{(0.99)} \quad (n_1 - 1 = 20)\ (n_2 - 1 = 18) = 3.00$$

$$F < F_{(0.99)}$$

The difference in the standard deviations is therefore not significant. Details of F-test obtained from Dick (110). The tabulated $F_{(0.99)}$ values were obtained from Snedecor (113).

The t-test was now applied to determine whether or not the two series showed any significant difference in the averages.*

$$s = \sqrt{\frac{\sum(x_{1,1} - \bar{x}_1)^2 + \sum(x_{1,2} - \bar{x}_2)^2}{n_1 + n_2 - 2}}$$

$$s = \pm 0.2^7$$

$$t^\circ = \frac{|\bar{x}_1 - \bar{x}_2|}{s}\sqrt{\frac{n_1 n_2}{n_1 + n_2}}$$

$$t = 2.92$$

$$t_{(0.99)} \quad (n_1 + n_2 - 2 = 30) = 2.75$$

$$t_{(\text{calculated})} > t_{(0.99)}$$

The calculated value for "t" is close enough to the t(0.99) value to make the following assumption. There is a 99 percent chance that the two means are the same and that any differences are random. The overall average and standard deviation for the 32 results involved is

Copper = $4.0^1 \pm 0.2^9$ ug Cu/g dried whole tissue

Unless specified, this same procedure for the testing of significance is applied for the remaining averages and standard deviations resulting from the analysis of the remaining metals.

* Details of the t-test obtained from Dick (112). The $t_{(0.99)}$ values were obtained from Snedecor (114).

Nickel

Appendix C-2 provides the experimental data for the analysis of dried whole rainbow trout tissue for nickel using the dry ashing pretreatment approach. The 22 values obtained during this experimental work yielded an average and standard deviation of:-

$$\text{Nickel} = 1.2^{7} \pm 0.2^{2} \text{ ug Ni/g dried whole tissue}$$

Appendix D-2 provides the data obtained during the analysis of dried whole rainbow trout tissue for nickel employing the wet ashing pressure/digestion method. The 24 values obtained yielded an average and standard deviation of:-

$$\text{Nickel} = 1.8^{2} \pm 0.2^{6} \text{ ug Ni/g dried whole tissue}$$

The application of the F-test resulted in,

$$F = \frac{(0.26)^2}{(0.22)^2} = 1.39$$

$$F_{(0.99)} \ (n_1 - 1 = 23) \ (n_2 - 1 = 21) = 2.82$$

$$F < F_{(0.99)}$$

The difference in the standard deviations is not significant.

Testing for significance in the difference of the two means (t-test) resulted in the following:-

$$s = \pm 0.24^4$$

$$t = \frac{|1.27 - 1.82|}{0.244} \sqrt{\frac{22 \times 24}{22 + 24}}$$

$$t = 7.64$$

$$t_{(0.99)} \ (n_1 + n_2 - 2 = 44) = 2.70$$

$$t_{(calculated)} > t_{(0.99)}$$

The difference between the two means is therefore significant. A possible source of determinate error may arise from some relatively low absorbance results obtained under both methods of pretreatment. These low numerical values (close to the sensitivity limit for nickel) without rejection requirements (2s criterion) were included in the final average result. The final result was obviously influenced by the inclusion of these highly uncertain values.

Thus, the determination of nickel in dried whole tissue must display two separate results as follows:-

Nickel (dry ashing) = $1.2^7 \pm 0.2^2$ ug Ni/g dried whole tissue

Nickel (wet ashing pressure/digestion) =

$1.8^2 \pm 0.2^6$ ug Ni/g dried whole tissue

Zinc

Appendix C-3 provides the experimental data for the analysis of dried whole rainbow trout tissue for zinc using the dry ashing pretreatment approach. The 25 values obtained during this experimental work yielded an average and standard deviation of:-

$$\text{Zinc} = 120.^{6} \pm 3.^{8} \text{ ug Zn/g dried whole tissue}$$

Appendix D-3 provides the experimental data obtained for the analysis of dried whole rainbow trout tissue for zinc employing the wet ashing pressure/digestion method. The 22 results obtained yielded an average and standard deviation of:-

$$\text{Zinc} = 115.^{1} \pm 9.^{1} \text{ ug Zn/g dried whole tissue}$$

The F-test was applied to determine whether or not the two series showed any significant difference in the standard deviations.

$$F = \frac{(9.1)^2}{(3.8)^2} = 5.73$$

$$F_{(0.99)} \; (n_1 - 1 = 21) \; (n_2 - 1 = 24) = 2.03$$

$$F \geq F_{(0.99)}$$

Since the two series showed a significant difference in the standard deviations, the conventional t-test could not be applied. Instead, a technique by Snedecor (111) was used, where the sum of

squares are not pooled but the variance of each mean is calculated separately and used as a weight value for each method in the approximation of t(0.99). By labelling the dry ashing method (A) and the wet ashing pressure/digestion method (B), the following was obtained:-

Method	No. of results	Deg. of freedom (d. f.)	Mean	Sum of $(\text{var})^2$ $\sum x^2$	$s^2 = \frac{\sum x^2}{\text{d.f.}}$	$s_{\bar{x}}^2 = s^2/n$	$t_{(0.99)}$
A	25	24	120.6	346	14.4	0.576	2.797
B	22	21	115.1	1739	82.8	3.76	2.831
	47	45	5.5			4.336	

$$s_{\bar{x}} = \sqrt{4.336} = 2.082$$

$$1\% \text{ level} = \frac{(s_{\bar{x}}^2)_A \, (t_{(0.99)})_A + (s_{\bar{x}}^2)_B \, (t_{(0.99)})_B}{(s_{\bar{x}}^2)_A + (s_{\bar{x}}^2)_B}$$

$$= \frac{(0.576)\,(2.797) + (3.76)\,(2.831)}{(0.576) + (3.76)}$$

$$= 2.82$$

$$\bar{x}/s_{\bar{x}} = (\text{Mean}_A - \text{Mean}_B)/s_{\bar{x}} = \frac{5.5}{2.082}$$

$$= 2.64$$

$$\bar{x}/s_{\bar{x}} < 1\% \text{ level } (t_{(0.99)})$$

Since the ratio $\bar{x}/s_{\bar{x}}$ is less than the value calculated for the 1 percent level, we can say that there is a 99 % chance that the two means are the same and that any difference is random. The overall average and standard deviation for the 47 results involved is

$$\text{Zinc} = 118.^{1} \pm 7.^{3} \text{ ug Zn/g dried whole tissue}$$

Lead

Appendix C-4 provides the experimental data for the analysis of dried whole rainbow trout tissue for lead using the dry ashing pretreatment approach. The 17 values obtained during this experimental work yielded an average and standard deviation of:-

$$\text{Lead} = 0.83 \pm 0.08 \text{ ug Pb/g dried whole tissue}$$

Appendix D-4 provides the data obtained during the analysis of dried whole rainbow trout tissue for lead employing the wet-ashing pressure/digestion method. The 19 results obtained yielded an average and standard deviation of:-

$$\text{Lead} = 0.78^{6} \pm 0.09^{2} \text{ ug Pb/g dried whole tissue}$$

The F-test was applied to determine whether or not the two series showed any significant difference in the standard deviations.

$$F = \frac{(0.092)^2}{(0.08)^2} = 1.32$$

$$F_{(0.99)} \ (n_1 - 1 = 18) \ (n_2 - 1 = 16) = 3.30$$

$$F < F_{(0.99)}$$

The difference in the standard deviations is not significant.

The conventional t-test was now applied to determine whether or not the two series showed any significance for the difference in their means.

$$s = \pm 0.085$$

$$t = \frac{|0.83 - 0.786|}{0.085} \sqrt{\frac{19 \times 17}{19 + 17}}$$

$$t = 1.55$$

$$t_{(0.99)} \ (n_1 + n_2 - 2 = 34) = 2.73$$

$$t_{(calculated)} < t_{(0.99)}$$

There is a 99 % chance that the two means are the same and that any differences are random. The overall average and standard deviation for the 36 results involved is

Lead = 0.81 ± 0.09 ug Pb/g dried whole tissue

Cadmium

Appendix C-5 provides the experimental data for the analysis of dried whole rainbow trout tissue for cadmium using the dry ashing pretreatment approach. The 20 values obtained during this experimental work yielded an average and standard deviation of:-

$$\text{Cadmium} = 0.032^{9} \pm 0.002^{3} \text{ ug Cd/g dried whole tissue}$$

Appendix D-5 provides the experimental data for the analysis of dried whole rainbow trout tissue for cadmium using the wet ashing pressure/digestion method. The 21 results obtained yielded an average and standard deviation of:-

$$\text{Cadmium} = 0.027^{6} \pm 0.003^{7} \text{ ug Cd/g dried whole tissue}$$

The F-test (significance in standard deviation differences) yielded the following results:-

$$F = \frac{(0.0037)^2}{(0.0023)^2} = 2.59$$

$$F_{(0.99)} \ (n_1 - 1 = 20) \ (n_2 - 1 = 19) = 3.00$$

$$F < F_{(0.99)}$$

The difference in the standard deviations in therefore not significant.

The t-test (significance in the difference in the means) yielded the following results:-

$$s = \pm 0.0032$$

$$t = \frac{|0.0329 - 0.0276|}{0.0032} \sqrt{\frac{21 \times 20}{21 + 20}}$$

$$t = 5.30$$

$$t_{(0.99)} \ (n_1 + n_2 = 39) = 2.71$$

$$t_{(calculated)} > t_{(0.99)}$$

As a result of the t-test, the difference between the two means is significant. However, in view of the overlap through the respective standard deviations and the very low numerical results, the two means can be considered to result from the same population.

The overall average and standard deviation for the 41 results involved is

Cadmium = $0.030^2 \pm 0.0041$ ug Cd/g dried whole tissue

Vanadium

Appendix C-6 provides the experimental data for the analysis of dried whole rainbow trout tissue for vanadium using the dry ashing pretreatment approach. The 23 values obtained during this experimental work yielded an average and standard deviation of:-

$$\text{Vanadium} = 0.27^{1} \pm 0.05^{3} \text{ ug V/g dried whole tissue}$$

Appendix D-6 provides the experimental data for the analysis of dried whole rainbow trout tissue for vanadium using the wet ashing pressure/digestion method. The 21 values obtained yielded an average and standard deviation of:-

$$\text{Vanadium} = 0.25^{3} \pm 0.04^{2} \text{ ug V/g dried whole tissue}$$

The F-test was applied to determine whether or not the two series showed any significant difference in their standard deviations.

$$F = \frac{(0.053)^2}{(0.042)^2} = 1.59$$

$$F_{(0.99)} \quad (n_1 - 1 = 22) \quad (n_2 - 1 = 20) = 2.90$$

$$F < F_{(0.99)}$$

The difference in the standard deviations is not significant.

The t-test was then applied to determine whether or not the two series showed any significant difference in their means.

$$s = \pm 0.04^8$$

$$t = \frac{|0.271 - 0.253|}{0.048} \sqrt{\frac{23 \times 21}{23 + 21}}$$

$$t = 1.24$$

$$t_{(0.99)} \ (n_1 + n_2 - 2 = 42) = 2.70$$

$$t_{(calculated)} < t_{(0.99)}$$

Thus, there is a 99 % chance that the two means are the same and that any differences are random.

The overall average and standard deviation for the 44 values involved is

Vanadium = $0.26^2 \pm 0.04^8$ ug V/g dried whole tissue

2.2.5 Recoveries of trace metals from dry and wet ashing pretreatments

Appendices C-1 to C-6 provide the experimental details and data for the recovery tests relative to the dry ashing pretreatment applied in the determination of copper, nickel, zinc, lead, cadmium and vanadium respectively. The experimental details and data for the recoveries of the same metals relative to the wet pressure/digestion pretreatment are given in Appendices D-1 to D-6. The average recovery values for each trace metal by both pretreatments are tabulated on Table 3. These values represent the percent recoveries for the mentioned metals from spiking on rainbow trout followed by a dry ashing or wet pressure/digestion with atomic absorption as the quantitative determination technique.

The results on Table 3 provide recovery percent averages satisfactory for the purpose of this investigation. All averages are within 10 percent of the optimum recovery percent value of 100. However an obvious general trend leans towards lower recovery, with the exception of vanadium values, for the wet pressure/digestion pretreatment technique. Also, the standard deviations tend to be generally higher for the wet ashing technique (lead is an exception). This observation is not surprising and is supported by previous

TABLE 3

ATOMIC ABSORPTION DETERMINATION OF TRACE METALS IN RAINBOW TROUT

PERCENT RECOVERIES AFTER DRY ASHING AND WET PRESSURE/DIGESTION PRETREATMENT.

Element	Dry ashing % recovery	Wet pressure/digestion % recovery
Copper	$103.^{2} \pm 3.^{0}$	$96.^{1} \pm 8.^{8}$
Nickel	$100.^{4} \pm 2.^{0}$	$97.^{2} \pm 4.^{0}$
Zinc	$101.^{3} \pm 6.^{7}$	$89.^{0} \pm 6.^{4}$
Lead	$100.^{3} \pm 10.5$	$93.^{2} \pm 5.^{6}$
Cadmium	$91.^{5} \pm 6.^{9}$	$92.^{4} \pm 8.^{2}$
Vanadium	$100.^{7} \pm 4.^{9}$	$107.^{2} \pm 4.^{8}$

experimentation by Anderson (79).

The generally higher variation in the average percent recoveries (standard deviation) for the wet ashing technique can be explained by the relatively higher sample manipulation over the dry ashing method. The possibility of handling errors and contamination is much greater for the wet technique, therefore relatively higher standard deviations in relation to the dry ashing technique would not be out of place.

The trend showing lower recovery precent averages for wet ashing techniques may arise from the loss of metal in the lengthy evaporation of perchloric acid. Possible volatilization may occur at this point expecially for the more volatile metal oxides of zinc, lead and cadmium. Vanadium recovery by wet digestion yielded a higher result than by the dry ashing method. The reason may be that the very stable metal oxide formed is not easily volatilized, and vanadium may not follow the trend established by the other metals.

As explained in Sub-section 1.5 the trace metals chosen represent a range of relative volatilities both high and low. This trend can be seen on Table 3 where zinc, lead and cadmium recoveries (high volatility oxides) were generally lower than that of copper, nickel and vanadium with relatively lower volatility metal oxides.

2.2.6 Efficiency of aliquoting process

Section 4 of each of the Appendices C-1 to C-6 provides a detailed procedure and data for the determination of aliquoting efficiency after pretreatment of tissue by the dry ashing technique. Likewise, Section 4 of the Appendices D-1 to D-6 provides the experimental details and data for aliquoting of sample solutions after wet pressure/digestion pretreatment. Table 4 lists the averaged recoveries from the determination of aliquot sample solutions pretreated by both dry and wet ashing methods.

The percent recoveries from aliquoting are satisfactory for the purpose of this investigation. Recoveries from Table 4 are all within the standard deviation limits of average recoveries from spiking tabulated on Table 3. This leads us to believe that the aliquoting process is adequate with no serious complications.

As in Sub-section 2.2.5, the wet pressure/digestion procedure yielded generally lower recovery percent results, with the exception of vanadium and cadmium. Again, the data seems to indicate the loss of metals in the wet pressure/digestion pretreatment technique. For an unknown reason cadmium seems to have behaved differently in this investigation. The wet pretreatment method yielded a higher recovery percent of cadmium from aliquoting. This may possibly be due to some determinate error in the procedure or a less representative sample of rainbow trout tissue used for the experimentation.

TABLE 4

ATOMIC ABSORPTION DETERMINATION OF TRACE METALS IN RAINBOW TROUT

PERCENT RECOVERIES FROM ALIQUOTING
OF DRY ASHED AND PRESSURE/DIGESTED SAMPLES

Element	Dry ashing* % recovery	Wet* pressure/digestion % recovery
Copper	104	96
Nickel	104	98
Zinc	101**	88
Lead	108	88
Cadmium	89	96
Vanadium	98	106

* These are average values from only two sets of results, therefore standard deviations are not indicative of the uncertainty in the procedure.

** Average of four results.

3. CONCLUSIONS

3.1 Atomic Absorption Spectrophotometry

Atomic absorption spectrophotometry proved to be an ideal method for the determination of trace metals in rainbow trout tissue. Both the flame and graphite furnace techniques lived up to their expectations as simple to operate and rapid techniques with almost instantaneous results (for flame methods).

The technique is an attractive one not only because of the speed of analysis but also because of the high sensitivities which were obtained in this investigation. Table 1 provides a list of sensitivities and linear range limits for the flame technique. For example, cadmium could be measured down to 0.048 ppm and zinc to 0.020 ppm while the less sensitive metals like vanadium still could be determined to 2.1 ppm. In general the sensitivities were excellent. However, much higher sensitivities resulted for the graphite furnace technique (Table 2). Sensitivities up to 1000 times higher than that of the flame were not uncommon. An example is lead which could be measured at 0.0006 ppm and cadmium at 0.00004 ppm with ease. Not many other techniques have been able to achieve such high sensitivities and still employ a simple operating procedure. In addition, all uncertainties in concentration within the linear

range values for both flame (Table 1) and graphite furnace (Table 2) techniques were below the sensitivity values. This indicates the precision in both methods. Again, cadmium is an exception, but at such low concentrations we can expect higher uncertainties.

The concentration linear response limits found by this investigation are reported in Table 1 for the flame technique and Table 2 for the graphite furnace approach. From both sets of results it can be seen that linearity covered a wide range for most metals either by flame or graphite furnace methods. However, it was extremely difficult to work at such small concentrations within the linear limits for cadmium.

3.2 Dry Ashing and Wet Pressure/Digestion Pretreatment Techniques

The methods of pretreatment of rainbow trout tissue were investigated for their adaptability and ease of operation. Both techniques involved very simple concepts. The ashing method by furnace heat required less manipulation of the sample and/or solutions when compared to the wet pressure/digestion technique. The dry ashing method was the simpler of the two. The wet pressure/digestion technique was sometimes messy, with pressure build-up and the splitting of faulty test tubes. Also, the evaporation of the final digested solution containing nitric and perchloric acid proved to be more time consuming than the evaporation of just nitric acid.

As explained later, the digestion completion step over a hot plate caused higher losses for the wet than for the dry ashing techniques.

In conclusion, in view of simplicity due to less sample manipulation, the dry ashing technique is preferred by the author over the wet pressure/digestion method.

3.3 Metal Concentration in Whole Rainbow Trout Tissue

The concentrations of trace metals in dried rainbow trout unexposed to experimentally high amounts of metals in the water and/or food are given in Table 5. These results could only produce a comparison between the wet and dry pretreatment techniques since no literature values are available. By comparing both pretreatment techniques a general trend was visualized. The results obtained by the wet ashing pretreatment are generally lower than those of the dry ashing with one exceptions (copper). Nickel cannot be considered an exception due to a possible determinate error suspected for both techniques. Various nickel concentration results, in spite of their high uncertainty (closeness to the sensitivity limit), were not rejected by the 2s test and therefore had to be included. These results obviously influenced the final averages from both pretreatment techniques.

TABLE 5

TRACE METAL CONCENTRATION IN DRIED BASE RAINBOW TROUT TISSUE

Element	Dry ashing ug/g fish base	Wet pressure/digestion ug/g fish base	Average result ug/g fish base
Copper	$3.8^{9} \pm 0.2^{9}$	$4.1^{4} \pm 0.2^{4}$	$4.0^{1} \pm 0.2^{9}$
Nickel*	$1.2^{7} \pm 0.2^{2}$	$1.8^{2} \pm 0.2^{6}$	--
Zinc	$120.^{6} \pm 3.^{8}$	$115.^{1} \pm 9.^{1}$	$118.^{1} \pm 7.^{3}$
Lead	0.83 ± 0.08	$0.78^{6} \pm 0.09^{2}$	0.81 ± 0.09
Cadmium	$0.032^{9} \pm 0.002^{3}$	$0.027^{6} \pm 0.003^{7}$	$0.030^{2} \pm 0.004^{1}$
Vanadium	$0.27^{1} \pm 0.05^{3}$	$0.25^{3} \pm 0.04^{2}$	$0.26^{2} \pm 0.04^{8}$

* Results were found to be significantly different, therefore no overall average was calculated.

3.4 Efficiency of Recovery from Spiking

As discussed in Sub-section 2.2.5, the metals involved in this investigation represent a range of both high and low relative volatilities. Zinc, lead and cadmium represent the high end while copper, nickel and vanadium the low volatility components. This volatility separation was observed on the results of percent recoveries for each metal as determined after each pretreatment methodology (Table 3). There was an obvious drop in percent recovery results from copper, nickel and vanadium to zinc, lead and cadmium, more so in the wet than the dry ashing technique. However, the most pronounced drop was in the cadmium recovery result for the dry ashing technique. With all other metals having recoveries from 100 to 103 percent, cadmium stood alone at 91.5%. This suggests that a lower ashing temperature than 500°C should have been used in the ashing of tissue samples for the determination of cadmium. Nevertheless, the recovery result is satisfactory for the purpose of this investigation.

For the wet pressure/digestion pretreatment approach, the differences between the suspected high and low volatility metal oxides is much more visible. Copper (96.1), nickel (97.2) and vanadium (107.2) yielded higher recoveries than zinc (89.0), lead (93.2) and cadmium (92.4).

Comparisom between the two pretreatment approaches shows a definite trend. Generally lower recovery percent averages with higher standard deviations were obtained from the wet ashing method in comparison to the dry ashing technique (Table 3). This may be attributed to the additional handling characteristics of the wet pressure/digestion technique. A possible source of error may also result from the lengthy evaporation of perchloric acid. The generally higher standard deviations for the wet ashing technique reflect the greater handling of samples involved.

In conclusion, both pretreatment techniques yielded satisfactory recovery percent results. However, the dry ashing technique is the method of choice due to the overall better percent recoveries obtained for the involved trace metals in rainbow trout tissue.

It should be pointed out that data from various facets of the determination of nickel suffered, first of all, from the fact that the base fish nickel content approached the sensitivity limits for the flame technique and second, from the fact that the spiking procedure applied involved quantities of added nickel too high for this base fish content. Although the graphite furnace method presents interference problems in the determination of nickel in fish tissue, further experimental work is required using this technique in order to eliminate the doubtful areas in this phase of the study.

3.5 Efficiency in Aliquoting

The average percent recoveries given in Table 4 represent satisfactory results on aliquoting of dry ashed and wet digested sample solutions of fish tissue. As mentioned in Sub-section 2.2.6 the average recoveries from aliquoting were all within the standard deviation limits of the recovery percent values from the spiking procedure of the method of additions (Table 3).

With the exception of cadmium and vanadium the general tendency is towards lower percent recovery results for the wet over the dry ashing pretreatment technique. This supports the previous conclusions on the efficiency of recovery from spiking and the results of metal concentration in whole dried rainbow trout tissue.

In spite of the lower results obtained for the wet pressure digestion pretreatment approach over the dry ashing method, the aliquoting process is adequate and should present no complications.

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APPENDIX A

APPENDIX A-1

Determination of the Linear Range of Copper in Aqueous Base Solutions by Flame Atomic Absorption Spectrophotometry

General operating conditions

Instrument:-	Perkin-Elmer Model 503 Atomic Absorption Spectrophotometer
Radiation source:-	Copper hollow cathode lamp
Wavelength:-	324.7 nm
Slit:-	0.7 nm
Background correction:-	D_2 beam corrector
Readout:-	3-seconds integration
Burner:-	4-inch, single slot
Fuel:-	Acetylene - 20 gauge units (7 lit/min.)
Oxidant:-	Air - 40 gauge units (14 lit/min.)
Water:-	Glass-distilled
Sample size:-	Continuous flow

Preparation of aqueous-base standard solutions

1. All standard solutions are prepared from a 1000.0 ± 0.2 ppm copper solution supplied by Fisher Scientific Company Limited as Certified Fisher Standard Stock Solution of Copper.

2. Dilution of the standard stock solution was carried out as follows:-

   (a) 25.00 ± 0.02 ml of 1000.0 ± 0.2 ppm copper solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 100.0 ± 0.1 ppm copper

(b) 25.00 ± 0.02 ml of 100.0 ± 0.1 ppm copper solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

Value:- 10.00 ± 0.02 ppm copper

3. The following indicates the preparation procedures for the copper working standard solutions. All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Solution preparation				Copper (ppm)
5.00 ± 0.02 ml	of	10.00 ± 0.02	ppm Cu	0.500 ± 0.003
10.00 ± 0.02 ml	"	"	" "	1.000 ± 0.004
2.00 ± 0.02 ml	"	100.0 ± 0.1	" "	2.00 ± 0.02
3.00 ± 0.02 ml	"	"	" "	3.00 ± 0.02
4.00 ± 0.02 ml	"	"	" "	4.00 ± 0.02
4.50 ± 0.02 ml	"	"	" "	4.50 ± 0.02
4.75 ± 0.02 ml	"	"	" "	4.75 ± 0.02
4.90 ± 0.02 ml	"	"	" "	4.90 ± 0.02
5.00 ± 0.02 ml	"	"	" "	5.00 ± 0.02
5.25 ± 0.02 ml	"	"	" "	5.25 ± 0.02
5.50 ± 0.02 ml	"	"	" "	5.50 ± 0.02
6.00 ± 0.02 ml	"	"	" "	6.00 ± 0.03

Testing of aqueous-base standard solutions

The set of working standards gave the following results:-

Copper (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.500 ± 0.003	0.025 ± 0.000
1.000 ± 0.004	0.049 ± 0.000
2.00 ± 0.02	0.104 ± 0.000
3.00 ± 0.02	0.156 ± 0.000
4.00 ± 0.02	0.208 ± 0.001
4.50 ± 0.02	0.234 ± 0.000
4.75 ± 0.02	0.245 ± 0.000
4.90 ± 0.02	0.252 ± 0.001
5.00 ± 0.02	0.260 ± 0.000
5.25 ± 0.02	0.267 ± 0.000
5.50 ± 0.02	0.274 ± 0.002
6.00 ± 0.03	0.285 ± 0.000

* All averages result of three 3-seconds integration readings. ± values represent standard deviations.

Note:- average standard deviation $< \pm 0.001$

Linear regression analysis

Concentrations included copper (ppm)	Linear regression* data
0.00 - 6.00	r = 0.9979 b = 0.002 m = 0.05013
0.00 - 5.50	r = 0.9995 b = 0.000 m = 0.05122
0.00 - 5.25	r = 0.9998 b = 0.000 m = 0.05171
0.00 - 5.00	r = 0.9999 b = -0.001 m = 0.05198

* r = correlation coefficient
b = intercept
m = slope

From the linear regression results for different ranges and the plot for Figure A-1, a loss of linearity after 5.50 ppm copper can be noted.

Therefore:-

Linear range regression analysis

0.00 - 5.50 ppm Cu — r = 0.9995
b = 0.000
m = 0.05122

Sensitivity (1% absorption) = 0.0044/0.05122 = 0.08^{6} ppm Cu

Note:- the value 0.0044 represents an arbitrary fixed absorbance for 1% absorption.

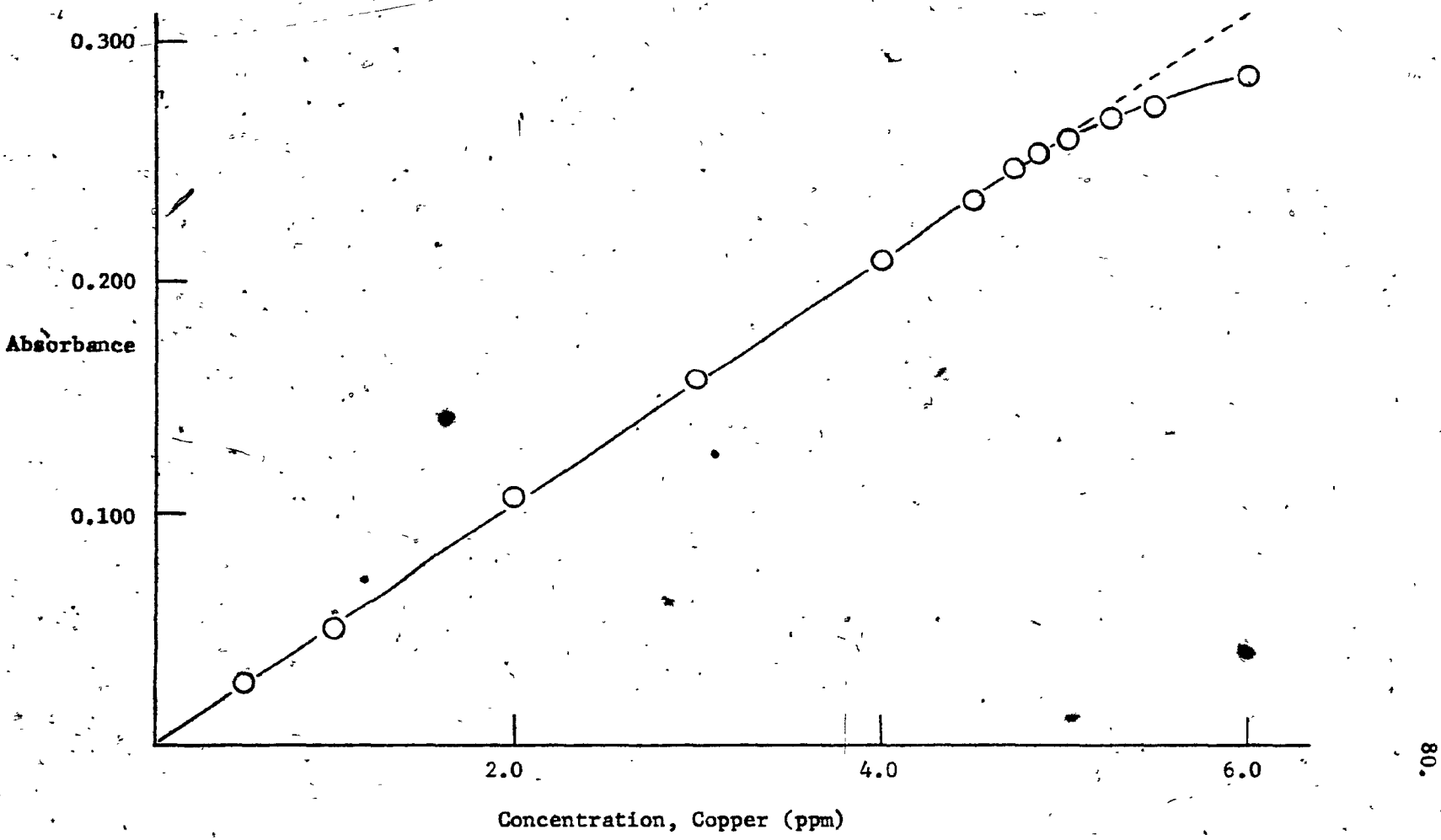


FIGURE A-1 ATOMIC ABSORPTION FLAME TECHNIQUE - CALIBRATION CURVE FOR COPPER

Reverse calculations for copper

Actual copper (ppm)	Calculated copper (ppm)	Absolute devn.
0.000	0.000	0.000
0.500 ± 0.003	0.488	0.012
1.000 ± 0.004	0.957	0.043
2.00 ± 0.02	2.03	0.03
3.00 ± 0.02	3.04	0.04
4.00 ± 0.02	4.06	0.06
4.50 ± 0.02	4.57	0.07
4.75 ± 0.02	4.78	0.03
4.90 ± 0.02	4.92	0.02
5.00 ± 0.02	5.08	0.08
5.25 ± 0.02	5.21	0.04
5.50 ± 0.02	5.35	0.15
	Average absolute deviation	0.05

Values of copper (ppm) from this equation subject to ± 0.05 ppm

APPENDIX A-2

Determination of the Linear Range of Nickel in Aqueous Base Solutions by Flame Atomic Absorption Spectrophotometry

General operating conditions

Instrument:-	Perkin-Elmer Model 503 Atomic Absorption Spectrophotometer
Radiation source:-	Nickel hollow cathode lamp
Wavelength:-	232.0 nm
Slit:-	0.2 nm
Background correction:-	D_2 beam corrector
Readout:-	3-seconds integration
Burner:-	4-inch, single slot
Fuel:-	Acetylene - 20 gauge units (7 lit/min.)
Oxidant:-	Air - 40 gauge units (14 lit/min.)
Water:-	Glass-distilled
Sample size:-	Continuous flow

Preparation of aqueous-base standard solutions

1. All standard solutions are prepared from a 1000.0 ± 0.2 ppm nickel solution supplied by Fisher Scientific Company Limited as Certified Fisher Standard Stock Solution of Nickel.

2. Dilution of the standard stock solution was carried out as follows:-

   (a) 25.00 ± 0.02 ml of 1000.0 ± 0.2 ppm Ni solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 100.0 ± 0.1 ppm nickel

(b) 25.00 ± 0.02 ml of 100.0 ± 0.1 ppm nickel solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

Value:- 10.00 ± 0.02 ppm nickel

3. The following indicates the preparation procedures for the nickel working standard solutions. All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Solution preparation		Nickel (ppm)
1.00 ± 0.02 ml of	10.00 ± 0.02 ppm Ni	0.100 ± 0.002
5.00 ± 0.02 ml "	" "	0.500 ± 0.003
10.00 ± 0.02 ml "	" "	1.000 ± 0.004
1.50 ± 0.02 ml "	100.0 ± 0.1 " "	1.50 ± 0.02
2.00 ± 0.02 ml "	" "	2.00 ± 0.02
2.50 ± 0.02 ml "	" "	2.50 ± 0.02
3.00 ± 0.02 ml "	" "	3.00 ± 0.02
3.50 ± 0.02 ml "	" "	3.50 ± 0.02
4.00 ± 0.02 ml "	" "	4.00 ± 0.02
4.50 ± 0.02 ml "	" "	4.50 ± 0.02
4.75 ± 0.02 ml "	" "	4.75 ± 0.02
5.00 ± 0.02 ml "	" "	5.00 ± 0.02
5.25 ± 0.02 ml "	" "	5.25 ± 0.03
5.50 ± 0.02 ml "	" "	5.50 ± 0.03
6.00 ± 0.02 ml "	" "	6.00 ± 0.03
7.00 ± 0.02 ml "	" "	7.00 ± 0.03
8.00 ± 0.02 ml "	" "	8.00 ± 0.03

Testing of aqueous-base standard solutions

The set of working standards gave the following results:-

Nickel (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.100 ± 0.002	0.003 ± 0.000
0.500 ± 0.003	0.015 ± 0.000
1.000 ± 0.004	0.030 ± 0.000
1.50 ± 0.02	0.045 ± 0.000
2.00 ± 0.02	0.059 ± 0.000
2.50 ± 0.02	0.073 ± 0.000
3.00 ± 0.02	0.086 ± 0.000
3.50 ± 0.02	0.100 ± 0.000
4.00 ± 0.02	0.114 ± 0.000
4.50 ± 0.02	0.126 ± 0.000
4.75 ± 0.02	0.132 ± 0.000

5.00 ± 0.02	0.139 ± 0.000
5.25 ± 0.03	0.143 ± 0.000
5.50 ± 0.03	0.151 ± 0.001
6.00 ± 0.03	0.162 ± 0.000
7.00 ± 0.03	0.188 ± 0.000
8.00 ± 0.03	0.211 ± 0.000

* All averages result of three 3-seconds integration readings. ± values represent standard deviations.

Note:- average standard deviation $< \pm 0.001$

Linear regression analysis

Concentrations included nickel (ppm)	Linear regression* data
0.00 - 8.00	r = 0.9990^9 b = 0.004 m = 0.02658
0.00 - 7.00	r = 0.9992^6 b = 0.003 m = 0.02693
0.00 - 6.00	r = 0.9993^1 b = 0.002^8 m = 0.0271^7
0.00 - 5.50	r = 0.9994^2 b = 0.002^4 m = 0.02739
0.00 - 5.25	r = 0.9994^1 b = 0.002^2 m = 0.02752
0.00 - 5.00	r = 0.9996 b = 0.001^8 m = 0.02778

* r = correlation coefficient
b = intercept
m = slope

From the linear regression results for different ranges and the plot for Figure A-2, a loss of linearity after 5.00 ppm nickel can be noted.

Therefore:-

Linear range regression analysis,

0.00 - 5.00 ppm Ni

$r = 0.9996$
$b = 0.001^8$
$m = 0.02778$

Sensitivity (1% absorption) = $0.0044/0.02778 = 0.1^5$ ppm Ni

Reverse calculations for nickel

Actual nickel (ppm)	Calculated nickel (ppm)	Absolute devn.
0.000	-0.065	0.065
0.100 ± 0.002	0.043	0.057
0.500 ± 0.003	0.475	0.025
1.000 ± 0.004	1.015	0.015
1.50 ± 0.02	1.555	0.055
2.00 ± 0.02	2.059	0.059
2.50 ± 0.02	2.563	0.063
3.00 ± 0.02	3.031	0.031
3.50 ± 0.02	3.535	0.035
4.00 ± 0.02	4.039	0.039
4.50 ± 0.02	4.471	0.029
4.75 ± 0.02	4.687	0.063
5.00 ± 0.02	4.939	0.061
	Average absolute deviation	0.04^6

Values of nickel (ppm) from this above equation subject to ± 0.04^6 ppm

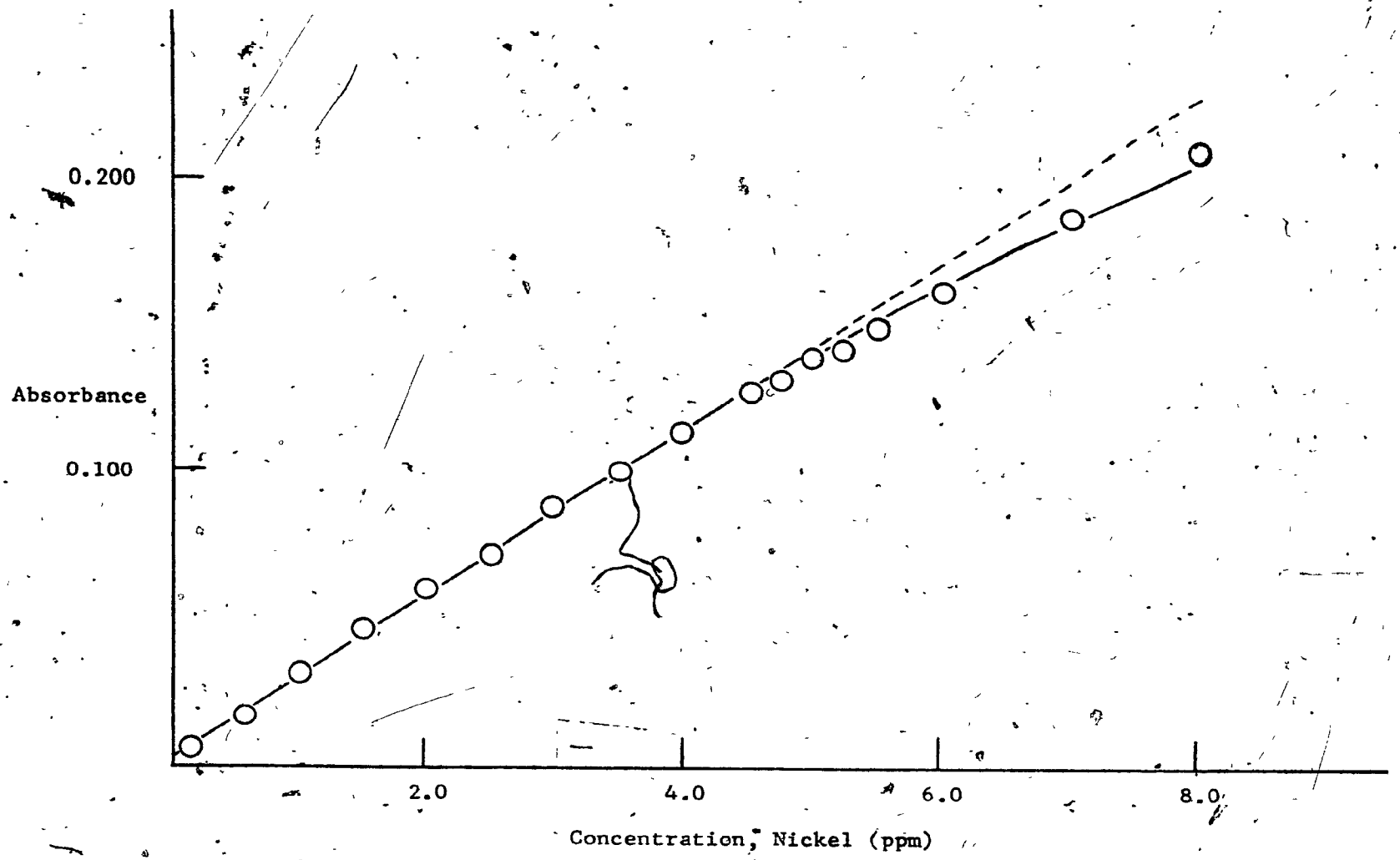


FIGURE A-2 ATOMIC ABSORPTION FLAME TECHNIQUE - CALIBRATION CURVE FOR NICKEL

APPENDIX A-3

Determination of the Linear Range of Zinc in Aqueous Base Solutions by Flame Atomic Absorption Spectrophotometry

General operating conditions

Instrument:-	Perkin-Elmer Model 503 Atomic Absorption Spectrophotometer
Radiation source:-	Zinc hollow cathode lamp
Wavelength:-	213.9 nm
Slit:-	0.7 nm
Background correction:-	D_2 beam corrector
Readout:-	3-seconds integration
Burner:-	4-inch, single slot
Fuel:-	Acetylene - 20 gauge units (7 lit/min.)
Oxidant:-	Air - 40 gauge units (14 lit/min.)
Water:-	Glass-distilled
Sample size:-	Continuous flow

Preparation of aqueous-base standard solutions

1. All standard solutions are prepared from a 1000.0 ± 0.2 ppm zinc solution supplied by Fisher Scientific Company Limited as Certified Fisher Standard Stock Solution of Zinc.

2. Dilution of the standard stock solution was carried out as follows:-

   (a) 25.00 ± 0.02 ml of 1000.0 ± 0.2 ppm Zn solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 100.0 ± 0.1 ppm zinc

(b) 25.00 ± 0.02 ml of 100.0 ± 0.1 ppm zinc solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

Value:- 10.00 ± 0.02 ppm zinc

3. The following indicates the preparation procedures for the zinc working standard solutions. All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Solution preparation	Zinc (ppm)
1.00 ± 0.02 ml of 10.00 ± 0.02 ppm Zn	0.100 ± 0.002
3.00 ± 0.02 ml " " " "	0.300 ± 0.003
5.00 ± 0.02 ml " " " "	0.500 ± 0.003
7.00 ± 0.02 ml " " " "	0.700 ± 0.003
8.00 ± 0.02 ml " " " "	0.800 ± 0.004
9.00 ± 0.02 ml " " " "	0.900 ± 0.004
9.50 ± 0.02 ml " " " "	0.950 ± 0.004
10.00 ± 0.02 ml " " " "	1.000 ± 0.004
10.50 ± 0.04 ml " " " "	1.050 ± 0.006
11.00 ± 0.04 ml " " " "	1.100 ± 0.006
12.00 ± 0.04 ml " " " "	1.200 ± 0.006
14.00 ± 0.04 ml " " " "	1.400 ± 0.007
15.00 ± 0.04 ml " " " "	1.500 ± 0.007

Testing of aqueous-base standard solutions

The set of working standards gave the following results:-

Zinc (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.100 ± 0.002	0.024 ± 0.000
0.300 ± 0.003	0.067 ± 0.000
0.500 ± 0.003	0.110 ± 0.000
0.700 ± 0.003	0.160 ± 0.000
0.800 ± 0.004	0.183 ± 0.001
0.900 ± 0.004	0.200 ± 0.000
0.950 ± 0.004	0.213 ± 0.001
1.000 ± 0.004	0.224 ± 0.001
1.050 ± 0.006	0.228 ± 0.000
1.100 ± 0.006	0.237 ± 0.000
1.200 ± 0.006	0.257 ± 0.000
1.400 ± 0.007	0.295 ± 0.002
1.500 ± 0.007	0.315 ± 0.002

* All averages result of three 3-seconds integration readings. ± values represent standard deviations.

Note:- average standard deviation $< \pm 0.001$

Linear regression analysis

Concentrations included zinc (ppm)	Linear regression* data
0.000 - 1.500	r = 0.9985 b = 0.006 m = 0.2112
0.000 - 1.400	r = 0.9986 b = 0.005 m = 0.2138
0.000 - 1.200	r = 0.9989 b = 0.003 m = 0.2172
0.000 - 1.100	r = 0.9991 b = 0.002 m = 0.2194

* r = correlation coefficient
b = intercept
m = slope

From the linear regression results for different ranges and the results on Figure A-3, a loss of linearity after the 1.100 ppm zinc value can be noted.

Therefore:-

Linear range regression analysis,

0.000 - 1.100 ppm Zn

r = 0.9991
b = 0.002
m = 0.2194

Sensitivity (1% absorption) = 0.0044/0.2194 = 0.020 ppm Zn

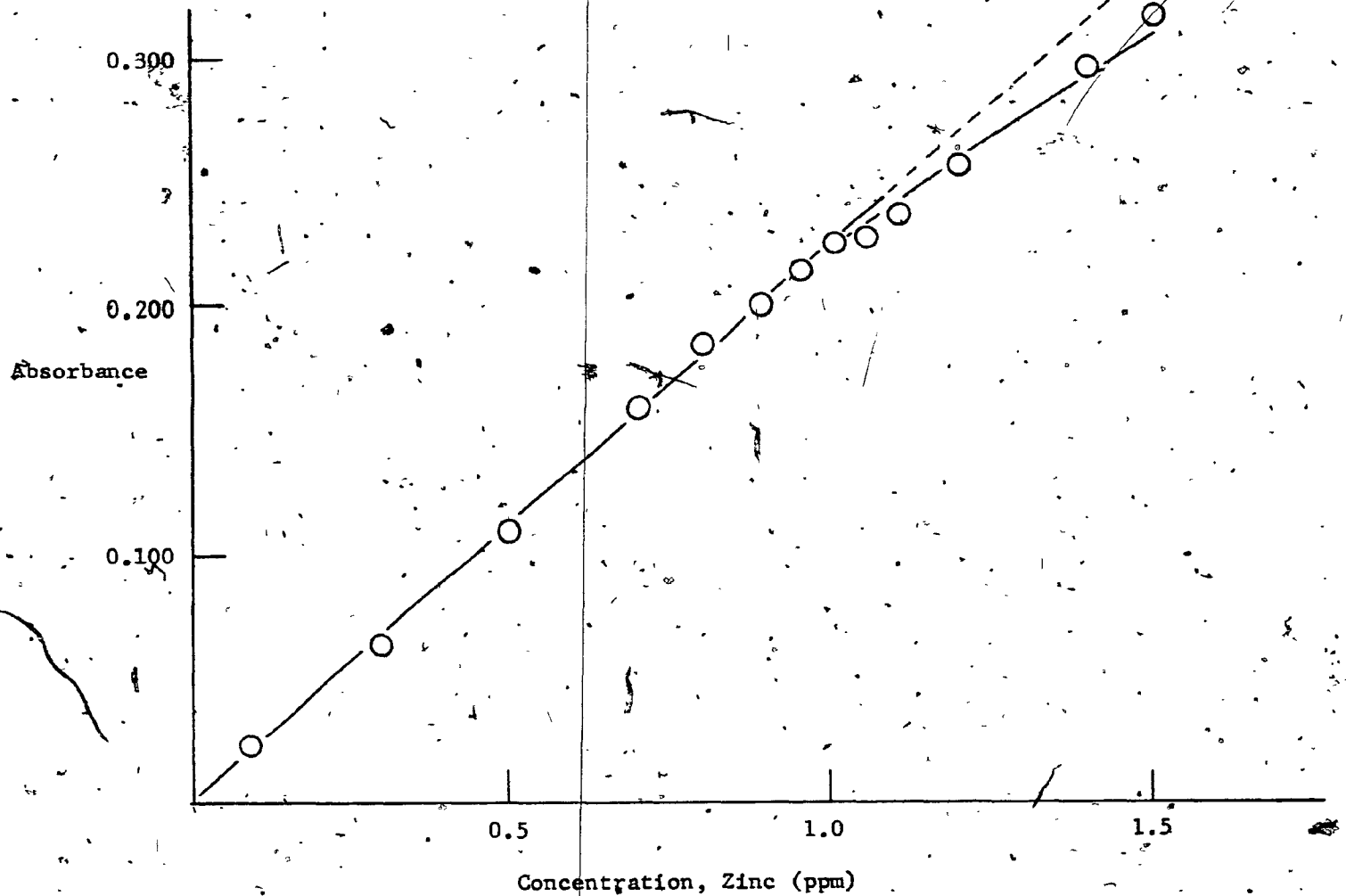


FIGURE A-3 ATOMIC ABSORPTION FLAME TECHNIQUE - CALIBRATION CURVE FOR ZINC

Reverse calculations for zinc.

Actual zinc (ppm)	Calculated zinc (ppm)	Absolute devn.
0.000	-0.009	0.009
0.100 ± 0.002	0.100	0.000
0.300 ± 0.003	0.296	0.004
0.500 ± 0.003	0.492	0.008
0.700 ± 0.003	0.720	0.020
0.800 ± 0.004	0.825	0.025
0.900 ± 0.004	0.902	0.002
0.950 ± 0.004	0.962	0.012
1.000 ± 0.004	1.012	0.012
1.050 ± 0.006	1.030	0.020
1.100 ± 0.006	1.071	0.029
	Average absolute deviation	0.01

Values for zinc (ppm) from the above equation subject to ±0.01 ppm

APPENDIX A-4

Determination of the Linear Range of Lead in Aqueous Base Solutions by Flame Atomic Absorption Spectrophotometry

General operating conditions

Instrument:-	Perkin-Elmer Model 503 Atomic Absorption Spectrophotometer.
Radiation source:-	Lead hollow cathode lamp
Wavelength:-	282.6 nm
Slit:-	0.7 nm
Background correction:-	D_2 beam corrector
Readout:-	3-seconds integration
Burner:-	4-inch, single slot
Fuel:-	Acetylene - 20 gauge units (7 lit/min.)
Oxidant:-	Air - 40 gauge units (14 lit/min.)
Water:-	Glass-distilled
Sample size:-	Continuous flow

Preparation of aqueous-base standard solutions

1. All standard solutions are prepared from a 1000.0 ± 0.2 ppm lead solution supplied by Fisher Scientific Company Limited as Certified Fisher Standard Stock Solution of Lead.

2. Dilution of the standard stock solution was carried out as follows:-

   (a) 25.00 ± 0.02 ml of 1000.0 ± 0.2 ppm Pb solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 100.0 ± 0.1 ppm lead

3. The following indicates the preparation procedures for the lead working standard solutions. All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Solution preparation	Lead (ppm)
1.00 ± 0.02 ml of 100.0 ± 0.1 ppm Pb	1.00 ± 0.02
2.00 ± 0.02 ml " " " "	2.00 ± 0.02
5.00 ± 0.02 ml " " " "	5.00 ± 0.02
8.00 ± 0.02 ml " " " "	8.00 ± 0.03
10.00 ± 0.02 ml " " " "	10.00 ± 0.03
12.00 ± 0.04 ml " " " "	12.00 ± 0.05
16.00 ± 0.04 ml " " " "	16.00 ± 0.06
19.00 ± 0.04 ml " " " "	19.00 ± 0.06
20.00 ± 0.04 ml " " " "	20.00 ± 0.06
21.00 ± 0.06 ml " " " "	21.00 ± 0.08
22.00 ± 0.06 ml " " " "	22.00 ± 0.08
23.00 ± 0.06 ml " " " "	23.00 ± 0.08
25.00 ± 0.06 ml " " " "	25.00 ± 0.08
26.00 ± 0.06 ml " " " "	26.00 ± 0.09
27.00 ± 0.06 ml " " " "	27.00 ± 0.09
28.00 ± 0.06 ml " " " "	28.00 ± 0.09
29.00 ± 0.06 ml " " " "	29.00 ± 0.09
30.00 ± 0.06 ml " " " "	30.00 ± 0.09

Testing of aqueous-base standard solutions

The set of working standards gave the following results:-

Lead (ppm)	Average absorbance*
0.000	0.000 ± 0.000
1.00 ± 0.02	0.005 ± 0.000
2.00 ± 0.02	0.011 ± 0.000
5.00 ± 0.02	0.027 ± 0.000
8.00 ± 0.03	0.043 ± 0.000
10.00 ± 0.03	0.054 ± 0.000
12.00 ± 0.05	0.065 ± 0.000
16.00 ± 0.06	0.087 ± 0.000
19.00 ± 0.06	0.101 ± 0.001
20.00 ± 0.06	0.107 ± 0.000
21.00 ± 0.08	0.110 ± 0.000
22.00 ± 0.08	0.113 ± 0.000
23.00 ± 0.08	0.117 ± 0.001
25.00 ± 0.08	0.126 ± 0.000
26.00 ± 0.09	0.130 ± 0.000
27.00 ± 0.09	0.134 ± 0.001
28.00 ± 0.09	0.139 ± 0.001
29.00 ± 0.09	0.144 ± 0.000
30.00 ± 0.09	0.150 ± 0.001

* All averages result of three 3-seconds integration readings.
± values represent standard deviations.
Note:- average standard deviation $< \pm 0.001$

Linear regression analysis

Concentrations included lead (ppm)	Linear regression* data
0.00 - 30.00	r = 0.9986 b = 0.002^{9} m = 0.00496^{0}
0.00 - 29.00	r = 0.9984 b = 0.002^{8} m = 0.00497^{4}
0.00 - 28.00	r = 0.9984 b = 0.002^{6} m = 0.00500^{2}
0.00 - 27.00	r = 0.9985 b = 0.002^{2} m = 0.00503^{8}
0.00 - 26.00	r = 0.9987 b = 0.001^{9} m = 0.00508^{5}

* r = correlation coefficient
b = intercept
m = slope

From the linear regression analysis results for different ranges and the plotted points on Figure A-4, a loss of linearity at the 26.00 ppm point can be noted.

Therefore:-

Linear range regression analysis,

0.00 - 26.00	r = 0.9987 b = 0.001^{9} m = 0.00508^{5}

Sensitivity (1% absorption) = 0.0044/0.005085 = 0.8^{6} ppm Pb

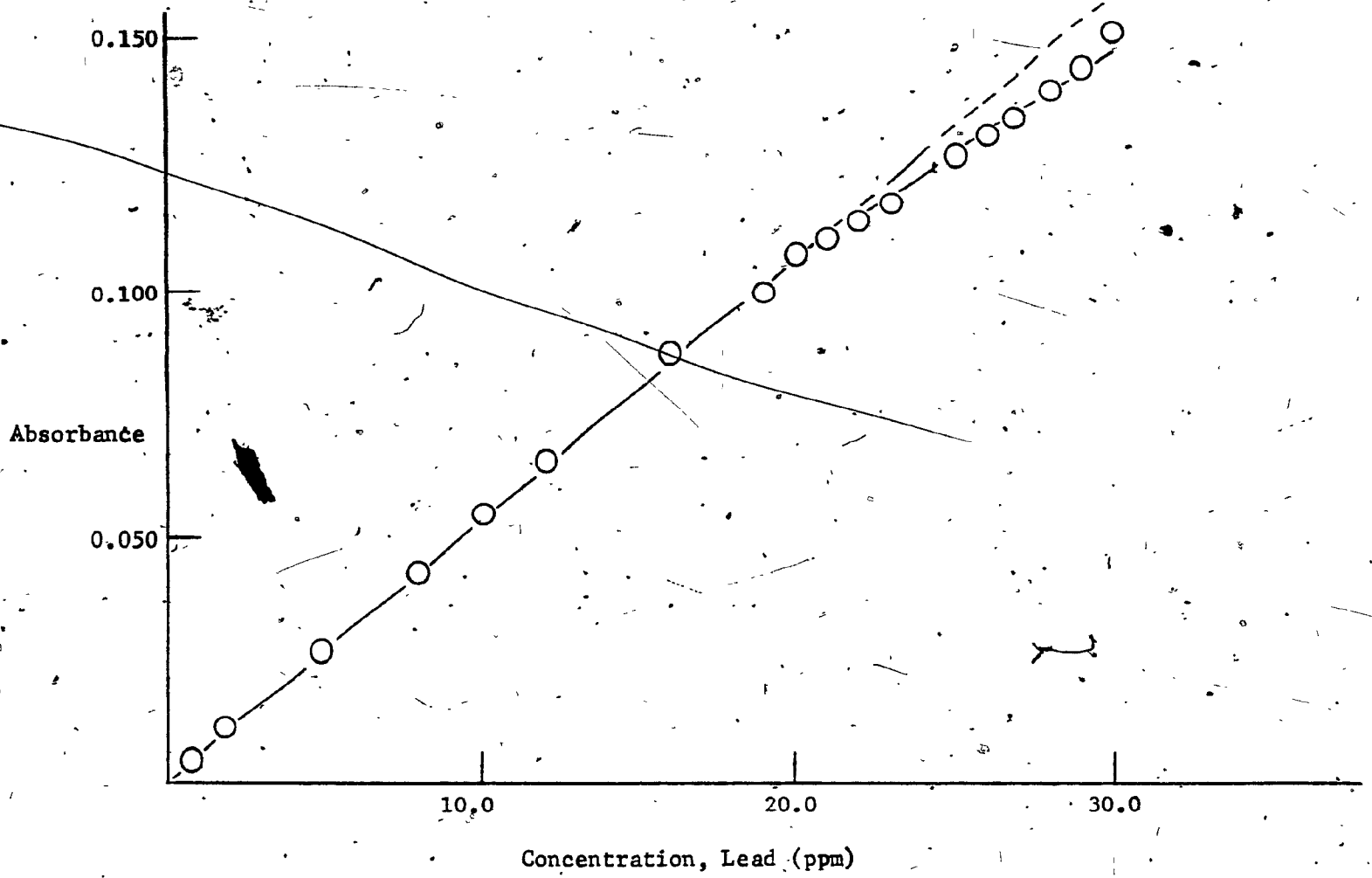


FIGURE A-4 ATOMIC ABSORPTION FLAME TECHNIQUE - CALIBRATION CURVE FOR LEAD

Reverse calculations for lead

Actual lead (ppm)	Calculated lead (ppm)	Absolute devn.
0.00	-0.37	0.37
1.00 ± 0.02	0.61	0.39
2.00 ± 0.02	1.79	0.21
5.00 ± 0.02	4.94	0.06
8.00 ± 0.03	8.08	0.08
10.00 ± 0.03	10.24	0.24
12.00 ± 0.05	12.41	0.41
16.00 ± 0.06	16.73	0.73
19.00 ± 0.06	19.49	0.49
20.00 ± 0.06	20.67	0.67
21.00 ± 0.08	21.26	0.26
22.00 ± 0.08	21.85	0.15
23.00 ± 0.08	22.64	0.36
25.00 ± 0.08	24.40	0.60
26.00 ± 0.09	25.19	0.81
	Average absolute deviation	0.4

Values for lead (ppm) from the above equation subject to ± 0.4 ppm

APPENDIX A-5

Determination of the Linear Range of Cadmium in Aqueous Base Solutions by Flame Atomic Absorption Spectrophotometry

General operating conditions

Instrument:-	Perkin-Elmer Model 503 Atomic Absorption Spectrophotometer
Radiation source:-	Cadmium hollow cathode lamp
Wavelength:-	228.2 nm
Slit:-	0.7 nm
Background correction:-	D_2 beam corrector
Readout:-	3-seconds integration
Burner:-	4-inch, single slot
Fuel:-	Acetylene - 20 gauge units (7 lit/min.)
Oxidant:-	Air - 40 gauge units (14 lit/min.)
Water:-	Glass-distilled
Sample size:-	Continuous flow

Preparation of aqueous-base standard solutions

1. All standard solutions are prepared from a 1000.0 ± 0.2 ppm cadmium solution supplied by Fisher Scientific Company Limited as Certified Fisher Standard Stock Solution of Cadmium.

2. Dilution of the standard stock solution was carried out as follows:-

   (a) 25.00 ± 0.02 ml of 1000.0 ± 0.2 ppm Cd solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 100.0 ± 0.1 ppm cadmium

(b) 25.00 ± 0.02 ml of 100.0 ± 0.1 ppm Cd solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

Value:- 10.00 ± 0.02 ppm cadmium

3. The following indicates the preparation procedures for the cadmium working standard solutions. All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Solution preparation	Cadmium (ppm)
0.50 ± 0.02 ml of 10.00 ± 0.02 ppm Cd	0.050 ± 0.002
1.00 ± 0.02 ml " " " "	0.100 ± 0.002
5.00 ± 0.02 ml " " " "	0.500 ± 0.003
10.00 ± 0.02 ml " " " "	1.000 ± 0.004
1.20 ± 0.02 ml of 100.0 ± 0.1 " "	1.20 ± 0.02
1.40 ± 0.02 ml " " " "	1.40 ± 0.02
1.60 ± 0.02 ml " " " "	1.60 ± 0.02
1.80 ± 0.02 ml " " " "	1.80 ± 0.02
2.00 ± 0.02 ml " " " "	2.00 ± 0.02
2.20 ± 0.02 ml " " " "	2.20 ± 0.02
2.40 ± 0.02 ml " " " "	2.40 ± 0.02
2.60 ± 0.02 ml " " " "	2.60 ± 0.02
3.00 ± 0.02 ml " " " "	3.00 ± 0.02
4.00 ± 0.02 ml " " " "	4.00 ± 0.02

Testing of aqueous-base standard solutions

The set of working standards gave the following results:-

Cadmium (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.050 ± 0.002	0.006 ± 0.000
0.100 ± 0.002	0.011 ± 0.000
0.500 ± 0.003	0.049 ± 0.000
1.000 ± 0.004	0.097 ± 0.000
1.20 ± 0.02	0.115 ± 0.000
1.40 ± 0.02	0.131 ± 0.002
1.60 ± 0.02	0.147 ± 0.000
1.80 ± 0.02	0.165 ± 0.001
2.00 ± 0.02	0.181 ± 0.000
2.20 ± 0.02	0.194 ± 0.001
2.40 ± 0.02	0.211 ± 0.000
2.60 ± 0.02	0.225 ± 0.001
3.00 ± 0.02	0.254 ± 0.000
4.00 ± 0.02	0.316 ± 0.000

* All averages result of three 3-seconds integration readings.
± values represent standard deviations.
Note:- average standard deviation $< \pm 0.001$

Linear regression analysis

Concentrations included cadmium (ppm)	Linear regression* data
0.00 - 4.00	r = 0.9961 b = 0.010 m = 0.08150
0.00 - 3.00	r = 0.9984 b = 0.006 m = 0.08569
0.00 - 2.60	r = 0.9987 b = 0.005 m = 0.08725
0.00 - 2.40	r = 0.9989 b = 0.004 m = 0.08840
0.00 - 2.20	r = 0.9990 b = 0.003 m = 0.08943
0.00 - 2.00	r = 0.9994 b = 0.002 m = 0.09088

* r = correlation coefficient
b = intercept
m = slope

From the linear regression results for different ranges and the plotted results on Figure A-5, a linear range to 2.00 ppm can be noted.

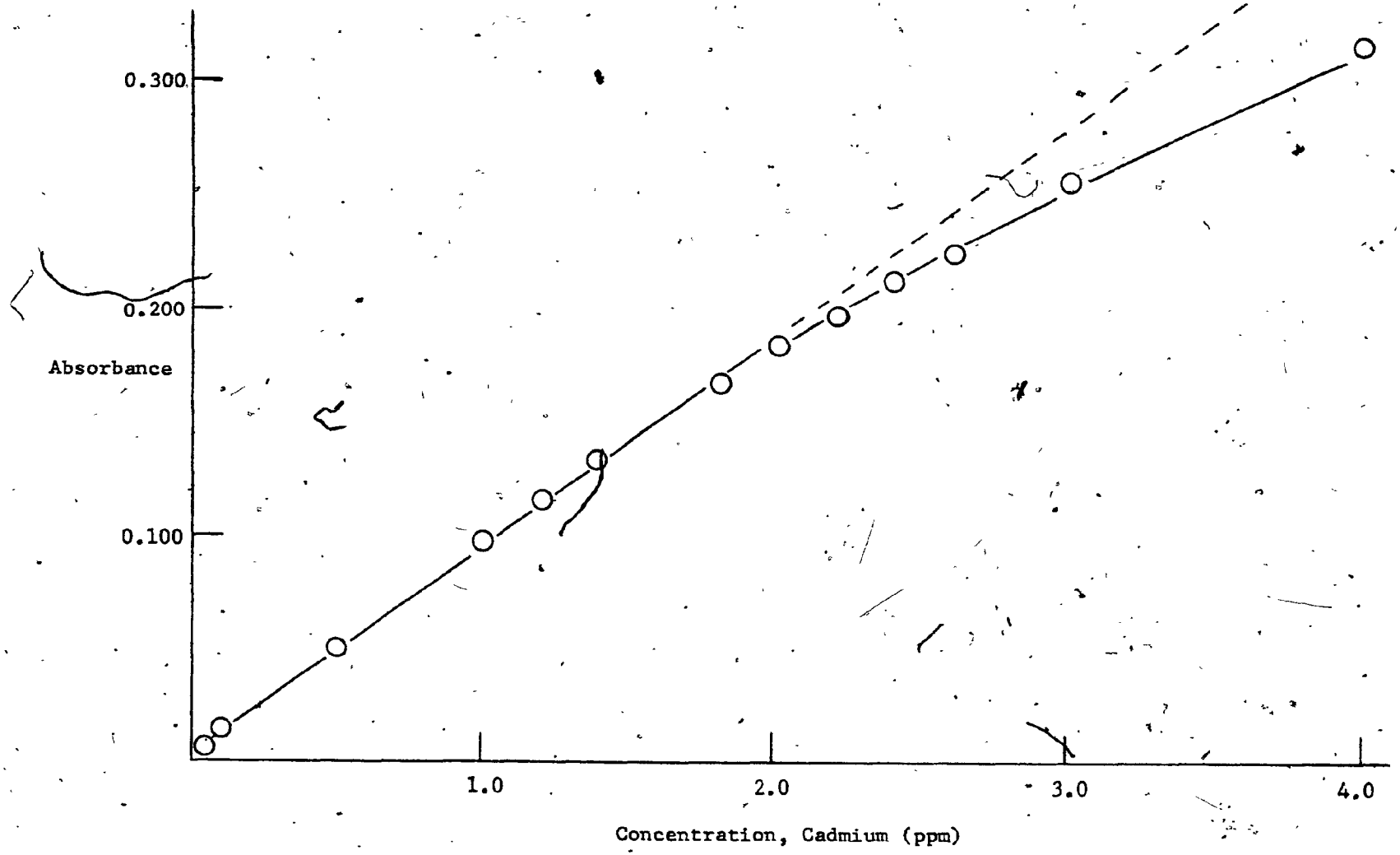


FIGURE A-5 ATOMIC ABSORPTION FLAME TECHNIQUE - CALIBRATION CURVE FOR CADMIUM

Therefore:-

Linear range regression analysis,

0.00 - 2.00 r = 0.9994
b = 0.002
m = 0.09088

Sensitivity (1% absorption) = 0.0044/0.09088 = 0.04^8 ppm Cd

Reverse calculations for cadmium

Actual cadmium (ppm)	Calculated cadmium (ppm)	Absolute devn.
0.000	-0.022	0.022
0.050 ± 0.002	0.044	0.006
0.100 ± 0.002	0.099	0.001
0.500 ± 0.003	0.517	0.017
1.000 ± 0.004	1.045	0.045
1.20 ± 0.02	1.243	0.043
1.40 ± 0.02	1.419	0.019
1.60 ± 0.02	1.596	0.004
1.80 ± 0.02	1.794	0.006
2.00 ± 0.02	1.970	0.030
	Average absolute deviation	0.019

Values of cadmium (ppm) from the above equation subject to $\pm 0.01^9$ ppm

APPENDIX A-6

Determination of the Linear Range of Vanadium in Aqueous Base Solutions by Flame Atomic Absorption Spectrophotometry

General operating conditions

Instrument:-	Perkin-Elmer Model 503 Atomic Absorption Spectrophotometer
Radiation source:-	Vanadium hollow cathode lamp
Wavelength:-	318 nm
Slit:-	0.7 nm
Background correction:-	D_2 beam corrector
Readout:-	3-seconds integration
Burner:-	2-inch, single slot
Fuel:-	Acetylene - 55 gauge units (19 lit/min.)
Oxidant:-	Nitrous Oxide - 35 gauge units (12 lit/min.)
Water:-	Glass-distilled
Sample size:-	Continuous flow

Preparation of aqueous-base standard solutions

1. All standard solutions are prepared from a 1000.0 ± 0.2 ppm vanadium solution supplied by Fisher Scientific Company Limited as Certified Fisher Standard Stock Solution of Vanadium.
2. Dilution of the standard stock solution was carried out as follows:-

   (a) 25.00 ± 0.02 ml of 1000.0 ± 0.2 ppm V solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 100.0 ± 0.1 ppm vanadium

3. The following indicates the preparation procedures for the vanadium working standard solutions. All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Solution preparation				Vanadium (ppm)
25.00 ± 0.02 ml	of	100.0 ± 0.1	ppm V	25.00 ± 0.04
50.00 ± 0.02 ml	"	"	" "	50.00 ± 0.07
7.50 ± 0.02 ml	"	1000.0 ± 0.2	" "	75.0 ± 0.2
10.00 ± 0.02 ml	"	"	" "	100.0 ± 0.2
12.00 ± 0.04 ml	"	"	" "	120.0 ± 0.4
13.00 ± 0.04 ml	"	"	" "	130.0 ± 0.4
14.00 ± 0.04 ml	"	"	" "	140.0 ± 0.4
15.00 ± 0.04 ml	"	"	" "	150.0 ± 0.4
16.00 ± 0.04 ml	"	"	" "	160.0 ± 0.4
17.50 ± 0.04 ml	"	"	" "	175.0 ± 0.4
20.00 ± 0.02 ml	"	"	" "	200.0 ± 0.2
25.00 ± 0.02 ml	"	"	" "	250.0 ± 0.2

Testing of aqueous-base standard solutions

The set of working standards gave the following results:-

Vanadium (ppm)	Average absorbance*
0.00	0.000 ± 0.000
25.00 ± 0.04	0.058 ± 0.000
50.00 ± 0.07	0.108 ± 0.000
75.0 ± 0.2	0.159 ± 0.000
100.0 ± 0.2	0.209 ± 0.000
120.0 ± 0.4	0.256 ± 0.001
130.0 ± 0.4	0.278 ± 0.000
140.0 ± 0.4	0.298 ± 0.000
150.0 ± 0.4	0.320 ± 0.000
160.0 ± 0.4	0.330 ± 0.001
175.0 ± 0.4	0.360 ± 0.000
200.0 ± 0.2	0.405 ± 0.002
250.0 ± 0.2	0.493 ± 0.002

* All averages result of three 3-seconds integration readings. ± values represent standard deviations.

Note:- average standard deviation $< \pm 0.001$

Linear regression analysis

Concentrations included vanadium (ppm)	Linear regression* data
0.0 - 250.0	r = 0.9986 b = 0.010 m = 0.001992
0.0 - 200.0	r = 0.9991 b = 0.006 m = 0.002044
0.00 - 175.0	r = 0.9994 b = 0.004 m = 0.002072
0.00 - 160.0	r = 0.9995 b = 0.003 m = 0.002092
0.00 - 150.0	r = 0.9998 b = 0.002 m = 0.002116

* r = correlation coefficient
b = intercept
m = slope

From the linear regression results for different ranges and the plotted results on Figure A-6, a loss of linearity after the 150.0 ppm point can be noted.

Therefore:-

Linear range regression analysis,

0.0 - 150.0

r = 0.9998
b = 0.002
m = 0.002116

Sensitivity (1% absorption) = 0.0044/0.002116 = 2.1 ppm V

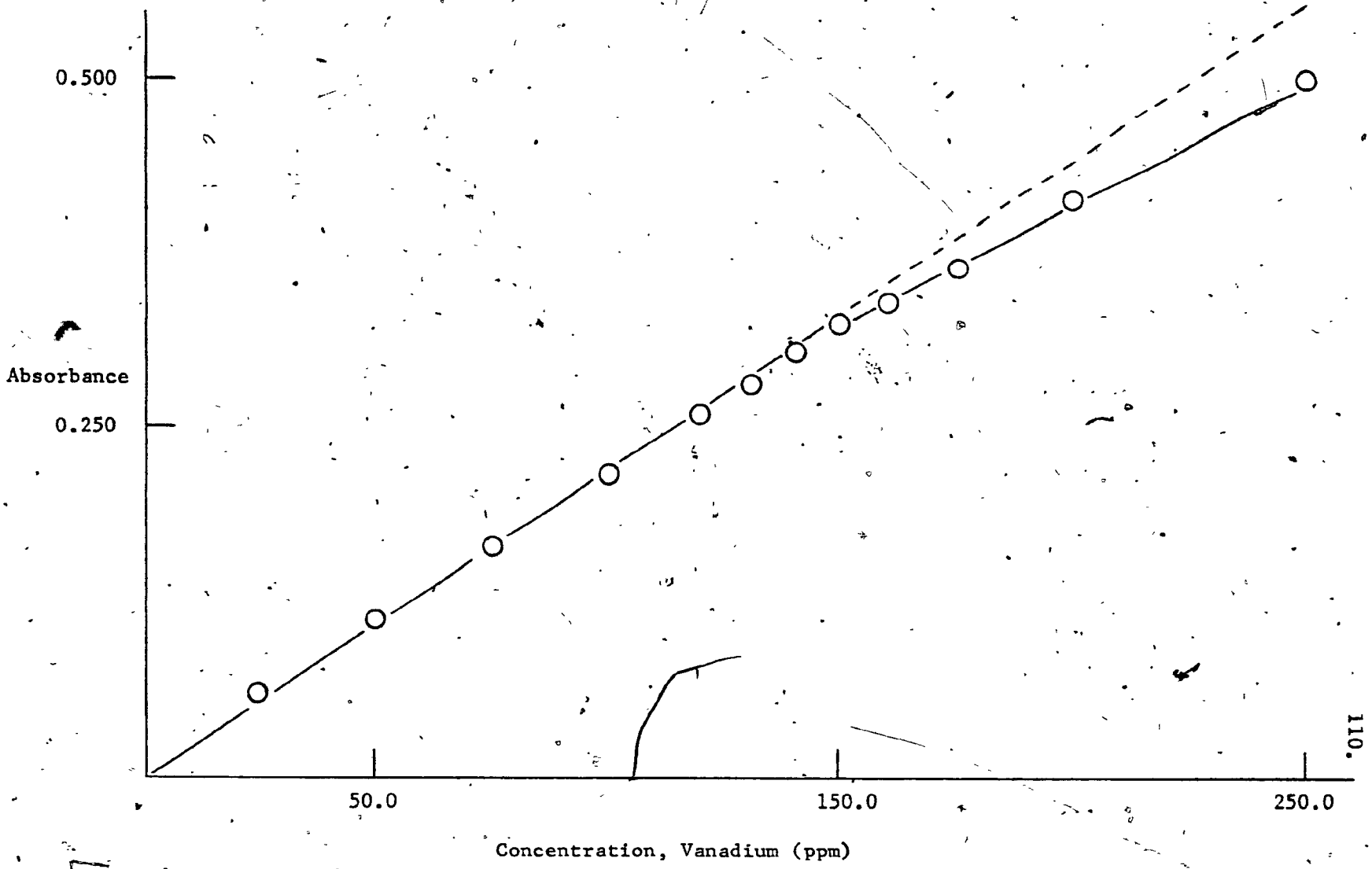


FIGURE A-6 ATOMIC ABSORPTION FLAME TECHNIQUE - CALIBRATION CURVE FOR VANADIUM

Reverse calculations for vanadium

Actual vanadium (ppm)	Calculated vanadium (ppm)	Absolute devn.
0.00	- 0.94	0.94
25.00 ± 0.04	26.46	1.46
50.00 ± 0.07	50.09	0.09
75.0 ± 0.2	74.2	0.8
100.0 ± 0.2	97.8	2.2
120.0 ± 0.4	120.0	0.0
130.0 ± 0.4	130.4	0.4
140.0 ± 0.4	139.9	0.1
150.0 ± 0.4	150.3	0.3
	Average absolute deviation	0.7

Values of vanadium (ppm) from the above equation subject to ± 0.7 ppm

APPENDIX B

APPENDIX B-1

Determination of Copper in Aqueous Base Solutions by Flameless Atomic Absorption Spectrophotometry

General operating conditions

Instrument:-	Perkin-Elmer Model 503 Atomic Absprption Spectrophotometer with HGA 2000 Graphite Furnace
Radiation source:-	Copper hollow cathode lamp
Wavelength:-	324.7 nm
Slit:-	0.7 nm
Background correction:-	D_2 beam corrector
Nitrogen flow:-	40 gauge units, normal mode (14 lit/min.)
Program:-	
Drying, temperature	120°C
time	20 sec
Charring, temperature	varied, 200 - 1700°C
time	30 sec
Atomizing, temperature	varied, 2000 - 2600°C
time	15 sec
Recorder:-	
Range	5 mV
Speed	10 mm/min
Readout:-	Peak integration mode
Water:-	Glass-distilled
Sample size:-	20 ul

Preparation of aqueous-base standard solutions

1. All standard solutions are prepared from a 1000.0 ± 0.2 ppm copper solution supplied by Fisher Scientific Company Limited

as Certified Fisher Standard Stock Solution of Copper.

2. Dilution of the standard stock solution was carried out as follows:-

(a) 25.00 ± 0.02 ml of 1000.0 ± 0.2 ppm copper solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

Value:- 100.0 ± 0.1 ppm copper

(b) 25.00 ± 0.02 ml of 100.0 ± 0.1 ppm copper solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

Value:- 10.00 ± 0.02 ppm copper

(c) 25.00 ± 0.02 ml of 10.00 ± 0.02 ppm copper solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

Value:- 1.00 ± 0.01 ppm copper

3. The following indicates the preparation procedures for the copper working standard solutions. All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Solution preparation				Copper (ppm)
0.50 ± 0.02 ml	of	1.00 ± 0.01	ppm Cu	0.0050 ± 0.0002
1.00 ± 0.02 ml	"	"	" "	0.0100 ± 0.0003
2.00 ± 0.02 ml	"	"	" "	0.0200 ± 0.0004
5.00 ± 0.02 ml	"	"	" "	0.0500 ± 0.0007
7.00 ± 0.02 ml	"	"	" "	0.0700 ± 0.0009
10.00 ± 0.02 ml	"	"	" "	0.100 ± 0.001
1.20 ± 0.02 ml	"	10.00 ± 0.02	" "	0.120 ± 0.002
1.50 ± 0.02 ml	"	"	" "	0.150 ± 0.002
1.80 ± 0.02 ml	"	"	" "	0.180 ± 0.002
2.00 ± 0.02 ml	"	"	" "	0.200 ± 0.002
2.20 ± 0.02 ml	"	"	" "	0.220 ± 0.002
2.50 ± 0.02 ml	"	"	" "	0.250 ± 0.002
3.00 ± 0.02 ml	"	"	" "	0.300 ± 0.003
3.50 ± 0.02 ml	"	"	" "	0.350 ± 0.003

Determination of optimum charring and atomizing temperatures

1. A standard aqueous solution containing 0.100 ± 0.001 ppm copper was prepared in order to establish the maximum possible charring temperature with no significant loss of copper. Repeated injections of this standard were carried out with varying charring temperatures.

The HGA program was set up as follows:-

	Drying	Charring	Atomizing
Temperature, °C	120	varied	2500
Time, sec	20	30	15

The following results were obtained:-

Charring temp., °C	Average* absorbance
200	0.175 ± 0.003
300	0.179 ± 0.001
400	0.175 ± 0.001
500	0.180 ± 0.001
600	0.176 ± 0.002
700	0.168 ± 0.002
800	0.172 ± 0.001
900	0.170 ± 0.002
1000	0.175 ± 0.002
1100	0.160 ± 0.002
1200	0.155 ± 0.001
1300	0.151 ± 0.001
1400	0.150 ± 0.002
1500	0.146 ± 0.001
1600	0.125 ± 0.000
1700	0.098 ± 0.001

* Average absorbance of three peak integration results and standard deviation

Figure B-1 shows the drop in absorbance after the 1100 °C point. It can be stated that the maximum allowable charring temperature for copper as analyzed under the general conditions given is 1100 °C.

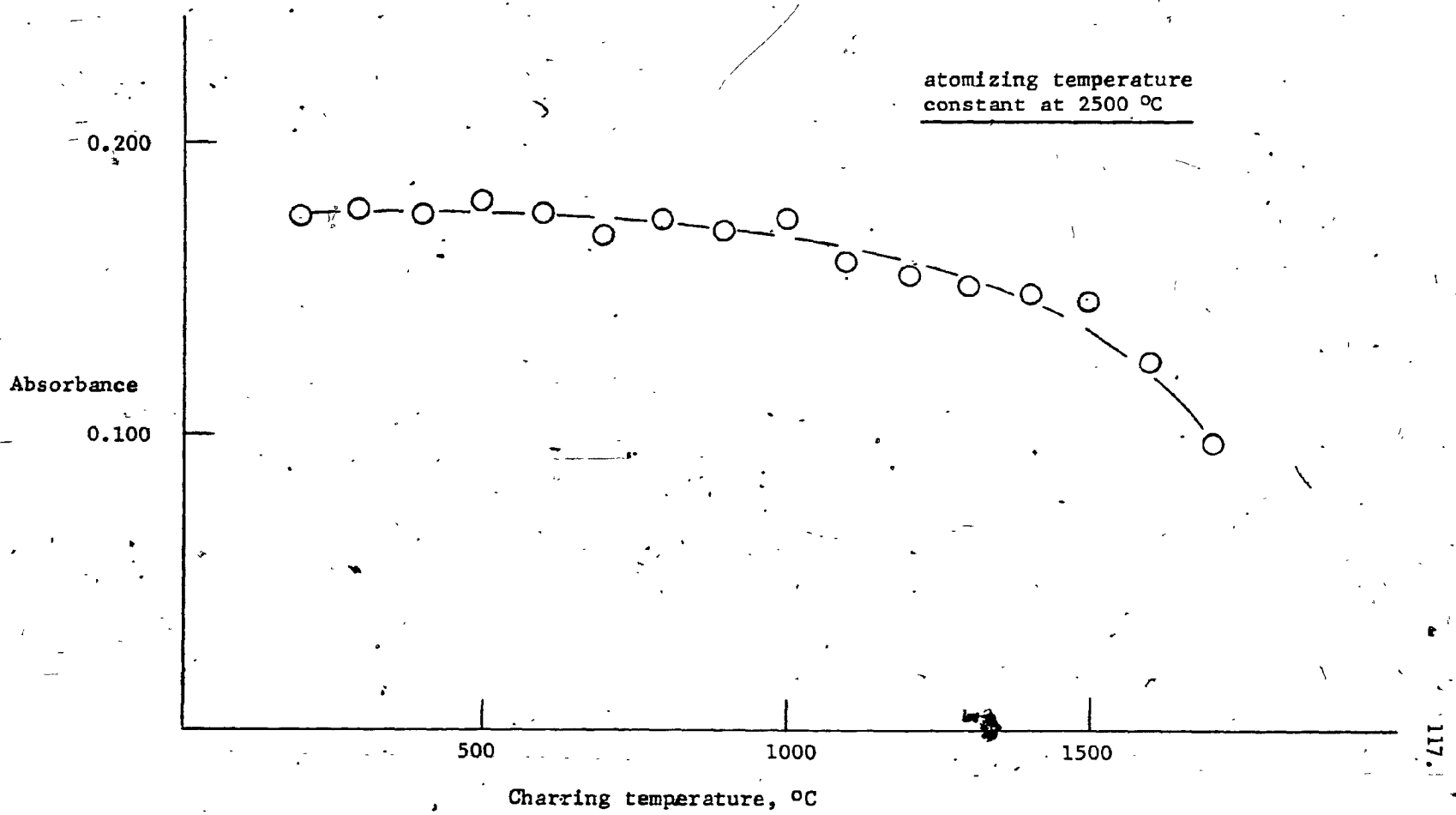


FIGURE B-1 GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE - MAXIMUM CHARRING TEMPERATURE, COPPER

A value of 750 °C was considered adequate for use in this investigation.

2. A standard aqueous solution containing 0.120 ± 0.002 ppm copper was prepared in order to establish the optimum atomizing temperature. Repeated injections of this standard were carried out with varying atomizing temperatures.

The HGA program was set up as follows:-

	Drying	Charring	Atomizing
Temperature, °C	120	750	varied
Time, sec	20	30	15

The following results were obtained:-

Atomizing temp., °C	Average* absorbance
2000	0.030 ± 0.000
2100	0.053 ± 0.002
2200	0.089 ± 0.001
2300	0.130 ± 0.002
2400	0.175 ± 0.003
2500	0.200 ± 0.003
2600	0.217 ± 0.003

* Average absorbance of three peak integration results and standard deviation

From the tabulated results above and Figure B-2, we can conclude that the optimum atomizing temperature was not quite attained. Under working conditions for the HGA 2000 furnace an atomizing temperature of 2500 °C can be set into the program with the expectation of satisfactory results.

Determination of the linear range for copper in aqueous base solutions

The general operating conditions remained the same, the HGA program was set up as follows:-

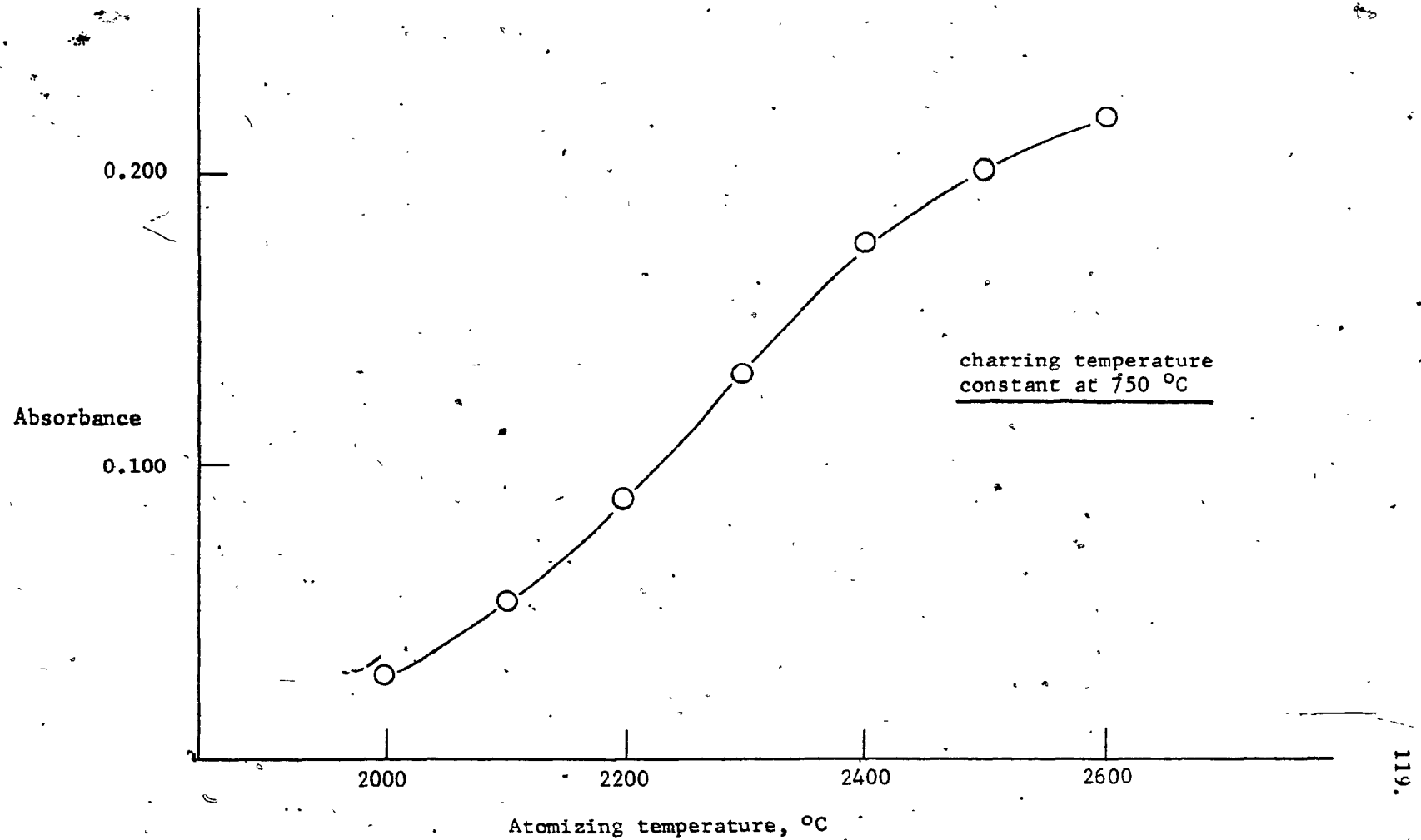


FIGURE B-2 GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE - OPTIMUM ATOMIZING TEMPERATURE, COPPER

	Drying	Charring	Atomizing
Temperature, °C	120	750	2500
Time, sec	20	30	15

The testing of the aqueous copper standards resulted in the following:-

Copper* (ppm)	Average** absorbance	Corrected average absorbance
0.000	0.001 ± 0.000	0.000 ± 0.000
0.0050 ± 0.0002	0.015 ± 0.000	0.014 ± 0.000
0.0100 ± 0.0003	0.021 ± 0.000	0.020 ± 0.000
0.0200 ± 0.0004	0.042 ± 0.001	0.041 ± 0.001
0.0500 ± 0.0007	0.086 ± 0.001	0.085 ± 0.001
0.0700 ± 0.0009	0.119 ± 0.002	0.118 ± 0.002
0.100 ± 0.001	0.172 ± 0.004	0.171 ± 0.004
0.120 ± 0.002	0.201 ± 0.001	0.200 ± 0.001
0.150 ± 0.002	0.253 ± 0.003	0.252 ± 0.003
0.180 ± 0.002	0.297 ± 0.003	0.296 ± 0.003
0.200 ± 0.002	0.331 ± 0.006	0.330 ± 0.006
0.220 ± 0.002	0.346 ± 0.004	0.345 ± 0.004
0.250 ± 0.002	0.389 ± 0.009	0.388 ± 0.009
0.300 ± 0.003	0.454 ± 0.005	0.453 ± 0.005
0.350 ± 0.003	0.52^{1} ± 0.01^{0}	0.52^{0} ± 0.01^{0}

* These results are plotted on Figure B-3

** Average of five peak integration readings with standard deviation

Note:- average standard deviation ± 0.003

Linear regression analysis:-

Concentrations included copper (ppm)	Linear regression* data
0.000 - 0.350	r = 0.9981 b = 0.013 m = 1.5014
0.000 - 0.300	r = 0.9984 b = 0.010 m = 1.5345

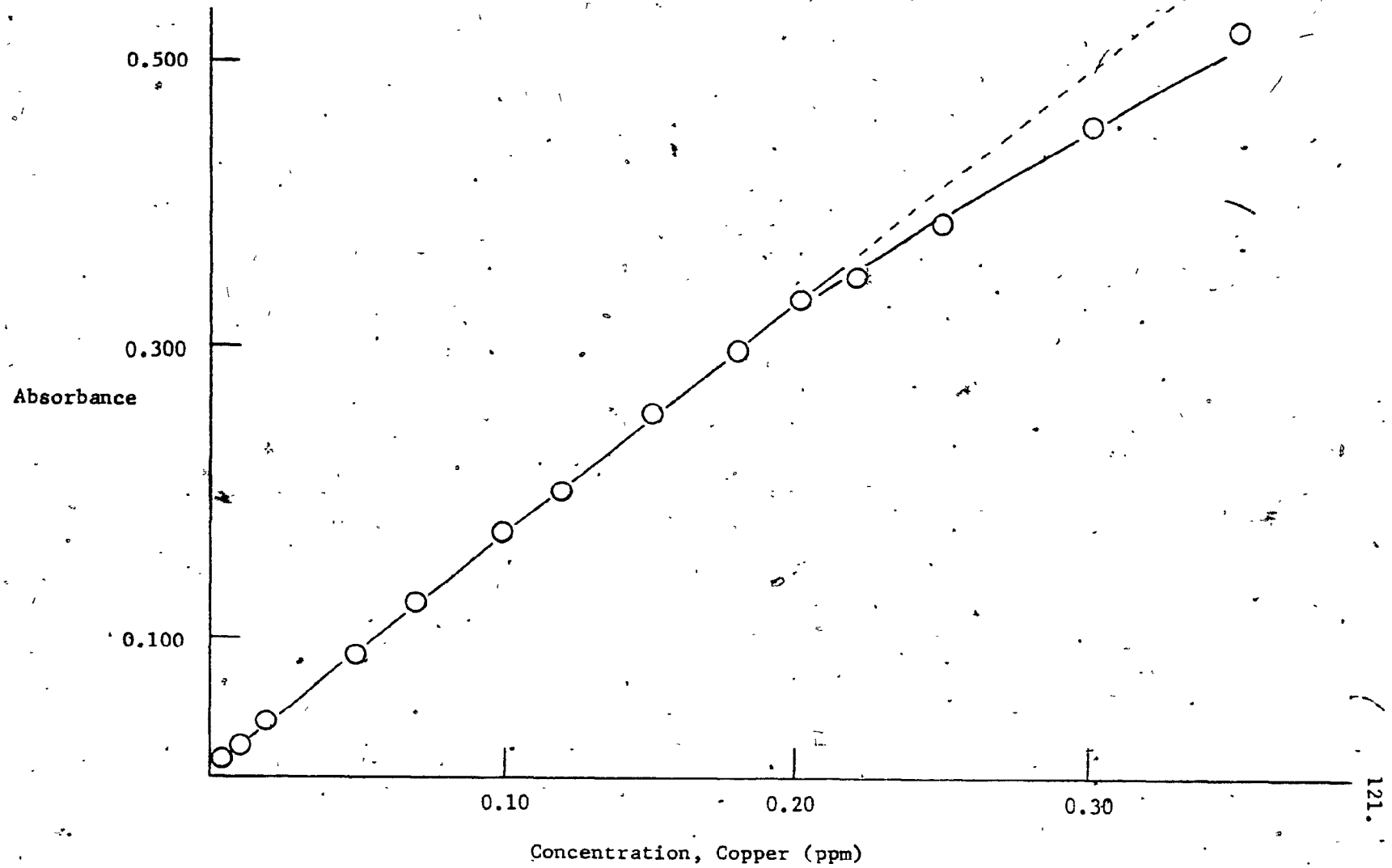


FIGURE B-3 GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE - CALIBRATION CURVE FOR COPPER

0.000 - 0.250	r = 0.9988 b = -0.008 m = 1.5712
0.000 - 0.220	r = 0.9992 b = 0.006 m = 1.5990

* r = correlation coefficient
b = intercept
m = slope

From the linear regression analysis and Figure B-3, a loss of linearity after the 0.220 ppm point can be noted. It can be assumed that the linear range for copper is 0.000 to 0.220 ppm.

Therefore:-

Linear regression analysis

0.000 - 0.220 ppm Cu	r = 0.9992 b = 0.006 m = 1.5990

Sensitivity (1% absorption) = 0.0044/1.5990 = 0.002^7 ppm Cu

Reverse calculations for copper using the above linear range equation are as follows:-

Actual copper (ppm)	Calculated copper (ppm)	Absolute devn.
0.000	-0.0038	0.0038
0.0050 ± 0.0002	0.0050	0.0000
0.0100 ± 0.0003	0.0088	0.0012
0.0200 ± 0.0004	0.0219	0.0019
0.0500 ± 0.0007	0.0494	0.0006
0.0700 ± 0.0009	0.0700	0.0000
0.100 ± 0.001	0.103	0.003
0.120 ± 0.002	0.121	0.001
0.150 ± 0.002	0.154	0.004
0.180 ± 0.002	0.181	0.001
0.200 ± 0.002	0.203	0.003
0.220 ± 0.002	0.212	0.008
	Average absolute deviation	0.002

Values of copper (ppm) from above equation subject to ± 0.002 ppm

APPENDIX B-2

Determination of Nickel in Aqueous Base Solutions by Flameless Atomic Absorption Spectrophotometry

General operating conditions

Instrument:-	Perkin-Elmer Model 503 Atomic Absorption Spectrophotometer with HGA 2000 Graphite Furnace
Radiation source:-	Nickel hollow cathode lamp
Wavelength:-	231.4 nm
Slit:-	0.2 nm
Background correction:-	D_2 beam corrector
Nitrogen flow:-	40 gauge units, normal mode (14 lit/min.)
Program:-	
Drying, temperature	120°C
time	20 sec
Charring, temperature	varied, 300 - 1800°C
time	30 sec
Atomizing, temperature	varied, 2000 - 2600°C
time	20 sec
Recorder:-	
Range	5 mV
Speed	20 mm/min
Readout:-	Peak integration mode
Water:-	Glass-distilled
Sample size:-	20 ul

Preparation of aqueous-base standard solutions

1. All standard solutions are prepared from a 1000.0 ± 0.2 ppm nickel solution supplied by Fisher Scientific Company Limited as Certified Fisher Standard Stock Solution of Nickel.

2. Dilution of the standard stock solution was carried out as follows:-

   (a) 25.00 ± 0.02 ml of 1000.0 ± 0.2 ppm nickel solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 100.0 ± 0.1 ppm nickel

   (b) 25.00 ± 0.02 ml of 100.0 ± 0.1 ppm nickel solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 10.00 ± 0.02 ppm nickel

3. The following indicates the preparation procedures for the nickel working standard solutions. All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Solution preparation				Nickel (ppm)
0.80 ± 0.02 ml of	10.00 ± 0.02	ppm	Ni	0.080 ± 0.002
1.00 ± 0.02 ml	"	"	"	0.100 ± 0.002
2.00 ± 0.02 ml	"	"	"	0.200 ± 0.002
2.50 ± 0.02 ml	"	"	"	0.250 ± 0.003
4.00 ± 0.02 ml	"	"	"	0.400 ± 0.003
5.00 ± 0.02 ml	"	"	"	0.500 ± 0.003
6.00 ± 0.02 ml	"	"	"	0.600 ± 0.003
8.00 ± 0.02 ml	"	"	"	0.800 ± 0.004
9.00 ± 0.02 ml	"	"	"	0.900 ± 0.004
10.00 ± 0.02 ml	"	"	"	1.000 ± 0.004
1.10 ± 0.02 ml	" 100.0 ± 0.1	"	"	1.10 ± 0.02
1.20 ± 0.02 ml	"	"	"	1.20 ± 0.02
1.30 ± 0.02 ml	"	"	"	1.30 ± 0.02
1.40 ± 0.02 ml	"	"	"	1.40 ± 0.02

Determination of optimum charring and atomizing temperatures

1. A standard aqueous solution containing 0.800 ± 0.004 ppm nickel was prepared in order to establish the maximum possible charring temperature with no significant loss of nickel. Repeated injections of this standard were carried out with varying charring temperatures.

   The HGA program was set up as follows:-

	Drying	Charring	Atomizing
Temperature, °C	120	varied	2500
Time, sec	20	30	20

   The following results were obtained:-

Charring temp., °C	Average* absorbance
300	0.190 ± 0.003
500	0.189 ± 0.004
700	0.184 ± 0.001
800	0.194 ± 0.001
900	0.190 ± 0.002
1000	0.188 ± 0.001
1100	0.190 ± 0.004
1200	0.190 ± 0.001
1300	0.191 ± 0.003
1400	0.187 ± 0.003
1500	0.187 ± 0.002
1600	0.178 ± 0.003
1700	0.160 ± 0.001
1800	0.138 ± 0.002

* Average absorbance of three peak integration results and standard deviation

Figure B-4 shows a drop in absorbance after the 1500 °C point. It can be stated that the maximum allowable temperature for nickel as analyzed under the general conditions given is 1500 °C. A value of 800 °C was considered adequate for use in this investigation.

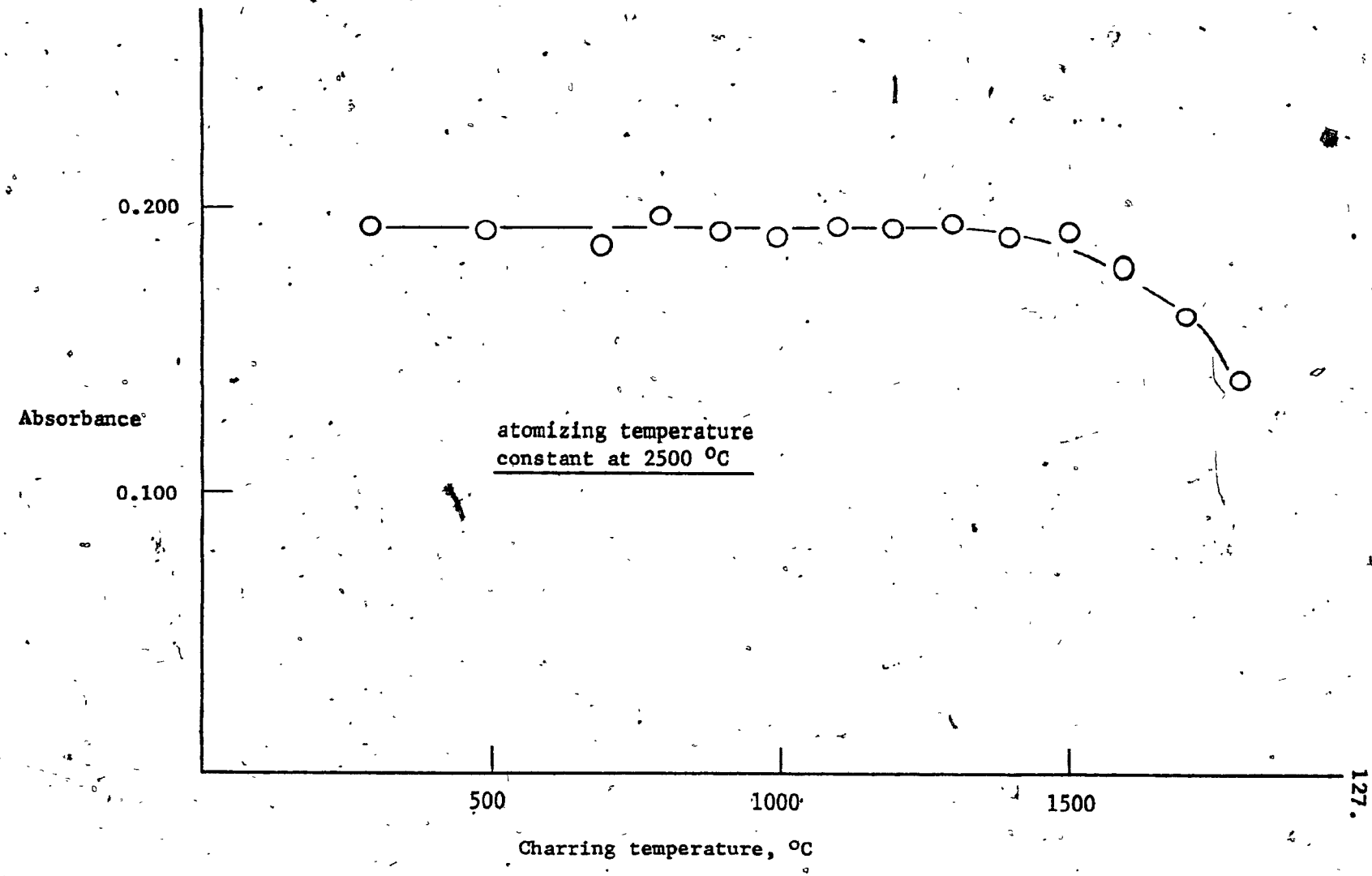


FIGURE B-4 GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE - MAXIMUM CHARRING TEMPERATURE, NICKEL

7. A standard aqueous solution containing 0.800 ± 0.004 ppm nickel was prepared in order to establish the optimum atomizing temperature. Repeated injections of this standard were carried out with varying atomizing temperatures.

The HGA program was set up as follows:-

	Drying	Charring	Atomizing
Temperature, °C	120	800	varied
Time, sec	20	30	20

The following results were obtained:-

Atomizing temp., °C	Average* absorbance
2000	0.043 ± 0.002
2100	0.076 ± 0.003
2200	0.102 ± 0.003
2350	0.164 ± 0.001
2500	0.240 ± 0.004
2600	0.288 ± 0.008

* Average absorbance of three peak integration results and standard deviation

From the tabulated results above and Figure B-5, it can be concluded that the optimum atomizing temperature was not attained. Under working conditions for the HGA 2000 furnace an atomizing temperature of 2500 °C can be set into the program with the expectation of satisfactory results.

Determination of the linear range for nickel in aqueous base solutions

The general operating conditions remained the same, the HGA program was set up as follows:-

	Drying	Charring	Atomizing
Temperature, °C	120	800	2500
Time, sec	20	30	20

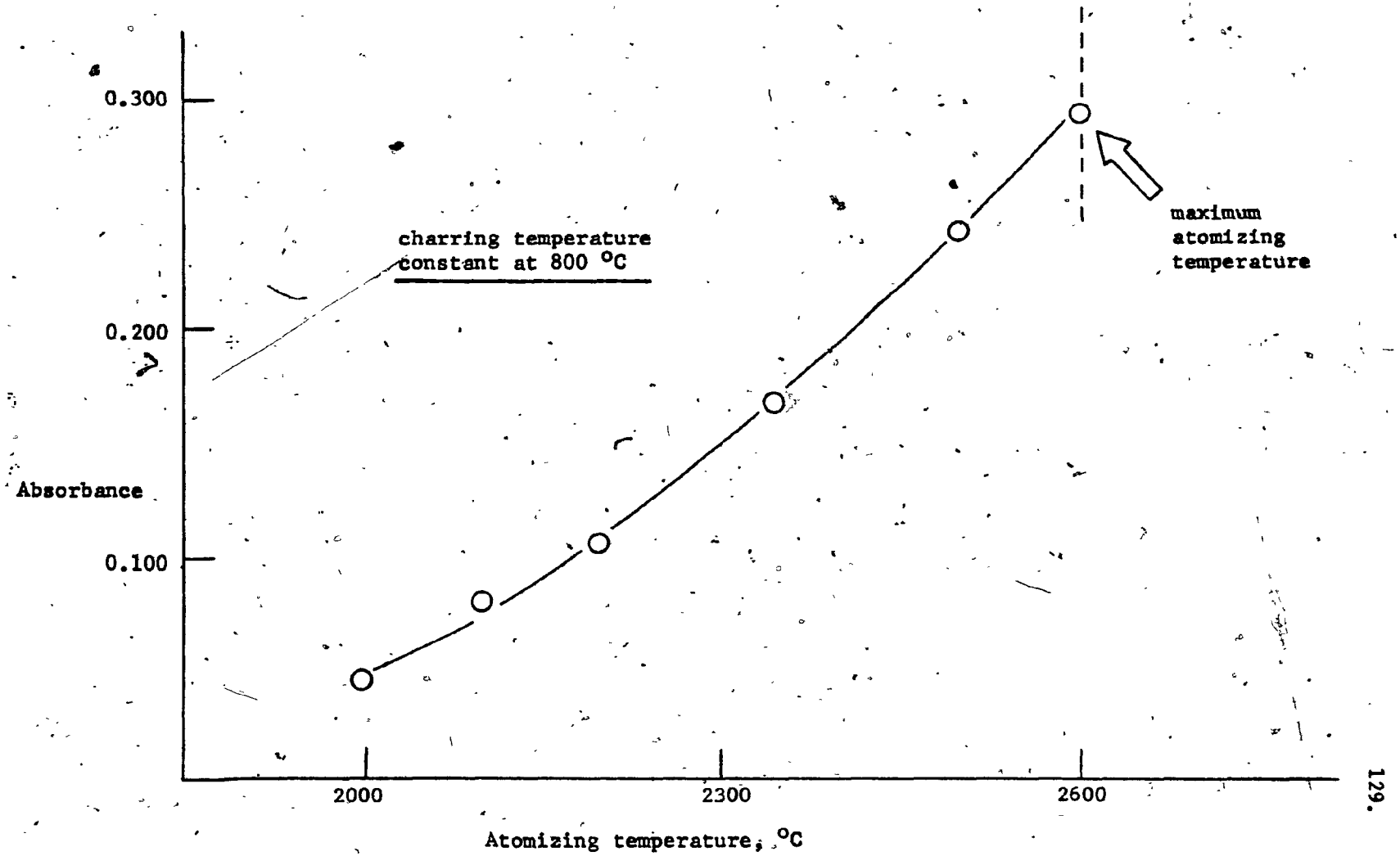


FIGURE B-5 GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE - OPTIMUM ATOMIZING TEMPERATURE, NICKEL

The testing of the aqueous nickel standards resulted in the following:-

Nickel* (ppm)	Average** absorbance	Corrected average absorbance
0.000	0.012 ± 0.000	0.000 ± 0.000
0.080 ± 0.002	0.029 ± 0.001	0.017 ± 0.001
0.100 ± 0.002	0.036 ± 0.001	0.024 ± 0.001
0.200 ± 0.002	0.065 ± 0.002	0.053 ± 0.002
0.250 ± 0.003	0.074 ± 0.003	0.062 ± 0.003
0.400 ± 0.003	0.112 ± 0.006	0.100 ± 0.006
0.500 ± 0.003	0.144 ± 0.003	0.132 ± 0.003
0.600 ± 0.003	0.166 ± 0.002	0.154 ± 0.002
0.800 ± 0.004	0.221 ± 0.003	0.209 ± 0.003
0.900 ± 0.004	0.247 ± 0.004	0.235 ± 0.004
1.000 ± 0.004	0.267 ± 0.005	0.255 ± 0.005
1.10 ± 0.02	0.290 ± 0.004	0.278 ± 0.004
1.20 ± 0.02	0.31^{0} ± 0.01^{0}	0.29^{8} ± 0.01^{0}
1.30 ± 0.02	0.327 ± 0.006	0.315 ± 0.006
1.40 ± 0.02	0.342 ± 0.004	0.330 ± 0.004

* These results are plotted on Figure B-6

** Average of five peak integration readings with standard deviation

Note:- average standard deviation ± 0.002

Linear regression analysis:-

Concentrations included nickel (ppm)	Linear regression* data
0.000 - 1.40	r = 0.9934 b = 0.006 m = 0.2380^{6}
0.000 - 1.30	r = 0.9981 b = 0.003^{5} m = 0.2450^{5}
0.000 - 1.20	r = 0.9994 b = 0.000^{2} m = 0.2544

* r = correlation coefficient
b = intercept
m = slope

From the linear regression analysis and Figure B-6, it is noted that linearity is lost between 1.20 and 1.30 ppm.

Therefore:-

Linear regression analysis

0.000 - 1.20 ppm Ni

r = 0.9994
b = 0.000^2
m = 0.2544

Sensitivity (1% absorption) = 0.0044/0.2544 = 0.01^7 ppm Ni

Reverse calculations for nickel using the above linear range equation are as follows:-

Actual nickel (ppm)	Calculated nickel (ppm)	Absolute devn.
0.000	0.000	0.000
0.080 ± 0.002	0.067	0.013
0.100 ± 0.002	0.094	0.006
0.200 ± 0.002	0.208	0.008
0.250 ± 0.003	0.244	0.006
0.400 ± 0.003	0.393	0.007
0.500 ± 0.003	0.519	0.019
0.600 ± 0.003	0.605	0.005
0.800 ± 0.004	0.822	0.022
0.900 ± 0.004	0.924	0.024
1.000 ± 0.004	1.002	0.002
1.10 ± 0.02	1.093	0.007
1.20 ± 0.02	1.171	0.029
	Average absolute deviation	0.009

Values of nickel (ppm) from the above equation subject to ± 0.009 ppm

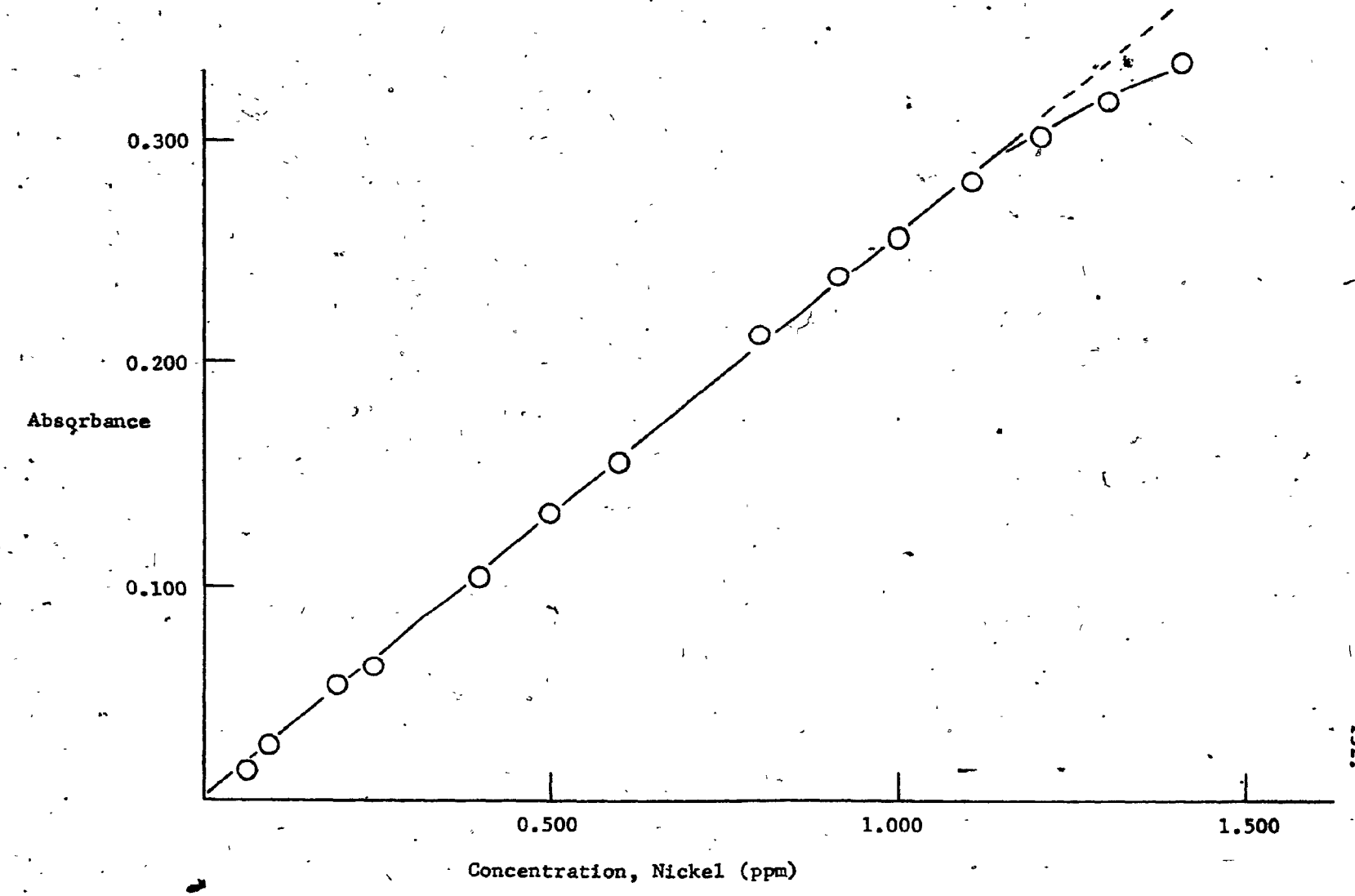


FIGURE B-6 GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE - CALIBRATION CURVE FOR NICKEL

APPENDIX B-3

Determination of Lead in Aqueous Base Solutions by Flameless Atomic Absorption Spectrophotometry

General operating conditions

Instrument:-	Perkin-Elmer Model 503 Atomic Absorption Spectrophotometer with HGA 2100 Graphite Furnace
Radiation source:-	Lead hollow cathode lamp
Wavelength:-	282.6 nm
Slit:-	0.7 nm
Background correction:-	D_2 beam corrector
Nitrogen flow:-	40 gauge units, normal mode (14 lit/min.)
Flush system:-	Auto-high temperature
Program:-	
Drying, temperature	120°C
time	20 sec
Charring, temperature	varied, 200 - 1400°C
time	30 sec
Atomizing, temperature	varied, 1700 - 2600°C
time	10 sec
Recorder:-	
Range	5 mV
Speed	20 mm/min
Readout:-	Peak integration mode
Water:-	Glass-distilled
Sample size:-	20 ul

Preparation of aqueous-base standard solutions

1. All standard solutions are prepared from a 1000.0 ± 0.2 ppm lead solution supplied by Fisher Scientific Company Limited as Certified Fisher Standard Stock Solution of Lead.

2. Dilution of the standard stock solution was carried out as follows:-

   (a) 25.00 ± 0.02 ml of 1000.0 ± 0.2 ppm lead solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 100.0 ± 0.1 ppm lead

   (b) 25.00 ± 0.02 ml of 100.0 ± 0.1 ppm lead solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 10.00 ± 0.02 ppm lead

   (c) 25.00 ± 0.02 ml of 10.00 ± 0.02 ppm lead solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 1.00 ± 0.01 ppm lead

   (d) 25.00 ± 0.02 ml of 1.00 ± 0.01 ppm lead solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 0.100 ± 0.001 ppm lead

3. The following indicates the preparation procedures for the lead working standard solutions. All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Solution preparation				Lead (ppm)
1.00 ± 0.02 ml	of	0.100 ± 0.001	ppm Pb	0.0010 ± 0.0001
5.00 ± 0.02 ml	"	"	" "	0.0050 ± 0.0001
10.00 ± 0.02 ml	"	"	" "	0.0100 ± 0.0001
1.50 ± 0.02 ml	"	1.00 ± 0.01	" "	0.0150 ± 0.0004
2.00 ± 0.02 ml	"	"	" "	0.0200 ± 0.0004
2.50 ± 0.02 ml	"	"	" "	0.0250 ± 0.0004
3.00 ± 0.02 ml	"	"	" "	0.0300 ± 0.0005
3.50 ± 0.02 ml	"	"	" "	0.0350 ± 0.0006

3.80 ± 0.02 ml	of	1.00 ± 0.01	ppm Pb	0.0380 ± 0.0006
3.90 ± 0.02 ml	"	"	" "	0.0390 ± 0.0006
4.00 ± 0.02 ml	"	"	" "	0.0400 ± 0.0006
4.10 ± 0.02 ml	"	"	" "	0.0410 ± 0.0006
4.20 ± 0.02 ml	"	"	" "	0.0420 ± 0.0006
4.40 ± 0.02 ml	"	"	" "	0.0440 ± 0.0006
4.80 ± 0.02 ml	"	"	" "	0.0480 ± 0.0007
5.00 ± 0.02 ml	"	"	" "	0.0500 ± 0.0007
5.20 ± 0.02 ml	"	"	" "	0.0520 ± 0.0007

Determination of optimum charring and atomizing temperatures

1. A standard aqueous solution containing 0.0300 ± 0.0005 ppm lead was prepared in order to establish the maximum possible charring temperature with no significant loss of lead. Repeated injections of this standard were carried out with varying charring temperatures.

The HGA program was set up as follows:-

	Drying	Charring	Atomizing
Temperature, °C	120	varied	2200
Time, sec	20	30	10

The following results were obtained:-

Charring temp., °C	Average* absorbance	Charring temp., °C	Average* absorbance
200	0.203 ± 0.002	900	0.199 ± 0.001
400	0.205 ± 0.001	1000	0.192 ± 0.002
500	0.210 ± 0.001	1100	0.180 ± 0.002
600	0.200 ± 0.002	1200	0.169 ± 0.001
700	0.199 ± 0.001	1300	0.154 ± 0.001
800	0.202 ± 0.003	1400	0.125 ± 0.001

* Average absorbance of three peak integration results and standard deviation

Figure B-7 shows a significant drop in absorbance after the 900 °C point. It can be assumed that the maximum allowable charring temperature is 900 °C. A charring temperature of 600 °C was considered adequate for use in this investigation.

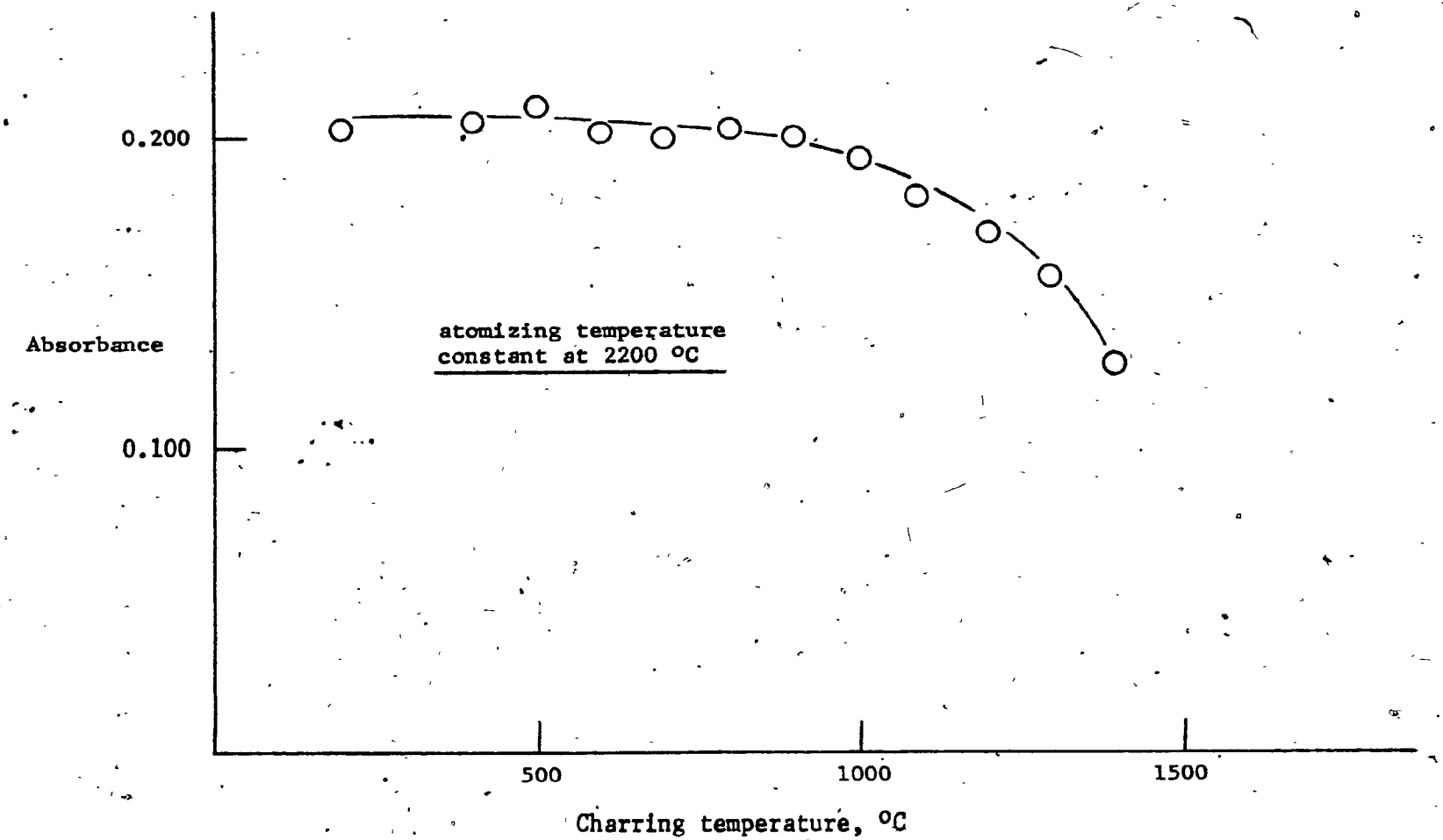


FIGURE B-7 GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE - MAXIMUM CHARRING TEMPERATURE, LEAD

2. A standard aqueous solution containing 0.0300 ± 0.0005 ppm lead was prepared in order to establish the optimum atomizing temperature. Repeated injections of this standard were carried out with varying atomizing temperatures.

The HGA program was set up as follows:-

	Drying	Charring	Atomizing
Temperature, °C	120	600	varied
Time, sec	20	30	10

The following results were obtained:-

Atomizing temp., °C	Average* absorbance
1800	0.082 ± 0.001
1900	0.153 ± 0.001
2000	0.193 ± 0.002
2100	0.216 ± 0.001
2200	0.220 ± 0.001
2300	0.223 ± 0.001
2400	0.228 ± 0.002
2500	0.232 ± 0.002
2600	0.228 ± 0.002

* Average absorbance of three peak integration results and standard deviation

From the tabulated results above and Figure B-8, the optimum atomizing temperature was noted to be in the 2100 to 2600 °C interval. Under working conditions, with the HGA 2100 furnace, an atomizing temperature of 2200 °C can be set into the program with expectation of satisfactory results.

Determination of the linear range for lead in aqueous base solutions

The general operating conditions remained the same, the HGA program was set up as follows:-

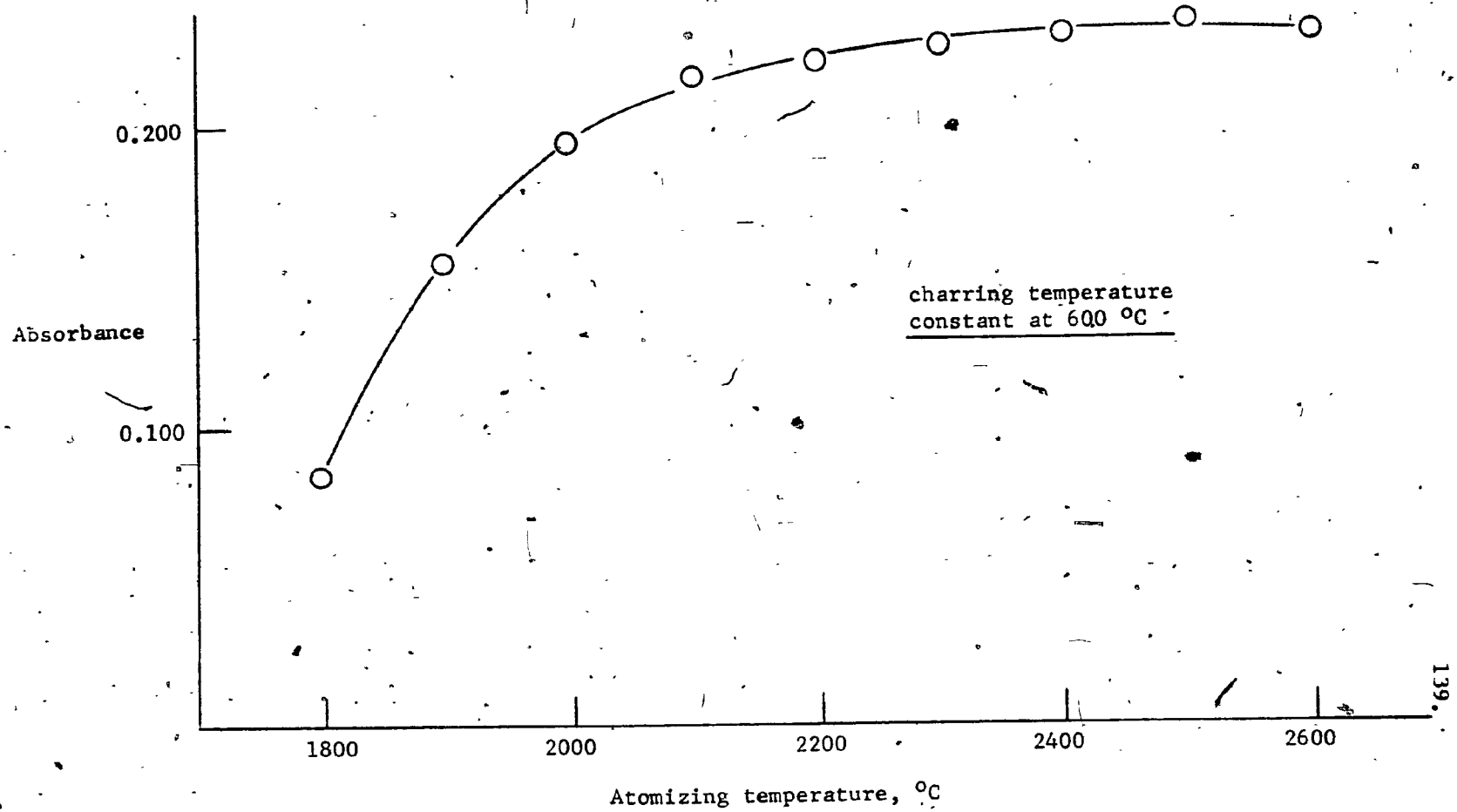


FIGURE B-8 GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE - OPTIMUM ATOMIZING TEMPERATURE, LEAD

	Drying	Charring	Atomizing
Temperature, °C	120	600	2200
Time, sec	20	30	10

The testing of the aqueous lead standards resulted in the following:-

Lead* (ppm)	Average** absorbance	Corrected average absorbance
0.0000	0.007 ± 0.000	0.000 ± 0.000
0.0010 ± 0.0001	0.015 ± 0.000	0.008 ± 0.000
0.0050 ± 0.0001	0.047 ± 0.001	0.040 ± 0.001
0.0100 ± 0.0001	0.076 ± 0.001	0.069 ± 0.001
0.0150 ± 0.0004	0.109 ± 0.000	0.102 ± 0.000
0.0200 ± 0.0004	0.145 ± 0.001	0.138 ± 0.001
0.0250 ± 0.0004	0.190 ± 0.002	0.183 ± 0.002
0.0300 ± 0.0005	0.226 ± 0.001	0.219 ± 0.001
0.0350 ± 0.0006	0.262 ± 0.002	0.255 ± 0.002
0.0380 ± 0.0006	0.282 ± 0.001	0.275 ± 0.001
0.0390 ± 0.0006	0.290 ± 0.000	0.283 ± 0.000
0.0400 ± 0.0006	0.292 ± 0.004	0.285 ± 0.004
0.0410 ± 0.0006	0.297 ± 0.003	0.290 ± 0.003
0.0420 ± 0.0006	0.304 ± 0.002	0.297 ± 0.002
0.0440 ± 0.0006	0.317 ± 0.002	0.310 ± 0.002
0.0480 ± 0.0007	0.342 ± 0.002	0.335 ± 0.002
0.0500 ± 0.0007	0.355 ± 0.002	0.348 ± 0.002
0.0520 ± 0.0007	0.364 ± 0.001	0.357 ± 0.001

* These results are plotted on Figure B-9

** Average of three peak integration readings with standard deviation

Note:- average standard deviation ± 0.001

Linear regression analysis:-

Concentrations included lead (ppm)	Linear regression* data
0.0000 - 0.0520	r = 0.9991 b = 0.0024 m = 7.010

0.0000 - 0.0500 ppm Pb

r = 0.9993
b = 0.0015
m = 7.062

* r = correlation coefficient
b = intercept
m = slope

From the linear regression analysis and Figure B-9, a deviation from linearity is noted at the 0.0500 ppm lead point.

Therefore:-

Linear regression analysis

0.0000 - 0.0500 ppm Pb

r = 0.9993
b = 0.0015
m = 7.062

Sensitivity (1% absorption) = 0.0044/7.062 = 0.0006 ppm Pb

Reverse calculations for lead using the above linear range equation are as follows:-

Actual lead (ppm)	Calculated lead (ppm)	Absolute devn.
0.0000	-0.0002	0.0002
0.0010 ± 0.0001	0.0009	0.0001
0.0050 ± 0.0001	0.0054	0.0004
0.0100 ± 0.0001	0.0096	0.0004
0.0150 ± 0.0004	0.0142	0.0008
0.0200 ± 0.0004	0.0193	0.0007
0.0250 ± 0.0004	0.0257	0.0007
0.0300 ± 0.0005	0.0308	0.0008
0.0350 ± 0.0006	0.0359	0.0009
0.0380 ± 0.0006	0.0387	0.0007
0.0390 ± 0.0006	0.0399	0.0009
0.0400 ± 0.0006	0.0401	0.0001
0.0410 ± 0.0006	0.0408	0.0002
0.0420 ± 0.0006	0.0418	0.0002
0.0440 ± 0.0006	0.0437	0.0003
0.0480 ± 0.0007	0.0472	0.0008
0.0500 ± 0.0007	0.0491	0.0009
	Average absolute deviation	0.0005

Values of lead (ppm) from the above equation subject to ± 0.0005 ppm

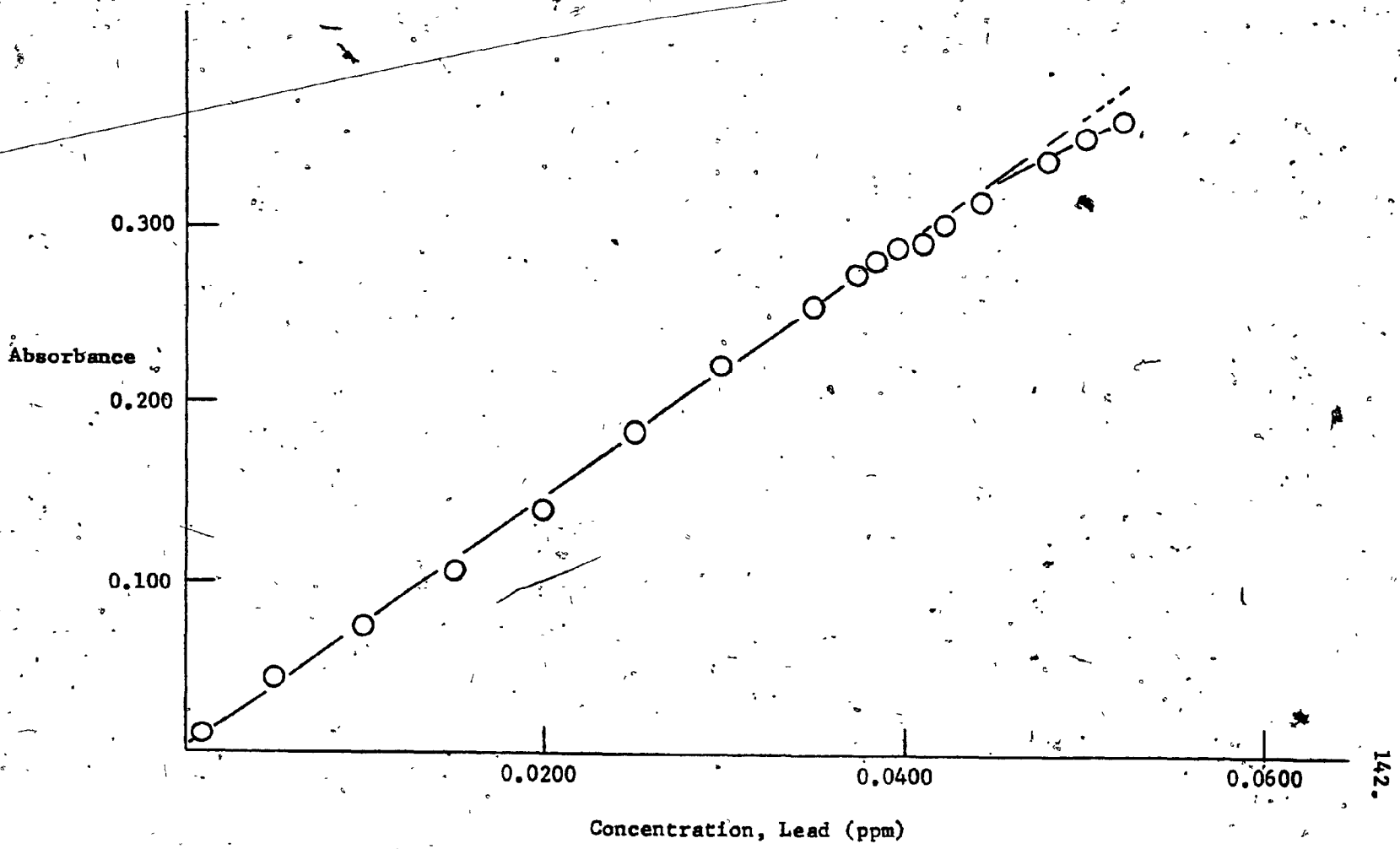


FIGURE B-9 GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE - CALIBRATION CURVE FOR LEAD

APPENDIX B-4

Determination of Cadmium in Aqueous Base Solutions by Flameless Atomic Absorption Spectrophotometry

General operating conditions

Instrument:-	Perkin-Elmer Model 503 Atomic Absorption Spectrophotometer with HGA 2100 Graphite Furnace
Radiation source:-	Cadmium hollow cathode lamp
Wavelength:-	228.2 nm
Slit:-	0.7 nm
Background correction	D_2 beam corrector
Nitrogen flow:-	40 gauge units, normal mode (14 lit/min.)
Flush system:-	Auto-high temperature
Program:-	
Drying, temperature	120 °C
time	20 sec
Charring, temperature	varied, 200 - 1400 °C
time	30 sec
Atomizing, temperature	varied, 1300 - 2600 °C
time	10 sec
Recorder:-	
Range	5 mV
Speed	20 mm/min
Readout:-	Peak integration mode
Water:-	Glass-distilled
Sample size:-	20 ul

Preparation of aqueous-base standard solutions

1. All standard solutions are prepared from a 1000.0 ± 0.2 ppm cadmium solution supplied by Fisher Scientific Company Limited as Certified Fisher Standard Stock Solution of Cadmium.

2. Dilution of the standard stock solution was carried out as follows:-

   (a) 25.00 ± 0.02 ml of 1000.0 ± 0.2 ppm cadmium solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 100.0 ± 0.1 ppm cadmium

   (b) 25.00 ± 0.02 ml of 100.0 ± 0.1 ppm cadmium solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 10.00 ± 0.02 ppm cadmium

   (c) 25.00 ± 0.02 ml of 10.00 ± 0.02 ppm cadmium solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 1.00 ± 0.01 ppm cadmium

   (d) 25.00 ± 0.02 ml of 1.00 ± 0.01 ppm cadmium solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 0.100 ± 0.001 ppm cadmium

   (e) 25.00 ± 0.02 ml of 0.100 ± 0.001 ppm cadmium solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 0.0100 ± 0.0001 ppm cadmium

3. The following indicates the preparation procedures for the cadmium working standard solutions. All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Solution preparation	Cadmium (ppm)
1.00 ± 0.02 ml of 0.0100 ± 0.0001 ppm Cd	0.00010 ± 0.00001
5.00 ± 0.02 ml " " " "	0.00050 ± 0.00001
7.50 ± 0.02 ml " " " "	0.00075 ± 0.00001
10.00 ± 0.02 ml " " " "	0.00100 ± 0.00001

1.50 ± 0.02 ml of	0.100 ± 0.001	ppm Cd	0.00150 ± 0.00004
2.00 ± 0.02 ml "	"	" "	0.00200 ± 0.00004
2.50 ± 0.02 ml "	"	" "	0.00250 ± 0.00004
2.75 ± 0.02 ml "	"	" "	0.00275 ± 0.00005
3.00 ± 0.02 ml "	"	" "	0.00300 ± 0.00005
3.25 ± 0.02 ml "	"	" "	0.00325 ± 0.00005
3.50 ± 0.02 ml "	"	" "	0.00350 ± 0.00006
4.00 ± 0.02 ml "	"	" "	0.00400 ± 0.00006

Determination of optimum charring and atomizing temperatures

1. A standard solution containing 0.00200 ± 0.00004 ppm cadmium was prepared in order to establish the maximum possible charring temperature with no significant loss of cadmium. Repeated injections of this aqueous standard were carried out with varying charring temperatures.

   The HGA program was set up as follows:-

	Drying	Charring	Atomizing
Temperature, °C	120	varied	2100
Time, sec	20	30	10

The following results were obtained:-

Charring temp., °C	Average* absorbance	Charring temp., °C	Average* absorbance
150	0.205 ± 0.003	600	0.208 ± 0.002
200	0.210 ± 0.001	700	0.190 ± 0.004
300	0.208 ± 0.001	800	0.175 ± 0.001
400	0.213 ± 0.002	1000	0.143 ± 0.001
500	0.203 ± 0.001	1200	0.050 ± 0.002

* Average absorbance of three peak integration results and standard deviation

Figure B-10 shows a significant drop in absorbance after the 600 °C point. It can be assumed that the maximum charring temperature is 600 °C. A value of 250 °C was considered adequate for use in this investigation.

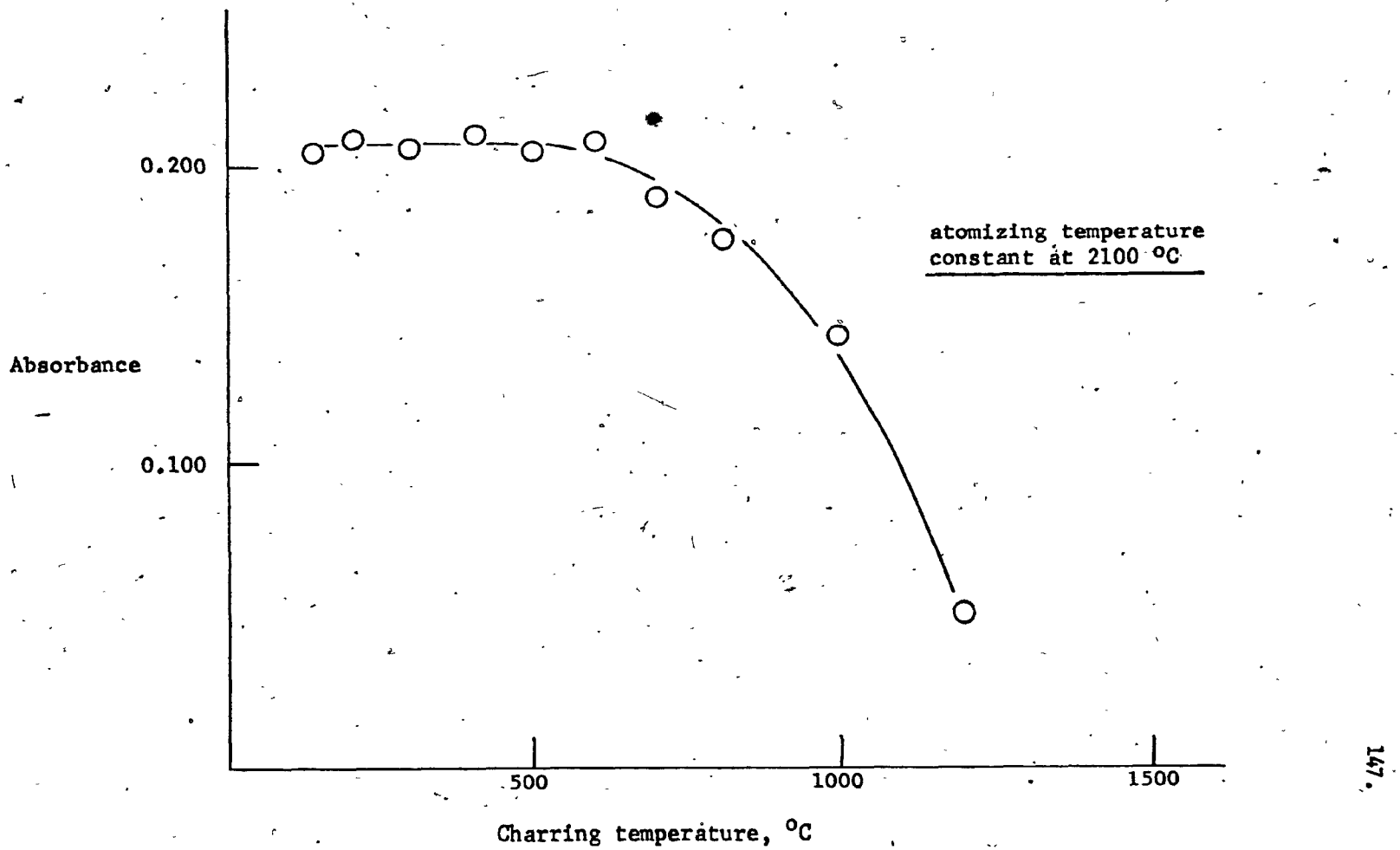


FIGURE B-10 GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE - MAXIMUM CHARRING TEMPERATURE, CADMIUM

2. A standard aqueous solution containing 0.00200 ± 0.00004 ppm cadmium was prepared in order to establish the optimum atomizing temperature. Repeated injections of this standard at different atomizing temperatures were carried out.

The HGA program was set up as follows:-

	Drying	Charring	Atomizing
Temperature, °C	120	250	varied
Time, sec	20	30	10

The following results were obtained:-

Atomizing temp., °C	Average* absorbance
1600	0.050 ± 0.002
1700	0.089 ± 0.001
1800	0.105 ± 0.003
1900	0.143 ± 0.003
2000	0.176 ± 0.004
2100	0.210 ± 0.002
2200	0.220 ± 0.004
2300	0.223 ± 0.005
2400	0.207 ± 0.003
2500	0.193 ± 0.006
2600	0.190 ± 0.004

* Averages of three peak integration absorbance results and standard deviations

From the tabulated results above and Figure B-11, the optimum atomizing temperature was noted in the range of 2100 - 2400 °C. Under working conditions, with the HGA 2100 furnace, an atomizing temperature of 2100 °C can be set into the program with the expectation of satisfactory results.

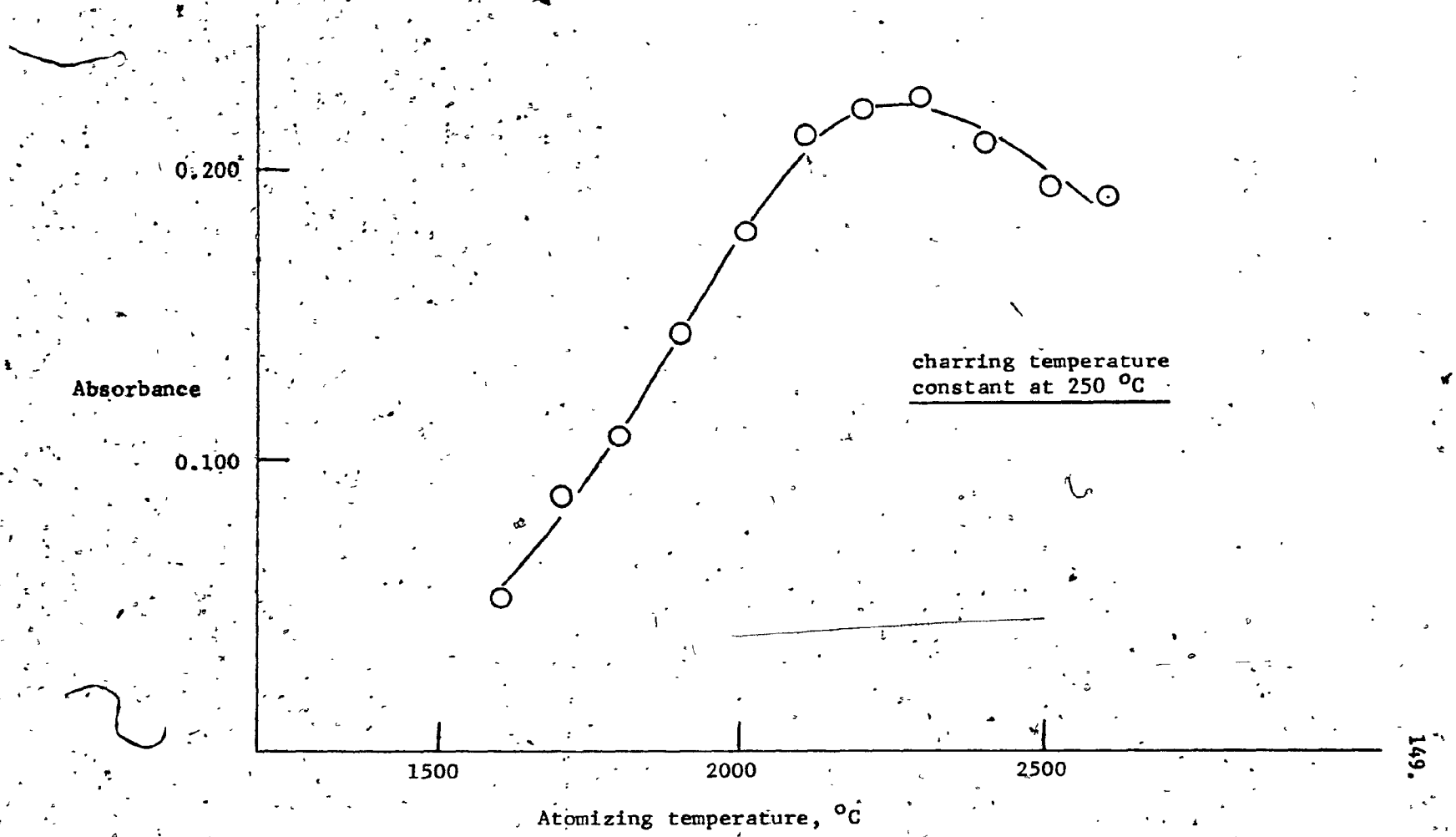


FIGURE B-11 GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE - OPTIMUM ATOMIZING TEMPERATURE, CADMIUM

Determination of the linear range for cadmium in aqueous base solutions

The general operating conditions remained the same, the HGA program was set up as follows:-

	Drying	Charring	Atomizing
Temperature, °C	120	250	2100
Time, sec	20	30	10

The testing of the aqueous cadmium standards resulted in the following:-

Cadmium* (ppm)	Average** absorbance	Corrected average absorbance
0.00000	0.011 ± 0.000	0.000 ± 0.000
0.00010 ± 0.00001	0.020 ± 0.000	0.009 ± 0.000
0.00050 ± 0.00001	0.061 ± 0.002	0.050 ± 0.002
0.00075 ± 0.00001	0.094 ± 0.001	0.083 ± 0.001
0.00100 ± 0.00001	0.116 ± 0.003	0.105 ± 0.003
0.00150 ± 0.00004	0.182 ± 0.003	0.171 ± 0.003
0.00200 ± 0.00004	0.220 ± 0.001	0.209 ± 0.001
0.00250 ± 0.00004	0.266 ± 0.004	0.255 ± 0.004
0.00275 ± 0.00005	0.303 ± 0.003	0.292 ± 0.003
0.00300 ± 0.00005	0.323 ± 0.004	0.312 ± 0.004
0.00325 ± 0.00005	0.341 ± 0.005	0.330 ± 0.005
0.00350 ± 0.00006	0.352 ± 0.008	0.341 ± 0.008
0.00400 ± 0.00006	0.371 ± 0.005	0.360 ± 0.005

* These results are plotted on Figure B-12

** Average of three peak integration readings with standard deviation

Note:- average standard deviation ± 0.003

Linear regression analysis:-

Concentrations included cadmium (ppm)	Linear regression* data
0.00000 - 0.00400	r = 0.9942 b = 0.009 m = 96.36

0.00000 - 0.00350

r = 0.9980
b = 0.004
m = 100.88

*
r = correlation coefficient
b = intercept
m = slope

From the linear regression analysis and Figure B-12, a deviation from linearity is noted between 0.00350 and 0.00400 ppm cadmium.

Therefore:-

Linear regression analysis

0.00000 - 0.00350 ppm Cd

r = 0.9980
b = 0.004
m = 100.88

Sensitivity (1% absorption) = 0.0044/100.88 = 0.00004 ppm Cd

Reverse calculations for cadmium using the above linear range equation are as follows:-

Actual cadmium (ppm)	Calculated cadmium (ppm)	Absolute devn.
0.00000	-0.00004	0.00004
0.00010 ± 0.00001	0.00005	0.00004
0.00050 ± 0.00001	0.00046	0.00004
0.00075 ± 0.00001	0.00078	0.00005
0.00100 ± 0.00001	0.00100	0.00000
0.00150 ± 0.00004	0.00166	0.00016
0.00200 ± 0.00004	0.00203	0.00003
0.00250 ± 0.00004	0.00249	0.00001
0.00275 ± 0.00005	0.00285	0.00010
0.00300 ± 0.00005	0.00305	0.00005
0.00325 ± 0.00005	0.00323	0.00002
0.00350 ± 0.00006	0.00334	0.00016
	Average absolute deviation	0.00006

Values of cadmium (ppm) from the above equation subject to ± 0.00006 ppm

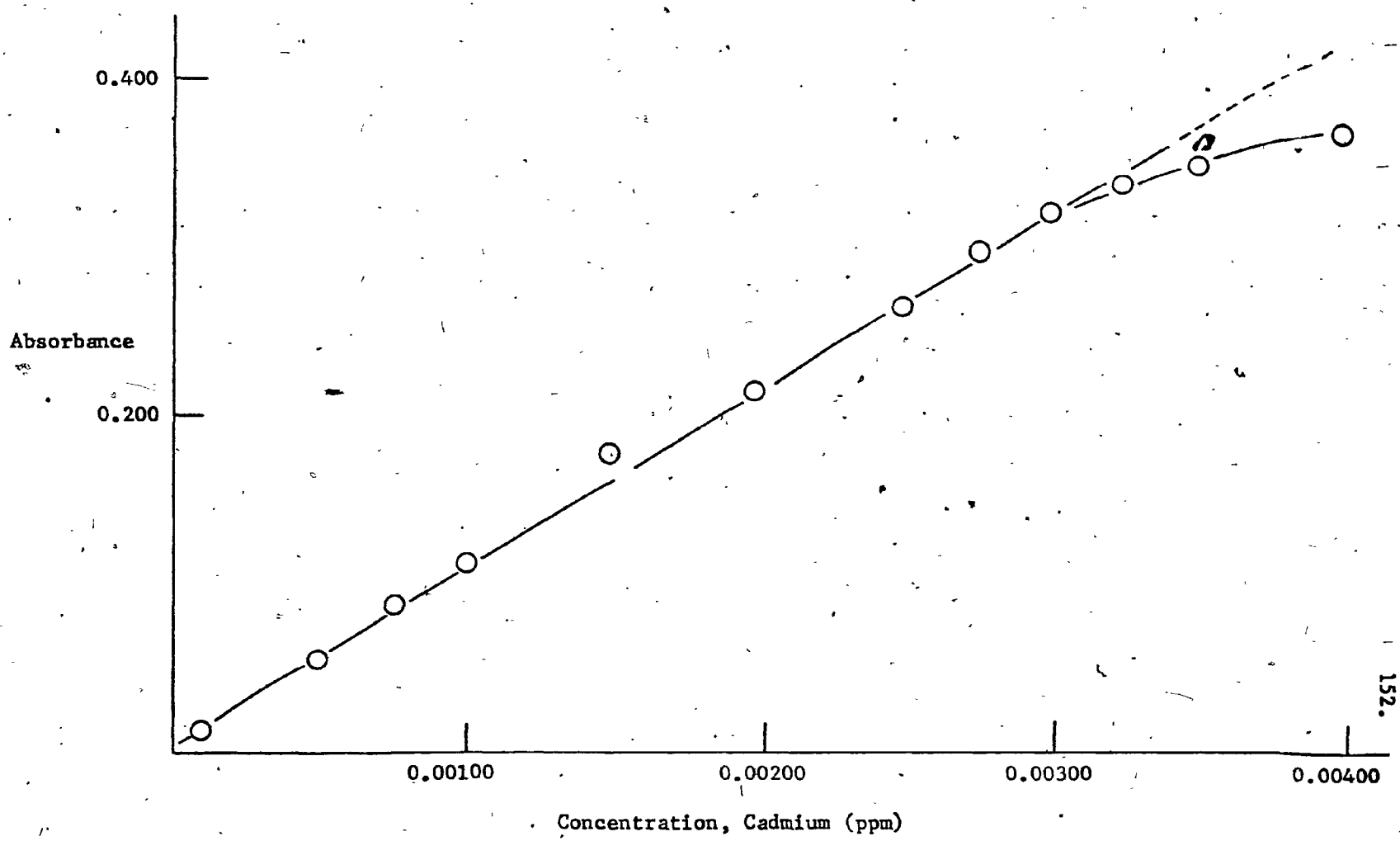


FIGURE B-12 GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE - CALIBRATION CURVE FOR CADMIUM

APPENDIX B-5

Determination of Vanadium in Aqueous Base Solutions by Flameless Atomic Absorption Spectrophotometry

General operating conditions

Instrument:-	Perkin-Elmer Model 503 Atomic Absorption Spectrophotometer with HGA 2100 Graphite Furnace and Ramp Assembly
Graphite tube:-	Pyrolytically coated
Radiation source:-	Vanadium hollow cathode lamp
Wavelength:-	318 nm
Slit:-	0.7 nm
Background correction:-	D_2 beam corrector
Nitrogen flow:-	40 gauge units, normal mode (14 lit/min.)
Flush system:-	Auto-high temperature
Program:-	
Drying, temperature	125 °C
time - HGA	30 sec
time - Ramp	10 sec
Charring, temperature	varied, 400 - 2200 °C
time - HGA	40 sec
time - Ramp	10 sec
Atomizing, temperature	varied, 2500 - 2800 °C
time - HGA	15 sec
time - Ramp	5 sec
Recorder:-	
Range	5 mV
Speed	20 mm/min
Readout:-	Peak integration mode
Water:-	Glass-distilled
Sample size:-	20 ul

Preparation of aqueous-base standard solutions

1. All standard solutions are prepared from a 1000.0 ± 0.2 ppm vanadium solution supplied by Fisher Scientific Company Limited as Certified Fisher Standard Stock Solution of Vanadium.

2. Dilution of the standard stock solution was carried out as follows:-

   (a) 25.00 ± 0.02 ml of 1000.0 ± 0.2 ppm vanadium solution pipetted into a 250.00 ± 0.05 ml volumetric flask and diluted to the mark.

   Value:- 100.0 ± 0.1 ppm vanadium

   (b) 25.00 ± 0.02 ml of 100.0 ± 0.1 ppm vanadium solution pipetted into a 250.00 ± 0.05 ml volumetric flask and dilute to the mark.

   Value:- 10.00 ± 0.02 ppm vanadium

3. The following indicates the preparation procedures for the vanadium working standard solutions. All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Solution preparation	Vanadium (ppm)
0.50 ± 0.02 ml of 10.00 ± 0.02 ppm V	0.050 ± 0.002
1.00 ± 0.02 ml " " " "	0.100 ± 0.002
2.00 ± 0.02 ml " " " "	0.200 ± 0.002
2.50 ± 0.02 ml " " " "	0.250 ± 0.002
5.00 ± 0.02 ml " " " "	0.500 ± 0.003
7.50 ± 0.02 ml " " " "	0.750 ± 0.004
10.00 ± 0.02 ml " " " "	1.000 ± 0.004

Determination of optimum charring and atomizing temperatures

1. A standard solution containing 1.000 ± 0.004 ppm vanadium was prepared in order to establish the maximum possible charring temperature with no significant loss of vanadium. Repeated injections of this aqueous standard were carried out with varying charring temperatures.

   The HGA - Ramp program was set up as follows:-

	Drying	Charring	Atomizing
Temperature, °C	125	varied	2800
Time - HGA, sec	30	40	15
Time - Ramp, sec	10	10	5

The following results were obtained:-

Charring temp., °C	Average* absorbance	Charring temp., °C	Average* absorbance
400	0.326 ± 0.009	1300	$0.34^{7} \pm 0.01^{4}$
500	0.325 ± 0.005	1400	$0.36^{4} \pm 0.01^{3}$
600	0.326 ± 0.004	1500	0.293 ± 0.007
700	0.344 ± 0.008	1600	0.287 ± 0.007
800	0.371 ± 0.003	1700	$0.28^{4} \pm 0.01^{4}$
1000	0.364 ± 0.004	1800	$0.27^{8} \pm 0.01^{5}$
1100	$0.35^{3} \pm 0.01^{5}$	2000	0.225 ± 0.004
1200	$0.36^{7} \pm 0.02^{2}$	2200	0.152 ± 0.003

* Average absorbance of three peak integration results and standard deviation

Figure B-13 shows a significant drop in absorbance after the 1400 °C point. It can be assumed that the maximum charring temperature is 1400 °C. A value of ramp charring temperature of 1400 °C (10/40 sec) was selected as being satisfactory for the conditions of the investigation.

2. A standard aqueous solution containing 0.500 ± 0.003 ppm vanadium was prepared in order to establish the optimum atomizing temperature. Repeated injections of this standard were carried out at various atomizing temperatures.

The HGA - Ramp program was set up as follows:-

	Drying	Charring	Atomizing
Temperature, °C	125	1400	varied
Time - HGA, sec	30	40	15
Time - Ramp, sec	10	10	5

The following results were obtained:-

Atomizing temp., °C	Average* absorbance
2500	0.045 ± 0.000
2600	0.081 ± 0.003
2650	0.104 ± 0.008

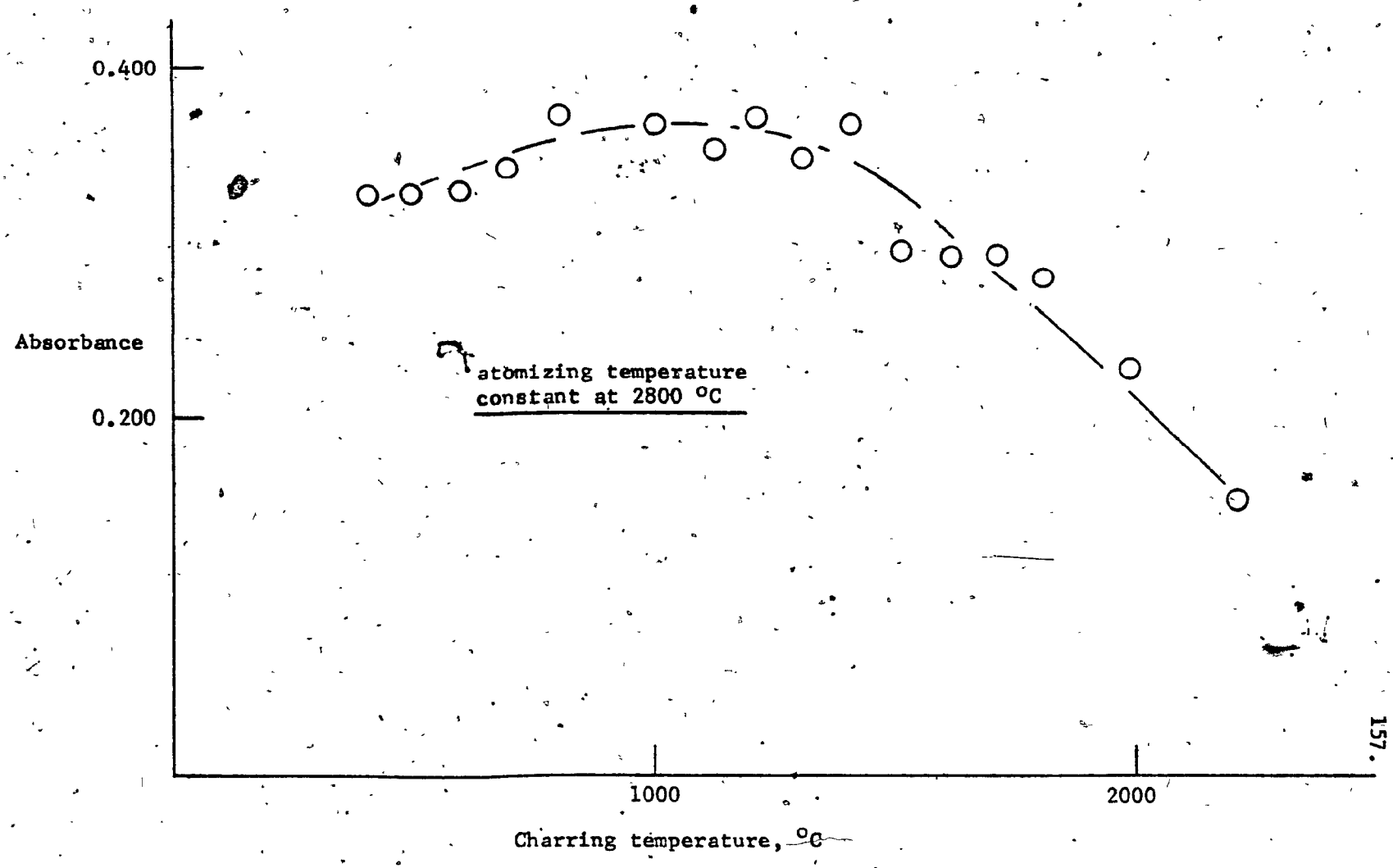


FIGURE B-13 GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE - MAXIMUM CHARRING TEMPERATURE, VANADIUM

2700	0.122 ± 0.003
2750	0.146 ± 0.002
2800	0.163 ± 0.002

* Averages of three peak integration absorbance results and standard deviations

As noted from the tabulated results above and Figure B-14, the optimum atomizing temperature was not attained. Under working conditions with the HGA 2100 furnace and pyro-coated graphite tubes, a ramp atomizing temperature of 2800 °C (5/15 sec) was applied and expected to give satisfactory results.

Determination of the linear range for vanadium in aqueous base solutions

The general operating conditions remained the same. The HGA - Ramp program was set up as follows:-

	Drying	Charring	Atomizing
Temperature, °C	125	1400	2800
Time - HGA, sec	30	40	15
Time - Ramp, sec	10	10	5

The testing of the aqueous vanadium standards resulted in the following:-

Vanadium* (ppm)	Average** absorbance	Corrected average absorbance
0.000	0.007 ± 0.000	0.000 ± 0.000
0.050 ± 0.002	0.022 ± 0.001	0.015 ± 0.001
0.100 ± 0.002	0.036 ± 0.002	0.029 ± 0.002
0.200 ± 0.002	0.073 ± 0.005	0.066 ± 0.005
0.250 ± 0.002	0.091 ± 0.001	0.084 ± 0.001
0.500 ± 0.003	0.161 ± 0.003	0.154 ± 0.003
0.750 ± 0.004	0.253 ± 0.005	0.246 ± 0.005
1.000 ± 0.004	0.316 ± 0.006	0.309 ± 0.006

* These results are plotted on Figure B-15

** Average of three peak integration readings with standard deviation

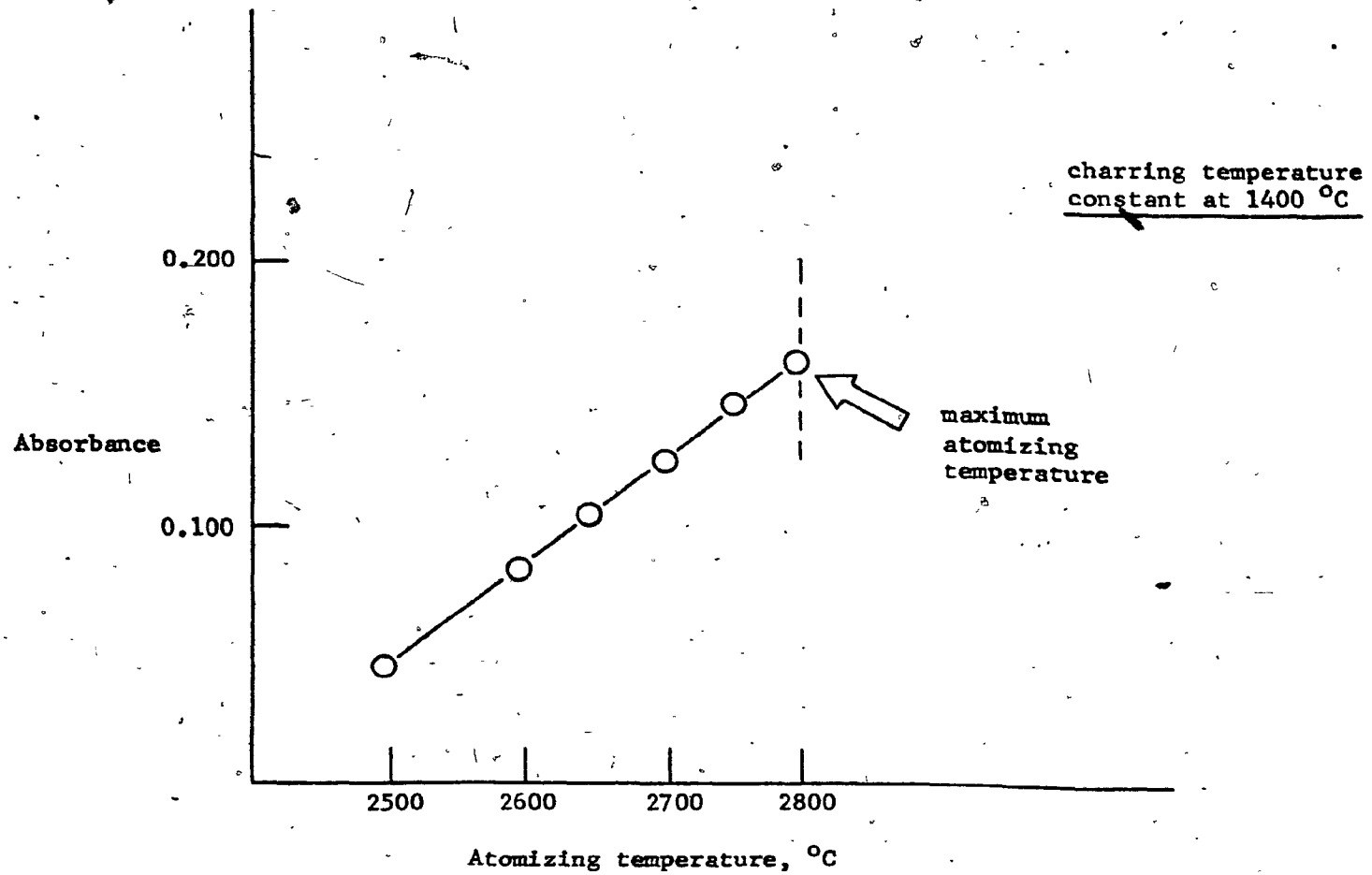


FIGURE B-14 GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE - OPTIMUM ATOMIZING TEMPERATURE, VANADIUM

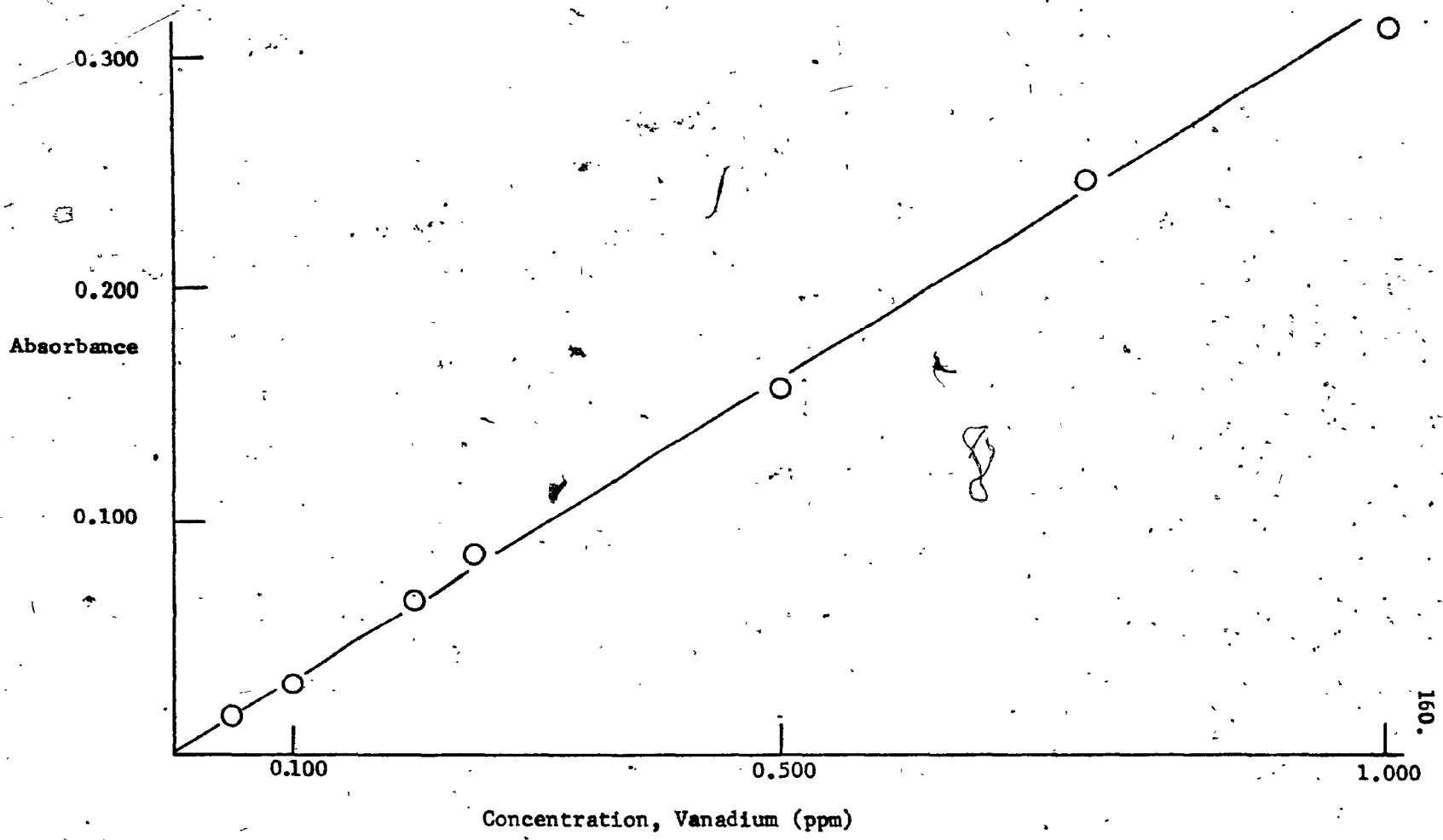


FIGURE B-15 GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE - CALIBRATION CURVE FOR VANADIUM

Linear regression analysis

0.000 ~ 1.000 ppm V

$r = 0.9990$
$b = 0.000^{9}$
$m = 0.3142^{5}$

Sensitivity (1% absorption) = $0.0044/0.3142^{5} = 0.01^{4}$ ppm V

Reverse calculations for vanadium using the above linear equation are as follows:-

Actual vanadium (ppm)	Calculated vanadium (ppm)	Absolute devn.
0.000	-0.002	0.002
0.050 ± 0.002	0.045	0.005
0.100 ± 0.002	0.089	0.011
0.200 ± 0.002	0.207	0.007
0.250 ± 0.002	0.264	0.014
0.500 ± 0.003	0.487	0.013
0.750 ± 0.004	0.780	0.030
1.000 ± 0.004	0.980	0.020
	Average absolute deviation	0.013

Values of vanadium (ppm) from the above linear equation subject to $\pm 0.01^{3}$ ppm

APPENDIX C

APPENDIX C-1

Determination of Copper in Rainbow Trout Tissue by the Dry-Ashing/ Flame Atomic Absorption Technique

1. General

The following sections outline the experimental approach applied to the testing, recovery from spiking and recovery from aliquoting of copper in rainbow trout tissue.

The flame method of atomic absorption ~~analysis~~ was applied, rather than the graphite furnace technique, because of the relatively high copper content found for rainbow trout unexposed to experimentally high copper levels in the water and/or food.

General operating conditions

Certain general operating conditions were maintained throughout the analytical procedures. These are outlined in Appendix A-1.

Glassware was washed several times with detergent soap solution, soaked overnight in a 1:1 HCl/HNO_3 solution and rinsed several times with glass-distilled water.

Preparation of aqueous-base standard solutions

Appendix A-1 outlines the preparation of the aqueous-base standard solutions. Additional working standard solutions were prepared as follows:-

Solution preparation*	Copper (ppm)
1.00 ± 0.02 ml of 10.00 ± 0.02 ppm Cu	0.100 ± 0.002
2.50 ± 0.02 ml " " " "	0.250 ± 0.003
1.50 ± 0.02 ml " 100.0 ± 0.1 " "	1.50 ± 0.02

* All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Preparation of fish tissue

The preparation of rainbow trout base tissue is described in Subsection 2.2.3 - (a).

2. Testing of Dry Ashed Rainbow Trout Base Tissue

Dry ashing of fish tissue

The general procedure is outlined in Sub-section 2.2.3 - (b). The specific details for the determination of copper are given below.

The sample weights were as follows:-

Sample No.	Fish weight (g)
1.	0.0000
2.	1.0000 ± 0.0002
3.	1.0000 ± 0.0002
4.	1.0000 ± 0.0002
5.	1.5000 ± 0.0002
6.	1.5000 ± 0.0002
7.	1.5000 ± 0.0002
8.	2.0000 ± 0.0002
9.	2.0000 ± 0.0002
10.	2.0000 ± 0.0002
11.	2.5000 ± 0.0002
12.	2.5000 ± 0.0002
13.	2.5000 ± 0.0002
14.	3.0000 ± 0.0002
15.	3.0000 ± 0.0002
16.	3.0000 ± 0.0002
17.	4.0000 ± 0.0002
18.	4.0000 ± 0.0002
19.	4.0000 ± 0.0002

The amount of HCl pipetted was,

0.50 ± 0.01 ml

The amount of water pipetted was,

19.50 ± 0.04 ml

Testing of working standards by flame atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Copper (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.100 ± 0.002	0.005 ± 0.000
0.250 ± 0.003	0.013 ± 0.000
0.500 ± 0.003	0.027 ± 0.000
1.000 ± 0.004	0.055 ± 0.000
1.50 ± 0.02	0.081 ± 0.000
2.00 ± 0.02	0.109 ± 0.000

* Average of five 3-seconds integration readings.
± values are standard deviations.

Linear regression analysis

0.000 - 2.00 ppm Cu

$r = 0.9999^4$
$b = -0.000^3$
$m = 0.0545^7$

Sensitivity (1% absorption) = $0.0044/0.0545^7$ = 0.080 ppm Cu

Reverse calculations for copper

Actual copper (ppm)	Calculated copper (ppm)	Absolute devn.
0.000	0.000	0.000
0.100 ± 0.002	0.092	0.008
0.250 ± 0.003	0.238	0.012
0.500 ± 0.003	0.495	0.005
1.000 ± 0.004	1.008	0.008
1.50 ± 0.02	1.48	0.02
2.00 ± 0.02	2.00	0.00
	Average absolute deviation	0.007

Values of copper (ppm) from the above equation subject to ± 0.007 ppm.

Results of copper determination in dry ashed fish base tissue

The results of the blank and fish base samples are tabulated in Table C-1.

The 18 values of copper in fish tissue resulted in an average of,

$$3.9^4 \pm 0.2^4 \text{ ppm Cu}$$

or

$$3.9^4 \pm 0.2^4 \text{ ug Cu/g dried rainbow trout}$$

3. Recovery of Copper from Spiked Fish Base Samples

Dry ashing of fish tissue

The weights of macerated and dried fish tissue weighed into 50-ml beakers are given in Table C-2.

These fish tissue samples were spiked with 10.00 ± 0.02 ppm Cu solution as follows:-

Series	Spike	ug copper added
A	none	0.0
B	1.50 ± 0.02 ml of 10.00 ppm Cu	15.0 ± 0.2
C	3.00 ± 0.02 ml " " " "	30.0 ± 0.3
D	5.00 ± 0.02 ml " " " "	50.0 ± 0.3
E	6.50 ± 0.02 ml " " " "	65.0 ± 0.3
F	8.00 ± 0.02 ml " " " "	80.0 ± 0.4

Subsequent to spiking each sample was placed in the oven and heated at just under 100 °C until the liquid spike volume had dried out. The subsequent ashing procedure duplicated the details of Sub-section 2.2.3 - (b). A blank with no spike was carried through the same procedure.

The amount of HCl pipetted was,

$$0.50 \pm 0.01 \text{ ml}$$

The amount of water pipetted was,

$$19.50 \pm 0.04 \text{ ml}$$

TABLE C-1

DETERMINATION OF COPPER IN RAINBOW TROUT BASE TISSUE
BY DRY ASHING/FLAME ATOMIC ABSORPTION TECHNIQUE

Sample No.	Average* absorbance	Corrected average absorbance	**Cu in 20 ml soln. (ppm)	Copper ug/20 ml	Copper in fish base (ppm)
1.	0.001 ± 0.000	0.000 ± 0.000	---	---	---
2.	0.012 ± 0.000	0.011 ± 0.000	0.202 ± 0.007	$4.0^4 \pm 0.1^4$	$4.0^4 \pm 0.1^4$
3.	0.011 ± 0.000	0.010 ± 0.000	0.183 ± 0.007	$3.6^6 \pm 0.1^4$	$3.6^6 \pm 0.1^4$
4.	0.012 ± 0.000	0.011 ± 0.000	0.202 ± 0.007	$4.0^4 \pm 0.1^4$	$4.0^4 \pm 0.1^4$
5.	0.017 ± 0.000	0.016 ± 0.000	0.293 ± 0.007	$5.8^6 \pm 0.1^4$	3.90 ± 0.09
6.	0.016 ± 0.000	0.015 ± 0.000	0.275 ± 0.007	$5.5^0 \pm 0.1^4$	3.66 ± 0.09
7.	0.017 ± 0.000	0.016 ± 0.000	0.293 ± 0.007	$5.8^6 \pm 0.1^4$	3.90 ± 0.09
8.	0.023 ± 0.000	0.022 ± 0.000	0.403 ± 0.007	$8.0^6 \pm 0.1^4$	4.03 ± 0.07
9.	0.025 ± 0.000	0.024 ± 0.000	0.440 ± 0.007	$8.8^0 \pm 0.1^4$	4.40 ± 0.07
10.	0.025 ± 0.000	0.024 ± 0.000	0.440 ± 0.007	$8.8^0 \pm 0.1^4$	4.40 ± 0.07
11.	0.028 ± 0.000	0.027 ± 0.000	0.495 ± 0.007	$9.9^0 \pm 0.1^4$	3.96 ± 0.06
12.	0.028 ± 0.000	0.027 ± 0.000	0.495 ± 0.007	$9.9^0 \pm 0.1^4$	3.96 ± 0.06
13.	0.028 ± 0.000	0.027 ± 0.000	0.495 ± 0.007	$9.9^0 \pm 0.1^4$	3.96 ± 0.06
14.	0.031 ± 0.000	0.030 ± 0.000	0.550 ± 0.007	$11.0^0 \pm 0.1^4$	3.67 ± 0.05
15.	0.031 ± 0.000	0.030 ± 0.000	0.550 ± 0.007	$11.0^0 \pm 0.1^4$	3.67 ± 0.05
16.	0.030 ± 0.000	0.029 ± 0.000	0.531 ± 0.007	$10.6^2 \pm 0.1^4$	3.54 ± 0.05
17.	0.044 ± 0.000	0.043 ± 0.000	0.788 ± 0.007	$15.7^6 \pm 0.1^4$	3.94 ± 0.04
18.	0.047 ± 0.000	0.046 ± 0.000	0.843 ± 0.007	$16.8^6 \pm 0.1^4$	4.21 ± 0.04
19.	0.045 ± 0.000	0.044 ± 0.000	0.806 ± 0.007	$16.1^2 \pm 0.1^4$	4.03 ± 0.04

* Each absorbance value represents the average of five 3-seconds integration readings. ± values are standard deviations.

** Using the linear equation from Page 166.

TABLE C-2

SAMPLE WEIGHTS OF BASE FISH TISSUE FOR
COPPER DRY ASHING SPIKING PROCEDURE

No.	Series A	Series B	Series C	Series D	Series E	Series F
1.	1.0060 ± 0.0002	1.0147 ± 0.0002	1.0100 ± 0.0002	1.0194 ± 0.0002	1.0439 ± 0.0002	1.0087 ± 0.0002
2.	1.0034 ± 0.0002	1.0158 ± 0.0002	1.0025 ± 0.0002	1.0153 ± 0.0002	1.0289 ± 0.0002	1.0064 ± 0.0002
3.	1.0004 ± 0.0002	1.0000 ± 0.0002	1.0046 ± 0.0002	1.0162 ± 0.0002	1.0015 ± 0.0002	1.0112 ± 0.0002
4.	1.0105 ± 0.0002	0.9991 ± 0.0002	1.0178 ± 0.0002	1.0043 ± 0.0002	1.0050 ± 0.0002	0.9977 ± 0.0002
5.	1.0031 ± 0.0002	1.0279 ± 0.0002	1.0094 ± 0.0002	1.0032 ± 0.0002	1.0420 ± 0.0002	0.9938 ± 0.0002

Testing of working standards by flame atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Copper (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.100 ± 0.002	0.005 ± 0.000
0.500 ± 0.003	0.026 ± 0.000
1.000 ± 0.004	0.052 ± 0.000
2.00 ± 0.02	0.105 ± 0.001
3.00 ± 0.02	0.155 ± 0.000
4.00 ± 0.02	0.206 ± 0.000
5.00 ± 0.02	0.259 ± 0.000

* Average of five 3-seconds integration readings
± values are standard deviations

Linear regression analysis

0.000 - 5.00 ppm Cu

$r = 0.9999^7$
$b = 0.000^2$
$m = 0.0516^8$

Sensitivity (1% absorption) = $0.0044/0.0516^8$ = 0.085 ppm Cu

Reverse calculations for copper

Actual copper (ppm)	Calculated copper (ppm)	Absolute devn.
0.000	0.000	0.000
0.100 ± 0.002	0.097	0.003
0.500 ± 0.003	0.503	0.003
1.000 ± 0.004	1.006	0.006
2.00 ± 0.02	2.03	0.03
3.00 ± 0.02	3.00	0.00
4.00 ± 0.02	3.99	0.01
5.00 ± 0.02	5.01	0.01
	Average absolute deviation	0.008

Values of copper (ppm) from the above equation subject to ± 0.008 ppm.

Results of copper determination of spiked dry ashed fish base tissue

The absorbance results of the blank and fish base samples spiked with copper (ug) are tabulated in Table C-3.

The calculated copper recoveries are shown in Table C-4.

The values for the 20 ml each Series A samples yield the following results:-

Series and No.	Cu in 20 ml soln. (ppm)	Cu (ug/g) in fish base
A-1	0.270 ± 0.008	5.3^{9} ± 0.1^{6}
2	0.309 ± 0.008	6.1^{8} ± 0.1^{6}
3	0.193 ± 0.008	3.8^{6} ± 0.1^{6}
4	0.193 ± 0.008	3.8^{2} ± 0.1^{6}
5	0.155 ± 0.008	3.0^{9} ± 0.1^{6}

These again represent fish base values and, combined with the 18 values obtained under Appendix C-1 - (2) Testing of Dry Ashed Rainbow Trout Base Tissue, provide a total of 23 values. Application of the 2s rejection criterion to this set of data results in the rejection of the two values, 5.3^{9} and 6.1^{8} ug Cu/g of fish base. The remaining 21 values yield a final value for the copper content of dried (65 ± 5 °C) rainbow trout unexposed to experimentally high copper contents in the water and/or food of:-

$3.8^{9} \pm 0.2^{9}$ ppm or $3.8^{9} \pm 0.2^{9}$ ug Cu/g dried rainbow trout base

Using the 2s rejection criterion to examine the recovery percent data of the last column in Table C-4, it was noted that rejection of the $128.^{7}$ % recovery value was required. The remaining values yield an overall recovery percent for copper spikes on rainbow trout dried base of up to 80 ug/g dried fish base of:-

$103.^{2} \pm 3.^{0}$ %

TABLE C-3

DRY ASHING/FLAME ATOMIC ABSORPTION TECHNIQUE

RESULTS OF COPPER DETERMINATION
ON SPIKED FISH BASE SAMPLES

Series and No.	Average* absorbance	Corrected average absorbance
blank	0.001 ± 0.000	0.000 ± 0.000
A-1	0.015 ± 0.000	0.014 ± 0.000
2	0.017 ± 0.000	0.016 ± 0.000
3	0.011 ± 0.000	0.010 ± 0.000
4	0.011 ± 0.000	0.010 ± 0.000
5	0.009 ± 0.000	0.008 ± 0.000
B-1	0.051 ± 0.000	0.050 ± 0.000
2	0.052 ± 0.000	0.051 ± 0.000
3	0.049 ± 0.000	0.048 ± 0.000
4	0.061 ± 0.000	0.060 ± 0.000
5	0.050 ± 0.000	0.049 ± 0.000
C-1	0.094 ± 0.000	0.093 ± 0.000
2	0.091 ± 0.000	0.090 ± 0.000
3	0.092 ± 0.000	0.091 ± 0.000
4	0.092 ± 0.001	0.091 ± 0.001
5	0.090 ± 0.000	0.089 ± 0.000
D-1	0.147 ± 0.000	0.146 ± 0.000
2	0.154 ± 0.001	0.153 ± 0.001
3	0.154 ± 0.001	0.153 ± 0.001
4	0.141 ± 0.000	0.140 ± 0.000
5	lost	
E-1	0.186 ± 0.001	0.185 ± 0.001
2	0.184 ± 0.001	0.183 ± 0.001
3	0.182 ± 0.001	0.181 ± 0.001
4	0.180 ± 0.000	0.179 ± 0.000
5	0.188 ± 0.000	0.187 ± 0.000
F-1	0.221 ± 0.001	0.220 ± 0.001
2	0.221 ± 0.001	0.220 ± 0.001
3	0.220 ± 0.001	0.219 ± 0.001
4	0.224 ± 0.000	0.223 ± 0.000
5	0.222 ± 0.001	0.221 ± 0.001

* Average of five 3-seconds integration readings.
± values are standard deviations.

TABLE C-4

DRY ASHING/FLAME ATOMIC ABSORPTION TECHNIQUE

RECOVERY OF COPPER IN SPIKED FISH BASE SAMPLES

Series and No.	Cu in 20 ml soln. (ppm)**	Total Cu (ug/20 ml)	*Cu (ug/20 ml) corr. fish base	Actual Cu spike (ug)	Recovery of Cu (%)
B-1	0.967 ± 0.008	$19.3^{4} \pm 0.1^{6}$	$15.3^{5} \pm 0.2^{9}$	15.0 ± 0.2	102.3 ± 1.9
2	0.987 ± 0.008	$19.7^{4} \pm 0.1^{6}$	$15.7^{9} \pm 0.2^{9}$	15.0 ± 0.2	105.3 ± 1.9
3	0.948 ± 0.008	$18.9^{6} \pm 0.1^{6}$	$15.0^{7} \pm 0.2^{9}$	15.0 ± 0.2	100.5 ± 1.9
4	1.160 ± 0.008	$23.2^{0} \pm 0.1^{6}$	$19.3^{1} \pm 0.2^{9}$	15.0 ± 0.2	128.7 ± 1.9
5	0.929 ± 0.008	$18.5^{8} \pm 0.1^{6}$	$14.5^{8} \pm 0.2^{9}$	15.0 ± 0.2	97.2 ± 1.9
C-1	1.800 ± 0.008	$36.0^{0} \pm 0.1^{6}$	$32.0^{7} \pm 0.2^{9}$	30.0 ± 0.3	106.9 ± 1.0
2	1.741 ± 0.008	$34.8^{2} \pm 0.1^{6}$	$30.9^{2} \pm 0.2^{9}$	30.0 ± 0.3	103.0 ± 1.0
3	1.761 ± 0.008	$35.2^{2} \pm 0.1^{6}$	$31.3^{1} \pm 0.2^{9}$	30.0 ± 0.3	104.3 ± 1.0
4	$1.76^{1} \pm 0.01^{9}$	$35.2^{2} \pm 0.3^{8}$	$31.2^{6} \pm 0.3^{8}$	30.0 ± 0.3	104.2 ± 1.3
5	1.722 ± 0.008	$34.4^{4} \pm 0.1^{6}$	$30.5^{1} \pm 0.2^{9}$	30.0 ± 0.3	101.7 ± 1.0
D-1	2.825 ± 0.008	$56.5^{0} \pm 0.1^{6}$	$52.5^{3} \pm 0.2^{9}$	50.0 ± 0.3	105.0 ± 0.6
2	$2.96^{0} \pm 0.01^{9}$	$59.2^{0} \pm 0.3^{8}$	$55.2^{5} \pm 0.3^{8}$	50.0 ± 0.3	110.5 ± 0.8
3	$2.96^{0} \pm 0.01^{9}$	$59.2^{0} \pm 0.3^{8}$	$55.2^{4} \pm 0.3^{8}$	50.0 ± 0.3	110.5 ± 0.8
4	2.709 ± 0.008	$54.1^{8} \pm 0.1^{6}$	$50.2^{7} \pm 0.2^{9}$	50.0 ± 0.3	100.5 ± 0.6
5	lost			50.0 ± 0.3	
E-1	$3.58^{0} \pm 0.01^{9}$	$71.6^{0} \pm 0.3^{8}$	$67.5^{4} \pm 0.3^{8}$	65.0 ± 0.3	103.9 ± 0.6
2	$3.54^{1} \pm 0.01^{9}$	$70.8^{2} \pm 0.3^{8}$	$66.8^{1} \pm 0.3^{8}$	65.0 ± 0.3	102.8 ± 0.6
3	$3.50^{2} \pm 0.01^{9}$	$70.0^{4} \pm 0.3^{8}$	$66.1^{4} \pm 0.3^{8}$	65.0 ± 0.3	101.8 ± 0.6
4	3.464 ± 0.008	$69.2^{8} \pm 0.1^{6}$	$65.3^{7} \pm 0.2^{9}$	65.0 ± 0.3	100.6 ± 0.4
5	3.618 ± 0.008	$72.3^{6} \pm 0.1^{6}$	$68.3^{0} \pm 0.2^{9}$	65.0 ± 0.3	105.0 ± 0.4
F-1	$4.25^{7} \pm 0.01^{9}$	$85.1^{4} \pm 0.3^{8}$	$81.2^{1} \pm 0.3^{8}$	80.0 ± 0.4	101.5 ± 0.5
2	$4.25^{7} \pm 0.01^{9}$	$85.1^{4} \pm 0.3^{8}$	$81.2^{2} \pm 0.3^{8}$	80.0 ± 0.4	101.5 ± 0.5
3	$4.23^{8} \pm 0.01^{9}$	$84.7^{6} \pm 0.3^{8}$	$80.8^{2} \pm 0.3^{8}$	80.0 ± 0.4	101.0 ± 0.5
4	4.315 ± 0.008	$86.3^{0} \pm 0.1^{6}$	$82.4^{1} \pm 0.2^{9}$	80.0 ± 0.4	103.0 ± 0.4
5	$4.29^{6} \pm 0.01^{9}$	$85.9^{2} \pm 0.3^{8}$	$82.0^{5} \pm 0.3^{8}$	80.0 ± 0.4	102.5 ± 0.5

* Based on the higher of the two uncertainties:- fish base copper or total copper in 20 ml.

** Using the linear equation from Page 170.

If the average for each of the Series B to F is taken with respect to the "Total Cu (ug/20 ml)" column data we have, omitting the B-4 result:-

Series	B	$19.1^{5} \pm 0.5^{0}$
	C	$35.1^{4} \pm 0.5^{8}$
	D	$57.^{3} \pm 2.4$
	E	70.8 ± 1.2
	F	$85.4^{5} \pm 0.6^{3}$

Linear regression analysis yields:- $r = 0.9993$, $b = 4.54$, $m = 1.021^{3}$

Thus the zero intercept on the ordinate axis yields a value of 4.54 ug Cu/20 ml of solution or, for the sample weights approximating 1.00 g of dried fish base, 4.54 ug Cu/g dried fish base. Carrying out reverse calculations indicates an uncertainty for this value of $\pm$ 0.6 ug, so that we have, from the method of additions, a calculated value of dried rainbow trout base content of copper of 4.5 $\pm$ 0.6 ug/g fish base. This compares, within the respective tolerance limits, with the value of $3.8^{9} \pm 0.2^{9}$ ug Cu/g dried fish base reported on Page 171.

Using the value of 4.5 $\pm$ 0.6 ug Cu/g dried fish base as the correction value to the "Total Cu (ug/20 ml)" data column from Table C-4 yields, omitting the B-4 value, an average recovery percent for spikes up to 80 ug on 1 g of dried fish base of $101.^{7} \pm 3.^{4}$. This also agrees well, within the respective tolerance limits, with the value of $103.^{2} \pm 3.^{0}$ percent reported on Page 171.

By including the average result of Series A to the average for each of the Series B to F with respect to the "Total Cu (ug/20 ml)" data column from Table C-4 in a graphical determination of copper in dried rainbow trout tissue by the method of additions, the plot on Figure C-1 was obtained. The copper content result can be extrapolated to the abscissa to 4.0 $\pm$ 0.2 ug Cu/g dried fish base. The value $\pm$ 0.2 represents the reading uncertainty on the graph. This compares (better than the calculated value above), within the tolerance limits, with the value of $3.8^{9} \pm 0.2^{9}$ reported on Page 171.

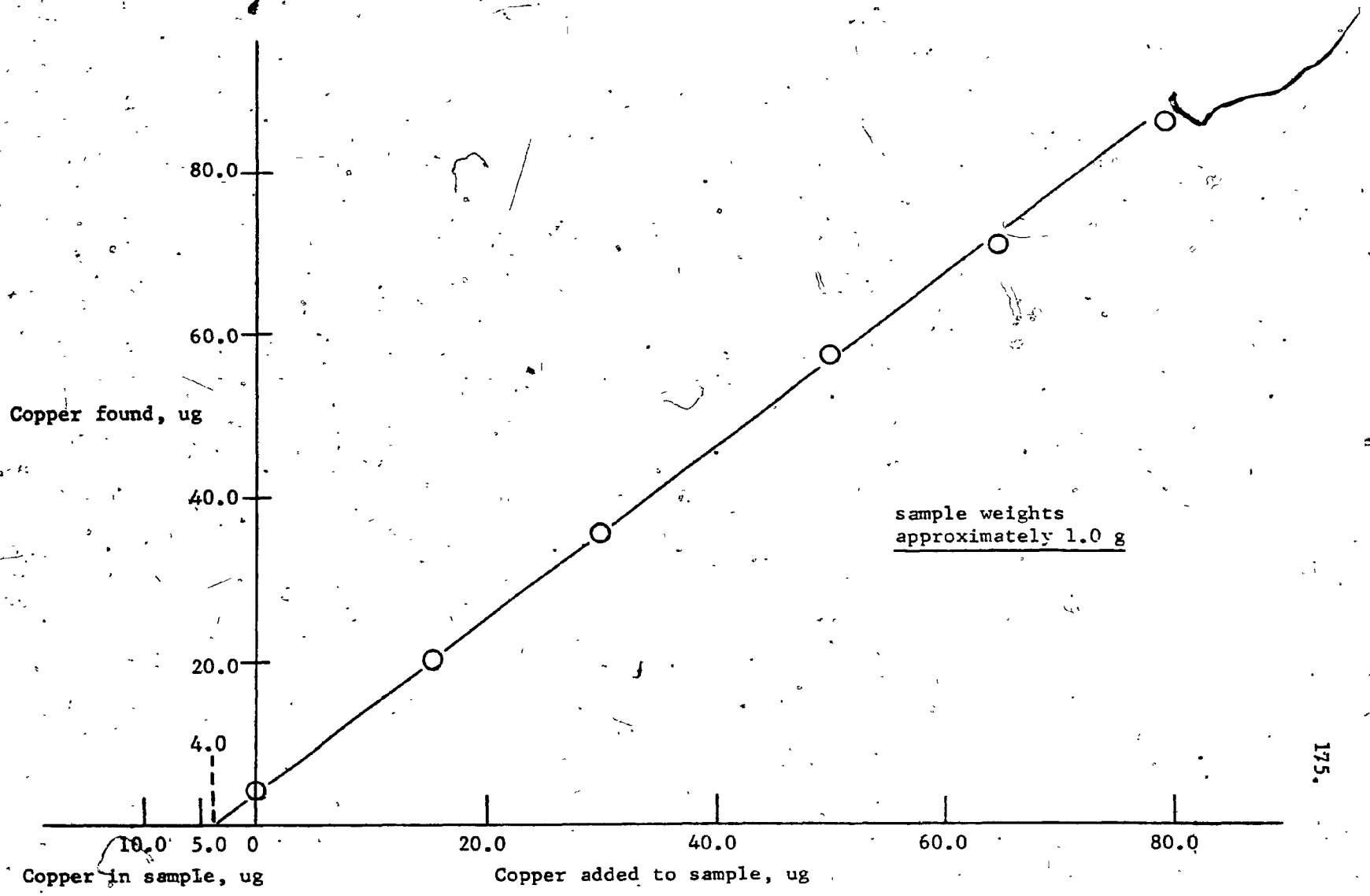


FIGURE C-1 GRAPHICAL METHOD OF ADDITIONS - COPPER DETERMINATION IN RAINBOW TROUT - DRY ASHING

4. Recovery of Copper from Aliquoted Spiked Fish Base Samples

Two sets of macerated and dried rainbow trout tissue were weighed out in duplicate. Each sample consisted of 2.0000 ± 0.0002 g and was placed in a 50-ml beaker.

One set was labeled A and was spiked with 70.0 ± 0.4 ug of copper each, while the second (B) was spiked with 90.0 ± 0.4 ug of copper each.

All samples were dried, ashed and extracted according to the details of Sub-section 2.2.3 - (b). A blank was carried through simultaneously. The amount of HCl pipetted was 0.50 ± 0.01 ml while the amount of water pipetted was 19.50 ± 0.04 ml.

For each set, 10 ml of the final solution was tested as-is, and 10 ml was diluted to 20 ml with 2.5 percent HCl and then tested. The following results were obtained:-

Set	Ave. absorbance corr. for blank		Total Cu* (ug/20 ml)		Cu corr. for** base fish (ug/g)		Actual spike (ug)	Recovery (%)	
	as-is	aliq.	as-is	aliq.x2	as-is	aliq.x2		as-is	aliq.
A	0.211	0.107	81.6	82.8	73.8	75.0	70.0	105	107
B	0.248	0.127	96.0	98.3	88.2	91.5	90.0	98	102

* Using the linear equation from Page 170.

** Using the correction factor of 3.9 ± 0.3 ug Cu/g dried rainbow trout base.

The recovery percent values agree within the expected tolerance limits for copper recovery on spiked samples, so that aliquoting of tissue sample solutions, suspected to contain copper in ppm beyond the linear range maximum, should present no difficulty.

APPENDIX C-2

Determination of Nickel in Rainbow Trout Tissue by the Dry-Ashing/ Flame Atomic Absorption Technique

1. General

The following sections outline the experimental approach applied to the testing, recovery from spiking and recovery from aliquoting of nickel in rainbow trout base tissue.

The flame method of atomic absorption analysis was applied, rather than the graphite furnace technique, because of the relatively high nickel content found for rainbow trout unexposed to experimentally high nickel levels in the water and/or food.

General operating conditions

Certain general operating conditions were maintained throughout the analytical procedures. These are outlined in Appendix A-2.

Glassware was washed several times with detergent soap solution, soaked overnight in a 1:1 HCl/HNO_3 solution and rinsed several times with glass-distilled water.

Preparation of aqueous-base standard solutions

Appendix A-2 outlines the preparation of the aqueous-base standard solutions. Additional working standard solutions were prepared as follows:-

Solution preparation*	Nickel (ppm)
2.50 ± 0.02 ml of 10.00 ± 0.02 ppm Ni	0.250 ± 0.003

* All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Preparation of fish tissue

The preparation of rainbow trout base tissue is described in Subsection 2.2.3 - (a).

2. Testing of Dry Ashed Rainbow Trout Base Tissue

Dry ashing of fish tissue

The general procedure is outlined in Sub-section 2.2.3 - (b). The specific details for the determination of nickel are given below.

The sample weights were as follows:-

Sample No.	Fish weight (g)
1.	0.0000
2.	1.0000 ± 0.0002
3.	1.0000 ± 0.0002
4.	1.0000 ± 0.0002
5.	1.5000 ± 0.0002
6.	1.5000 ± 0.0002
7.	1.5000 ± 0.0002
8.	2.0000 ± 0.0002
9.	2.0000 ± 0.0002
10.	2.0000 ± 0.0002
11.	2.5000 ± 0.0002
12.	2.5000 ± 0.0002
13.	2.5000 ± 0.0002
14.	3.0000 ± 0.0002
15.	3.0000 ± 0.0002
16.	3.0000 ± 0.0002
17.	4.0000 ± 0.0002
18.	4.0000 ± 0.0002
19.	4.0000 ± 0.0002

The amount of HCl pipetted was,

$$0.50 \pm 0.01 \text{ ml}$$

The amount of glass-distilled water pipetted was,

$$19.50 \pm 0.04 \text{ ml}$$

Testing of working standards by flame atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Nickel (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.100 ± 0.002	0.004 ± 0.001
0.250 ± 0.003	0.010 ± 0.000
0.500 ± 0.003	0.020 ± 0.000
1.000 ± 0.004	0.040 ± 0.000
1.50 ± 0.02	0.059 ± 0.000
2.00 ± 0.02	0.079 ± 0.001

* Average of five 3-seconds integration readings.
± values are standard deviations.

Linear regression analysis

0.000 - 2.00 ppm Ni

$r = 0.9999^7$
$b = 0.000^1$
$m = 0.0394^3$

Sensitivity (1% absorption) = 0.0044/0.03943 = 0.11 ppm Ni

Reverse calculations for nickel

Actual nickel (ppm)	Calculated nickel (ppm)	Absolute devn.
0.000	0.000	0.000
0.100 ± 0.002	0.101	0.001
0.250 ± 0.003	0.254	0.004
0.500 ± 0.003	0.507	0.007
1.000 ± 0.004	1.014	0.014
1.50 ± 0.02	1.50	0.00
2.00 ± 0.02	2.00	0.00
	Average absolute deviation	0.003

Values of nickel (ppm) from the above equation subject to ± 0.003 ppm.

Results of nickel determination in dry ashed fish base tissue

The results of the blank and fish base samples are tabulated in Table C-5.

The 18 values of nickel in fish tissue resulted in an average of,

$$1.3^2 \pm 0.2^2 \text{ ppm Ni}$$

or,

$$1.3^2 \pm 0.2^2 \text{ ug Ni/g dried rainbow trout base}$$

3. Recovery of Nickel from Spiked Fish Base Samples

Dry ashing of fish tissue

The weights of macerated and dried fish tissue weighed into 50-ml beakers are given in Table C-6.

These fish tissue samples were spiked with 10.00 ± 0.02 ppm Ni solution as follows:-

Series	Spike	ug Ni added
A	none	0.00
B	1.50 ± 0.02 ml of 10.00 ppm Ni	15.0 ± 0.2
C	3.00 ± 0.02 ml " " " "	30.0 ± 0.3
D	5.00 ± 0.02 ml " " " "	50.0 ± 0.3
E	7.50 ± 0.02 ml " " " "	75.0 ± 0.4
F	10.00 ± 0.02 ml " " " "	100.0 ± 0.4

Subsequent to spiking each sample was placed in the oven and heated at just under 100 °C until the liquid spike volume had dried out. The subsequent ashing procedure duplicated the details of Sub-section 2.2.3 - (b). A blank with no spike was carried through the same procedure.

The amount of HCl pipetted was,

$$0.50 \pm 0.01 \text{ ml}$$

The amount of water pipetted was,

$$19.50 \pm 0.04 \text{ ml}$$

TABLE C-5

DETERMINATION OF NICKEL IN RAINBOW TROUT BASE TISSUE
BY DRY ASHING/FLAME ATOMIC ABSORPTION TECHNIQUE

Sample No.	Average* absorbance	Corrected average absorbance	Ni in 20 ml soln. (ppm)**	Nickel ug/20 ml	Nickel in fish base (ppm)
1.	0.003 ± 0.000	0.000 ± 0.000	---	---	---
2.	0.006 ± 0.000	0.003 ± 0.000	0.076 ± 0.003	1.52 ± 0.06	1.52 ± 0.06
3.	0.005 ± 0.000	0.002 ± 0.000	0.050 ± 0.003	1.00 ± 0.06	1.00 ± 0.06
4.	0.006 ± 0.000	0.003 ± 0.000	0.076 ± 0.003	1.52 ± 0.06	1.52 ± 0.06
5.	0.008 ± 0.000	0.005 ± 0.000	0.127 ± 0.003	2.54 ± 0.06	1.69 ± 0.04
6.	0.007 ± 0.000	0.004 ± 0.000	0.101 ± 0.003	2.02 ± 0.06	1.35 ± 0.04
7.	0.008 ± 0.000	0.005 ± 0.000	0.127 ± 0.003	2.54 ± 0.06	1.69 ± 0.04
8.	0.009 ± 0.000	0.006 ± 0.000	0.152 ± 0.003	3.04 ± 0.06	1.52 ± 0.03
9.	0.007 ± 0.000	0.004 ± 0.000	0.101 ± 0.003	2.02 ± 0.06	1.01 ± 0.03
10.	0.008 ± 0.000	0.005 ± 0.000	0.127 ± 0.003	2.54 ± 0.06	1.27 ± 0.03
11.	0.009 ± 0.000	0.006 ± 0.000	0.152 ± 0.003	2.54 ± 0.06	1.02 ± 0.02
12.	0.010 ± 0.000	0.007 ± 0.000	0.177 ± 0.003	3.54 ± 0.06	1.42 ± 0.02
13.	0.010 ± 0.000	0.007 ± 0.000	0.177 ± 0.003	3.54 ± 0.06	1.42 ± 0.02
14.	0.011 ± 0.000	0.008 ± 0.000	0.203 ± 0.003	4.06 ± 0.06	1.35 ± 0.02
15.	0.010 ± 0.000	0.007 ± 0.000	0.177 ± 0.003	3.54 ± 0.06	1.15 ± 0.02
16.	0.011 ± 0.000	0.008 ± 0.000	0.203 ± 0.003	4.06 ± 0.06	1.35 ± 0.02
17.	0.012 ± 0.000	0.009 ± 0.000	0.228 ± 0.003	4.56 ± 0.06	1.14 ± 0.02
18.	0.012 ± 0.000	0.009 ± 0.000	0.228 ± 0.003	4.56 ± 0.06	1.14 ± 0.02
19.	0.012 ± 0.000	0.009 ± 0.000	0.228 ± 0.003	4.56 ± 0.06	1.14 ± 0.02

* Average represents five 3-seconds integration readings.
± values are standard deviations.

** Using the linear equation from Page 180.

TABLE C-6

SAMPLE WEIGHTS OF BASE FISH TISSUE FOR NICKEL DRY ASHING SPIKING PROCEDURE,

No.	Series A	Series B	Series C	Series D	Series E	Series F
1.	1.5036 ± 0.0002	1.4898 ± 0.0002	1.4688 ± 0.0002	1.5068 ± 0.0002	1.1014 ± 0.0002	1.4253 ± 0.0002
2.	1.5288 ± 0.0002	1.4263 ± 0.0002	1.4240 ± 0.0002	1.4392 ± 0.0002	1.3119 ± 0.0002	1.5082 ± 0.0002
3.	1.5274 ± 0.0002	1.5234 ± 0.0002	1.4433 ± 0.0002	1.4511 ± 0.0002	1.3651 ± 0.0002	1.5726 ± 0.0002
4.	1.5011 ± 0.0002	1.5029 ± 0.0002	1.4517 ± 0.0002	1.3970 ± 0.0002	1.4847 ± 0.0002	1.4474 ± 0.0002
5.	1.4737 ± 0.0002	1.4602 ± 0.0002	1.4567 ± 0.0002	1.2963 ± 0.0002	1.3997 ± 0.0002	1.5561 ± 0.0002

Testing of working standards by flame atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Nickel (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.100 ± 0.002	0.003 ± 0.000
0.250 ± 0.003	0.009 ± 0.000
0.500 ± 0.003	0.019 ± 0.000
1.000 ± 0.004	0.037 ± 0.000
1.50 ± 0.02	0.057 ± 0.001
2.00 ± 0.02	0.075 ± 0.001
3.00 ± 0.02	0.112 ± 0.000
3.50 ± 0.02	0.130 ± 0.001
4.00 ± 0.02	0.147 ± 0.000
5.00 ± 0.02	0.184 ± 0.001

* Average and standard deviation of five 3-seconds integration readings.

Linear regression analysis

0.000 - 5.00 ppm Ni

$r = 0.9999^1$

$b = 0.000^3$

$m = 0.0369^0$

Sensitivity (1% absorption) = $0.0044/0.0369^0$ = 0.12 ppm Ni

Reverse calculations for nickel

Actual nickel (ppm)	Calculated nickel (ppm)	Absolute devn.
0.000	0.000	0.000
0.100 ± 0.002	0.081	0.019
0.250 ± 0.003	0.244	0.006
0.500 ± 0.003	0.515	0.015
1.000 ± 0.004	1.003	0.003
1.50 ± 0.02	1.54	0.04
2.00 ± 0.02	2.03	0.03
3.00 ± 0.02	3.03	0.03
3.50 ± 0.02	3.52	0.02
4.00 ± 0.02	3.98	0.02
5.00 ± 0.02	4.98	0.02
	Average absolute deviation	0.018

Values of nickel (ppm) from the above equation subject to $\pm 0.01^8$ ppm.

Results of nickel determination of spiked dry ashed fish base tissue

The absorbance results of the blank and fish base samples spiked with nickel (ug) are shown in Table C-7.

The calculated nickel recoveries for Series B to F are tabulated in Table C-8.

The values for the 20 ml each Series A samples yield the following results:-

Series and No.	Ni in 20 ml soln. (ppm)	Ni (ug/g) in fish base
A-1	$0.13^{5} \pm 0.01^{8}$	$1.7^{9} \pm 0.2^{4}$
2	$0.08^{1} \pm 0.01^{8}$	$1.0^{6} \pm 0.2^{4}$
3	$0.08^{1} \pm 0.01^{8}$	$1.0^{6} \pm 0.2^{4}$
4	$0.08^{1} \pm 0.02^{7}$	$1.0^{8} \pm 0.3^{6}$
5	$0.08^{1} \pm 0.02^{7}$	$1.1^{0} \pm 0.3^{6}$

Application of the 2s rejection criterion to this set of data results in the rejection of the value 1.7^{9} ug Ni/g of fish base.

These again represent fish base values and, combined with the 18 values obtained under Appendix C-2 - (2) Testing of Dry Ashed Rainbow Trout Base Tissue, provide a total of 22 results (excluding the 1.7^{9} value). These results yield a final value for the nickel content of dried rainbow trout unexposed to experimentally high nickel contents in the water and/or food of:-

$1.2^{7} \pm 0.2^{2}$ ppm or $1.2^{7} \pm 0.2^{2}$ ug Ni/g dried rainbow trout base

Using the 2s rejection criterion to examine the recovery percent data of the last column in Table C-8, it was noted that rejection of the $106.^{5}$ and $106.^{3}$ percent values was required. The remaining results yielded an overall average recovery percent for nickel spikes on rainbow trout dried base of up to 100 ug/1.5 g dried fish base of:-

$100.^{4} \pm 2.^{0}$ %

TABLE C-7

DRY ASHING/FLAME ATOMIC ABSORPTION TECHNIQUE

RESULTS OF NICKEL DETERMINATION
ON SPIKED FISH BASE SAMPLES

Series and No.	Average* absorbance	Corrected average absorbance
blank	0.002 ± 0.000	0.000 ± 0.000
A-1	0.007 ± 0.000	0.005 ± 0.000
2	0.005 ± 0.000	0.003 ± 0.000
3	0.005 ± 0.000	0.003 ± 0.000
4	0.005 ± 0.001	0.003 ± 0.001
5	0.005 ± 0.001	0.003 ± 0.001
B-1	0.035 ± 0.000	0.033 ± 0.000
2	lost	
3	0.035 ± 0.000	0.033 ± 0.000
4	0.034 ± 0.000	0.032 ± 0.000
5	0.034 ± 0.001	0.032 ± 0.001
C-1	0.062 ± 0.000	0.060 ± 0.000
2	0.061 ± 0.000	0.059 ± 0.000
3	0.062 ± 0.000	0.060 ± 0.000
4	0.062 ± 0.000	0.060 ± 0.000
5	lost	
D-1	0.097 ± 0.000	0.095 ± 0.000
2	0.100 ± 0.001	0.098 ± 0.001
3	0.099 ± 0.000	0.097 ± 0.000
4	0.101 ± 0.000	0.099 ± 0.000
5	0.098 ± 0.000	0.096 ± 0.000
E-1	0.143 ± 0.000	0.141 ± 0.000
2	0.143 ± 0.000	0.141 ± 0.000
3	0.142 ± 0.000	0.140 ± 0.000
4	0.143 ± 0.001	0.141 ± 0.001
5	0.145 ± 0.000	0.143 ± 0.000
F-1	0.187 ± 0.000	0.185 ± 0.000
2	0.185 ± 0.001	0.183 ± 0.001
3	0.188 ± 0.000	0.186 ± 0.000
4	0.185 ± 0.000	0.183 ± 0.000
5	0.185 ± 0.001	0.183 ± 0.001

*Average and standard deviation of five 3-seconds integration readings.

TABLE C-8

DRY ASHING/FLAME ATOMIC ABSORPTION TECHNIQUE

RECOVERY OF NICKEL IN SPIKED FISH BASE SAMPLES

Series and No.	**Ni in 20 ml soln. (ppm)	Total Ni (ug/20 ml)	*Ni (ug/20 ml) corr. fish base	Actual Ni spike (ug)	Recovery of Ni (%)
B-1	$0.89^{4} \pm 0.01^{8}$	$17.8^{8} \pm 0.3^{6}$	$15.9^{8} \pm 0.3^{6}$	15.0 ± 0.2	$106.^{5} \pm 2.^{4}$
2	lost				
3	$0.89^{4} \pm 0.01^{8}$	$17.8^{8} \pm 0.3^{6}$	$15.9^{4} \pm 0.3^{6}$	15.0 ± 0.2	$106.^{3} \pm 2.^{4}$
4	$0.86^{7} \pm 0.01^{8}$	$17.3^{4} \pm 0.3^{6}$	$15.4^{3} \pm 0.3^{6}$	15.0 ± 0.2	$102.^{9} \pm 2.^{4}$
5	$0.86^{7} \pm 0.02^{7}$	$17.3^{4} \pm 0.5^{4}$	$15.4^{8} \pm 0.5^{4}$	15.0 ± 0.2	$103.^{2} \pm 3.^{6}$
C-1	$1.62^{6} \pm 0.01^{8}$	$32.5^{2} \pm 0.3^{6}$	$30.6^{5} \pm 0.3^{6}$	30.0 ± 0.3	$102.^{2} \pm 1.^{2}$
2	$1.59^{9} \pm 0.01^{8}$	$31.9^{8} \pm 0.3^{6}$	$30.1^{7} \pm 0.3^{6}$	30.0 ± 0.3	$100.^{6} \pm 1.^{2}$
3	$1.62^{6} \pm 0.01^{8}$	$32.5^{2} \pm 0.3^{6}$	$30.6^{8} \pm 0.3^{6}$	30.0 ± 0.3	$102.^{3} \pm 1.^{2}$
4	$1.62^{6} \pm 0.01^{8}$	$32.5^{2} \pm 0.3^{6}$	$30.6^{7} \pm 0.3^{6}$	30.0 ± 0.3	$102.^{2} \pm 1.^{2}$
5	lost				
D-1	$2.57^{4} \pm 0.01^{8}$	$51.4^{8} \pm 0.3^{6}$	$49.5^{7} \pm 0.3^{6}$	50.0 ± 0.3	99.1 ± 0.7
2	$2.65^{6} \pm 0.02^{7}$	$53.1^{2} \pm 0.5^{4}$	$51.2^{9} \pm 0.5^{4}$	50.0 ± 0.3	$102.^{6} \pm 1.^{1}$
3	$2.62^{9} \pm 0.01^{8}$	$52.5^{8} \pm 0.3^{6}$	$50.7^{3} \pm 0.3^{6}$	50.0 ± 0.3	101.5 ± 0.7
4	$2.68^{3} \pm 0.01^{8}$	$53.6^{6} \pm 0.3^{6}$	$51.8^{8} \pm 0.3^{6}$	50.0 ± 0.3	103.8 ± 0.7
5	$2.60^{2} \pm 0.01^{8}$	$52.0^{4} \pm 0.3^{6}$	$50.3^{9} \pm 0.3^{6}$	50.0 ± 0.3	100.8 ± 0.7
E-1	$3.82^{1} \pm 0.01^{8}$	$76.4^{2} \pm 0.3^{6}$	$75.0^{2} \pm 0.3^{6}$	75.0 ± 0.4	100.0 ± 0.4
2	$3.82^{1} \pm 0.01^{8}$	$76.4^{2} \pm 0.3^{6}$	$74.7^{5} \pm 0.3^{6}$	75.0 ± 0.4	99.7 ± 0.4
3	$3.79^{4} \pm 0.01^{8}$	$75.8^{8} \pm 0.3^{6}$	$74.1^{4} \pm 0.3^{6}$	75.0 ± 0.4	98.8 ± 0.4
4	$3.82^{1} \pm 0.02^{7}$	$76.4^{2} \pm 0.5^{4}$	$74.5^{3} \pm 0.5^{4}$	75.0 ± 0.4	99.4 ± 0.7
5	$3.87^{5} \pm 0.01^{8}$	$77.5^{0} \pm 0.3^{6}$	$75.7^{2} \pm 0.3^{6}$	75.0 ± 0.4	101.0 ± 0.4
F-1	$5.01^{3} \pm 0.01^{8}$	$100.2^{6} \pm 0.3^{6}$	$98.4^{5} \pm 0.3^{6}$	100.0 ± 0.4	98.4 ± 0.3
2	$4.95^{9} \pm 0.02^{7}$	$99.1^{8} \pm 0.5^{4}$	$97.2^{6} \pm 0.5^{4}$	100.0 ± 0.4	97.3 ± 0.5
3	$5.04^{1} \pm 0.01^{8}$	$100.9^{6} \pm 0.3^{6}$	$98.9^{6} \pm 0.3^{6}$	100.0 ± 0.4	99.0 ± 0.3
4	$4.95^{9} \pm 0.01^{8}$	$99.1^{8} \pm 0.3^{6}$	$97.3^{4} \pm 0.3^{6}$	100.0 ± 0.4	97.3 ± 0.3
5	$4.95^{9} \pm 0.02^{7}$	$99.1^{8} \pm 0.5^{4}$	$97.2^{0} \pm 0.5^{4}$	100.0 ± 0.4	97.2 ± 0.5

* Based on the higher of the two uncertainties:- fish base nickel or total nickel in 20 ml.

** Using the linear equation from Page 184.

If the average value for each of the Series B to F is taken with respect to the "Total Ni (ug/20 ml)" column data we have, omitting samples B-1 and B-3:-

Series	B	$17.3^4 \pm 0.3^6$
	C	$32.3^8 \pm 0.3^6$
	D	$52.5^7 \pm 0.8^6$
	E	$76.5^2 \pm 0.5^9$
	F	$99.7^5 \pm 0.8^2$

Linear regression analysis yields:- $r = 0.9998^2$, $b = 3.31$, $m = 0.970^3$

Thus the zero intercept on the ordinate yields a value of 3.31 ug Ni/20 ml solution or, for sample weights approximating 1.50 g of dried fish base, 2.21 ug Ni/g dried fish base. Carrying out the reverse calculations indicates an uncertainty for this value of $\pm$ 0.5 ug Ni/g fish, so that we have, from the method of additions, a calculated value of dried rainbow trout base content of nickel of 2.2 ± 0.5 ug/g fish base. This does not quite compare, within the respective tolerance limits, with the value of $1.2^7 \pm 0.2^2$ ug Ni/g dried fish base reported above. This difference can be attributed to the wide variation in sample weights used in the spiking determination. The difference although significant, is not large.

Using the value of 2.2 ± 0.5 ug Ni/g dried fish base as the correction value to the "Total Ni (ug/20 ml)" data column yields, omitting results B-1 and B-3; an average recovery percent for spikes up to 100 ug on 1.5 g of dried fish base of $97.^0 \pm 1.^9$. This compares well, within the respective tolerance limits, with the value of $100.^4 \pm 2.^0$ % reported on the previous page.

By including the average result of Series A to the average for each of the Series B to F with respect to the "Total Ni (ug/20 ml)" data column from Table C-8 in a graphical determination of nickel in dried rainbow trout by the method of additions, the plot on Figure C-2 was obtained. The nickel content in fish tissue can be extrapolated to the abscissa to 2.4 ± 0.3 ug Ni/1.5 g dried fish base. The $\pm$ 0.3 value represents the reading uncertainty on the graph. The extrapolated result corresponds to 1.6 ± 0.2 ug Ni/g dried fish base. This compares well, within the respective tolerance limits, with the value of $1.2^7 \pm 0.2^2$ ug Ni/g dried fish base reported on Page 185.

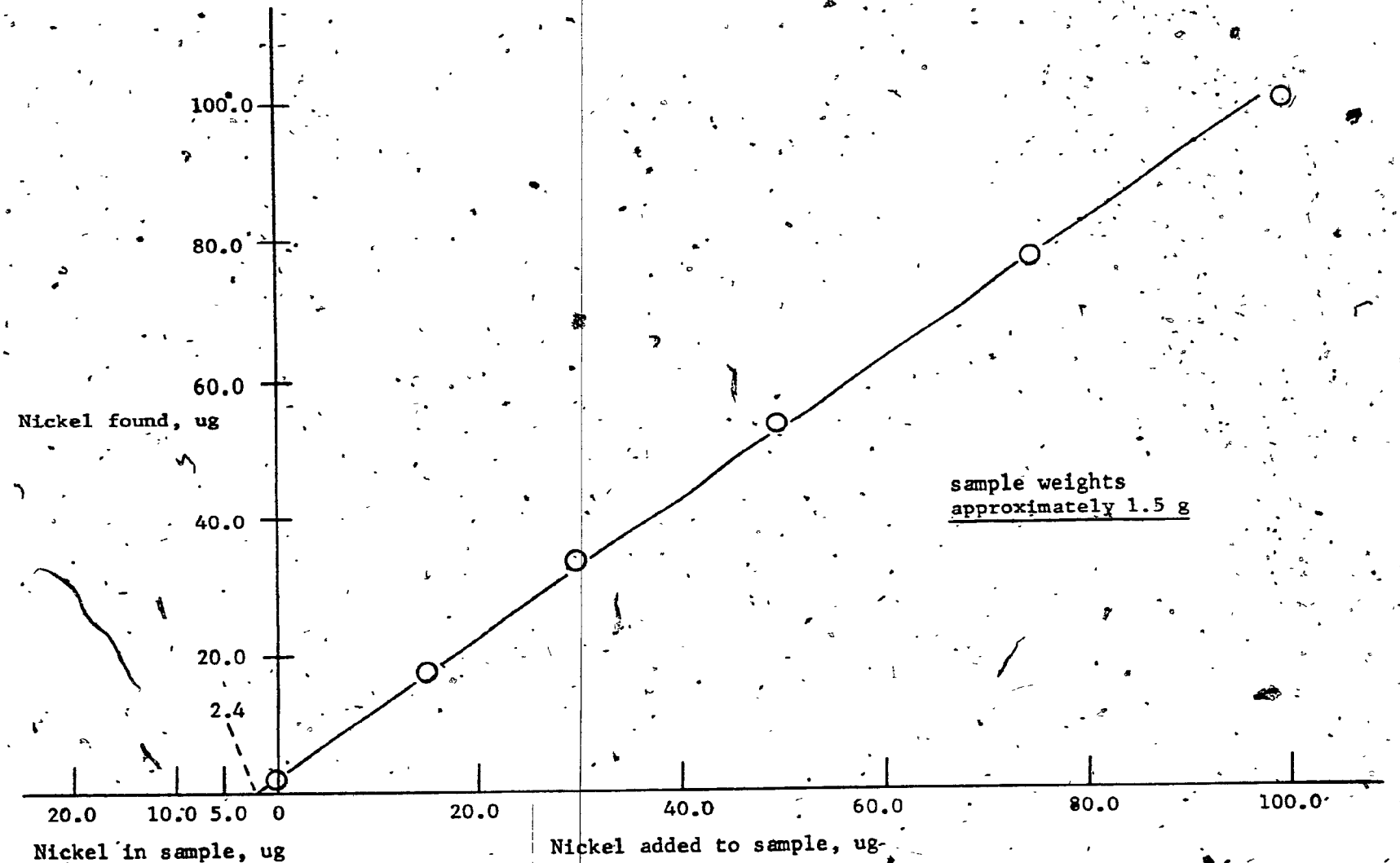


FIGURE C-2 GRAPHICAL METHOD OF ADDITIONS - NICKEL DETERMINATION IN RAINBOW TROUT - DRY ASHING

4. Recovery of Nickel from Aliquoted Spiked Fish Base Samples

Two sets of macerated and dried rainbow trout tissue were weighed out in duplicate. Each sample consisted of 2.0000 ± 0.0002 g and was placed in a 50-ml beaker.

One set (A) was spiked with 70.0 ± 0.4 ug Ni each, while the second set (B) was spiked with 90.0 ± 0.4 ug Ni each.

All samples were dried, ashed and extracted according to the details of Sub-section 2.2.3 - (b). A blank was carried through simultaneously. The amount of HCl pipetted was 0.50 ± 0.01 ml while the amount of water pipetted was 19.50 ± 0.04 ml.

For each set, 10 ml of the final solution was tested as-is, and 10 ml was diluted to 20 ml with 2.5 percent HCl and then tested. The following results were obtained:-

Set	Ave. absorbance corr. for blank		Total Ni* (ug/20 ml)		Ni corr. for** base fish (ug/g)		Actual spike (ug)	Recovery (%)	
	as-is	aliq.	as-is	aliqx2	as-is	aliqx2		as-is	aliq.
A	0.140	0.071	75.9	77.0	73.3	74.4	70.0	105	106
B	0.170	0.086	92.1	93.2	89.5	90.6	90.0	99	101

* Using the linear equation from Page 184.

** Using the correction factor of 1.3 ug Ni/g dried rainbow trout base.

The recovery percent values showed limited but reasonable agreement, within the expected tolerance ranges for nickel recovery on spiked samples, so that aliquoting of tissue sample solutions, suspected to contain nickel in ppm beyond the linear range maximum, should present no difficulty.

APPENDIX C-3

Determination of Zinc in Rainbow Trout Tissue by the Dry-Ashing/Flame Atomic Absorption Technique

1. General

The following sections outline the experimental approach applied to the testing, recovery from spiking and recovery from aliquoting of zinc in rainbow trout base tissue.

The flame method of atomic absorption analysis was applied, rather than the graphite furnace technique, because of the relatively high zinc content found in rainbow trout unexposed to experimentally high zinc levels in the water and/or food.

General operating conditions

Certain general operating conditions were maintained throughout the analytical procedures. These are outlined in Appendix A-3.

Glassware was washed several times with detergent soap solution, soaked overnight in a 1:1 HCl/HNO_3 solution and rinsed several times with glass-distilled water.

Preparation of aqueous-base standard solutions

Appendix A-3 outlines the preparation of the aqueous-base standard solutions. Additional working standard solutions were prepared as follows:-

Solution preparation*	Zinc (ppm)
2.00 ± 0.02 ml of 1.000 ± 0.004 ppm Zn	0.0200 ± 0.0003
3.00 ± 0.02 ml " " " "	0.0300 ± 0.0003
5.00 ± 0.02 ml " " " "	0.0500 ± 0.0004
7.50 ± 0.02 ml " 10.00 ± 0.02 " "	0.750 ± 0.004

* All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Preparation of fish tissue

The preparation of rainbow trout base tissue is described in Sub-section 2.2.3 - (a).

2. Testing of Dry Ashed Rainbow Trout Base Tissue

Dry ashing of fish tissue

The general procedure is outlined in Sub-section 2.2.3 - (b). The specific details for the determination of zinc are given below.

The sample weights were as follows:-

Sample No.	Fish weight (g)
1.	0.0000
2.	0.1005 ± 0.0002
3.	0.1000 ± 0.0002
4.	0.1004 ± 0.0002
5.	0.1502 ± 0.0002
6.	0.1505 ± 0.0002
7.	0.1497 ± 0.0002
8.	0.3016 ± 0.0002
9.	0.3006 ± 0.0002
10.	0.3042 ± 0.0002
11.	0.5000 ± 0.0002
12.	0.4999 ± 0.0002
13.	0.5013 ± 0.0002
14.	0.6000 ± 0.0002
15.	0.5987 ± 0.0002
16.	0.5989 ± 0.0002
17.	0.7530 ± 0.0002
18.	0.7514 ± 0.0002
19.	0.7504 ± 0.0002
20.	1.0007 ± 0.0002
21.	1.0040 ± 0.0002
22.	1.0032 ± 0.0002

The amount of HCl pipetted was,

$$2.00 \pm 0.02 \text{ ml}$$

Each sample was made up to 100 ml in a 100.00 ± 0.04 ml volumetric flask.

Testing of working standards by flame atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Zinc (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.0200 ± 0.0003	0.003 ± 0.000
0.0500 ± 0.0004	0.009 ± 0.000
0.100 ± 0.002	0.021 ± 0.000
0.300 ± 0.003	0.065 ± 0.000
0.500 ± 0.003	0.108 ± 0.000
0.700 ± 0.004	0.151 ± 0.001
0.900 ± 0.004	0.194 ± 0.001
1.000 ± 0.004	0.217 ± 0.001

* Average of five 3-seconds integration readings.
± values are standard deviations.

Linear regression analysis

0.0000 - 1.000 ppm Zn

$r = 0.9999^7$
$b = -0.000^8$
$m = 0.2173^1$

Sensitivity (1% absorption) = $0.0044/0.2173^1$ = 0.02 ppm Zn

Reverse calculations for zinc

Actual zinc (ppm)	Calculated zinc (ppm)	Absolute devn.
0.000	0.003	0.003
0.0200 ± 0.0003	0.017	0.003
0.0500 ± 0.0004	0.045	0.005
0.100 ± 0.002	0.100	0.000
0.300 ± 0.003	0.303	0.003
0.500 ± 0.003	0.501	0.001
0.700 ± 0.004	0.698	0.002
0.900 ± 0.004	0.896	0.004
1.000 ± 0.004	1.002	0.002
	Average absolute deviation	0.002

Values of zinc (ppm) from the above equation subject to ± 0.002 ppm.

Results of zinc determination in dry ashed fish base tissue

The results of the blank and fish base samples are tabulated in Table C-9.

The 2s criterion rejects the $140.^5$ value of sample 5. The remaining 21 values recorded in the last column yield an average of,

$$121.^1 \pm 3.^9 \text{ ppm Zn}$$

or,

$$121.^1 \pm 3.^9 \text{ ug Zn/g dried rainbow trout base}$$

3. Recovery of Zinc from Spiked Fish Base Samples

Dry ashing of fish tissue

The weights of macerated and dried fish tissue weighed into 50-ml beakers are given in Table C-10.

These fish tissue samples were spiked with 10.00 ± 0.02 ppm Zn solution as follows:-

Series	Spike	ug Zn added
A	none	0.0
B	1.00 ± 0.02 ml of 10.00 ppm Zn	10.0 ± 0.2
C	2.00 ± 0.02 ml " " " "	20.0 ± 0.2
D	3.00 ± 0.02 ml " " " "	30.0 ± 0.3
E	5.00 ± 0.02 ml " " " "	50.0 ± 0.3
F	6.00 ± 0.02 ml " " " "	60.0 ± 0.3
G	7.00 ± 0.02 ml " " " "	70.0 ± 0.3

Subsequent to spiking each sample was placed in the oven and heated at just under 100 °C until the liquid spike volume had dried out. The subsequent ashing procedure duplicated the details of Sub-section 2.2.3 - (b). A blank with no spike was carried through the same procedure.

The amount of HCl pipetted was,

$$2.00 \pm 0.02 \text{ ml}$$

The samples were made up to 100 ml in a 100.00 ± 0.04 ml volumetric flask.

TABLE C-9

DETERMINATION OF ZINC IN RAINBOW TROUT BASE TISSUE
BY DRY ASHING/FLAME ATOMIC ABSORPTION TECHNIQUE

Sample No.	Average* absorbance	Corrected average absorbance	**Zn in 100 ml soln. (ppm)	Zinc ug/100 ml	Zinc in fish base (ppm)
1.	0.005 ± 0.000	0.000 ± 0.000	---	---	---
2.	0.030 ± 0.000	0.025 ± 0.000	0.119 ± 0.002	11.9 ± 0.2	118.4 ± 2.2
3.	0.030 ± 0.000	0.025 ± 0.000	0.119 ± 0.002	11.9 ± 0.2	119.0 ± 2.2
4.	0.030 ± 0.000	0.025 ± 0.000	0.119 ± 0.002	11.9 ± 0.2	118.5 ± 2.2
5.	0.050 ± 0.000	0.045 ± 0.000	0.211 ± 0.002	21.1 ± 0.2	140.5 ± 1.5
6.	0.045 ± 0.000	0.040 ± 0.000	0.188 ± 0.002	18.8 ± 0.2	124.9 ± 1.5
7.	0.043 ± 0.000	0.038 ± 0.000	0.179 ± 0.002	17.9 ± 0.2	119.3 ± 1.5
8.	0.082 ± 0.000	0.077 ± 0.000	0.358 ± 0.002	35.8 ± 0.2	118.7 ± 0.7
9.	0.086 ± 0.000	0.081 ± 0.000	0.376 ± 0.002	37.6 ± 0.2	125.2 ± 0.7
10.	0.084 ± 0.000	0.079 ± 0.000	0.367 ± 0.002	36.7 ± 0.2	120.6 ± 0.7
11.	0.140 ± 0.000	0.135 ± 0.000	0.625 ± 0.002	62.5 ± 0.2	125.0 ± 0.4
12.	0.143 ± 0.000	0.138 ± 0.000	0.639 ± 0.002	63.9 ± 0.2	127.8 ± 0.4
13.	0.142 ± 0.000	0.137 ± 0.000	0.634 ± 0.002	63.4 ± 0.2	126.5 ± 0.4
14.	0.161 ± 0.000	0.156 ± 0.000	0.722 ± 0.002	72.2 ± 0.2	120.3 ± 0.4
15.	0.168 ± 0.000	0.163 ± 0.000	0.754 ± 0.002	75.4 ± 0.2	125.7 ± 0.4
16.	0.167 ± 0.000	0.162 ± 0.000	0.749 ± 0.002	74.9 ± 0.2	125.1 ± 0.4
17.	0.200 ± 0.000	0.195 ± 0.000	0.901 ± 0.002	90.1 ± 0.2	119.6 ± 0.3
18.	0.205 ± 0.000	0.200 ± 0.000	0.924 ± 0.002	92.4 ± 0.2	123.0 ± 0.3
19.	0.195 ± 0.000	0.190 ± 0.000	0.878 ± 0.002	87.8 ± 0.2	117.0 ± 0.3
20.	0.256 ± 0.000	0.251 ± 0.000	1.159 ± 0.002	115.9 ± 0.2	115.8 ± 0.2
21.	0.262 ± 0.000	0.257 ± 0.000	1.186 ± 0.002	118.6 ± 0.2	118.1 ± 0.2
22.	0.253 ± 0.000	0.248 ± 0.000	1.145 ± 0.002	114.5 ± 0.2	114.1 ± 0.2

* Average and standard deviation of five 3-seconds integration readings.

** Using the linear equation from Page 194.

TABLE C-10

SAMPLE WEIGHTS OF BASE FISH TISSUE FOR ZINC DRY ASHING SPIKING PROCEDURE

No.	Series A	Series B	Series C	Series D
1.	0.2000 ± 0.0002	0.2000 ± 0.0002	0.2000 ± 0.0002	0.2010 ± 0.0002
2.	0.2000 ± 0.0002	0.2000 ± 0.0002	0.2000 ± 0.0002	0.2009 ± 0.0002
3.	0.2000 ± 0.0002	0.2000 ± 0.0002	0.2000 ± 0.0002	0.2000 ± 0.0002
4.	0.2000 ± 0.0002	0.2002 ± 0.0002	0.2000 ± 0.0002	0.2000 ± 0.0002
5.	0.2000 ± 0.0002	0.2003 ± 0.0002	0.2000 ± 0.0002	0.1998 ± 0.0002

No.	Series E	Series F	Series G
1.	0.2004 ± 0.0002	0.2000 ± 0.0002	0.2007 ± 0.0002
2.	0.2000 ± 0.0002	0.2000 ± 0.0002	0.1998 ± 0.0002
3.	0.2000 ± 0.0002	0.2000 ± 0.0002	0.2000 ± 0.0002
4.	0.2000 ± 0.0002	0.2000 ± 0.0002	0.2000 ± 0.0002
5.	0.2000 ± 0.0002	0.2000 ± 0.0002	0.2002 ± 0.0002

Testing of working standards by flame atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Zinc (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.0300 ± 0.0003	0.004 ± 0.000
0.0500 ± 0.0004	0.009 ± 0.001
0.100 ± 0.002	0.020 ± 0.000
0.300 ± 0.003	0.064 ± 0.001
0.500 ± 0.003	0.111 ± 0.001
0.750 ± 0.004	0.166 ± 0.001
0.900 ± 0.004	0.200 ± 0.001
1.000 ± 0.004	0.224 ± 0.001

* Average and standard deviation of five 3-seconds integration readings.

Linear regression analysis

0.000 - 1.000 ppm Zn

$r = 0.9999^2$
$b = -0.002$
$m = 0.2250^0$

Sensitivity (1% absorption) = $0.0044/0.2250^0$ = 0.02 ppm Zn

Reverse calculations for zinc

Actual zinc (ppm)	Calculated zinc (ppm)	Absolute devn.
0.000	0.009	0.009
0.0300 ± 0.0003	0.027	0.003
0.0500 ± 0.0004	0.049	0.001
0.100 ± 0.002	0.098	0.002
0.300 ± 0.003	0.293	0.007
0.500 ± 0.003	0.502	0.002
0.750 ± 0.004	0.747	0.003
0.900 ± 0.004	0.898	0.002
1.000 ± 0.004	1.004	0.004
	Average absolute deviation	0.004

Values of zinc (ppm) from the above equation subject to ± 0.004 ppm.

Results of zinc determination of spiked dry ashed fish base tissue

The absorbance results of the blank and fish base samples spiked with zinc (ug) are shown in Table C-11.

The calculated zinc recoveries for Series B to C are tabulated in Table C-12.

The values for the 100 ml each Series A samples yield the following results:-

Series and No.	Zn in 100 ml soln. (ppm)	Zn (ug/g) in fish base
A-1	0.236 ± 0.004	$118.^{0} \pm 2.^{1}$
2	0.240 ± 0.008	$120.^{0} \pm 4.^{1}$
3	0.244 ± 0.008	$122.^{0} \pm 4.^{1}$
4	0.236 ± 0.004	$118.^{0} \pm 2.^{1}$
5	0.231 ± 0.008	$115.^{5} \pm 4.^{1}$

These again represent fish base values and, combined with the 20 values (excluding the $140.^{5}$ ug Zn/g result) obtained under Appendix C-3 - (2) Testing of Dry Ashed Rainbow Trout Base Tissue, provide a total of 25 results. These results yield a final value for the zinc content of dried (65 ± 5 °C) rainbow trout unexposed to experimentally high zinc contents in the water and/or food of:-

$$120.^{6} \pm 3.^{8} \text{ ppm or } 120.^{6} \pm 3.^{8} \text{ ug Zn/g dried rainbow trout base}$$

An overall average recovery percent was obtained for zinc spikes on rainbow trout dried base of up to 70.0 ug/0.2 g of:-

$$101.^{3} \pm 6.^{7} \%$$

TABLE C-11

DRY ASHING/FLAME ATOMIC ABSORPTION TECHNIQUE

RESULTS OF ZINC DETERMINATION
ON SPIKED FISH BASE SAMPLES

Series and No.	Average* absorbance	Series and No.	Average* absorbance
blank	0.000 ± 0.000	D-3	0.116 ± 0.000
A-1	0.051 ± 0.000	4	0.119 ± 0.000
2	0.052 ± 0.001	5	0.127 ± 0.000
3	0.053 ± 0.001	E-1	0.166 ± 0.001
4	0.051 ± 0.000	2	0.162 ± 0.000
5	0.050 ± 0.001	3	0.156 ± 0.001
B-1	0.074 ± 0.000	4	0.163 ± 0.000
2	0.073 ± 0.000	5	0.166 ± 0.001
3	0.075 ± 0.000	F-1	0.184 ± 0.000
4	0.077 ± 0.000	2	0.186 ± 0.001
5	0.078 ± 0.000	3	0.188 ± 0.000
C-1	0.096 ± 0.000	4	0.189 ± 0.000
2	0.096 ± 0.000	5	0.183 ± 0.001
3	0.097 ± 0.000	G-1	0.206 ± 0.001
4	0.103 ± 0.000	2	0.204 ± 0.000
5	0.095 ± 0.000	3	0.212 ± 0.000
D-1	0.119 ± 0.001	4	0.225 ± 0.001
2	0.130 ± 0.001	5	0.231 ± 0.001

* Absorbance same as Corrected absorbance

Average and standard deviation of five 3-seconds integration readings.

TABLE C-12

DRY ASHING/FLAME ATOMIC ABSORPTION TECHNIQUE

RECOVERY OF ZINC IN SPIKED FISH BASE SAMPLES

Series and No.	Zn in 100 ml soln. (ppm)**	Total Zn (ug/100ml)	Zn C. F. B.* (ug/100 ml)	Actual Zn spike (ug)	Recovery of Zn (%)
B-1	0.338 ± 0.004	33.8 ± 0.4	9.6^{8} ± 0.8	10.0 ± 0.2	$96.^{8}$ ± $8.^{0}$
2	0.333 ± 0.004	33.3 ± 0.4	9.1^{8} ± 0.8	10.0 ± 0.2	$91.^{8}$ ± $8.^{0}$
3	0.342 ± 0.004	34.2 ± 0.4	10.0^{8} ± 0.8	10.0 ± 0.2	$100.^{8}$ ± $8.^{0}$
4	0.351 ± 0.004	35.1 ± 0.4	10.9^{5} ± 0.8	10.0 ± 0.2	$109.^{5}$ ± $8.^{0}$
5	0.356 ± 0.004	35.6 ± 0.4	11.4^{4} ± 0.8	10.0 ± 0.2	$114.^{4}$ ± $8.^{0}$
C-1	0.436 ± 0.004	43.6 ± 0.4	19.4^{8} ± 0.8	20.0 ± 0.2	$97.^{4}$ ± $4.^{0}$
2	0.436 ± 0.004	43.6 ± 0.4	19.4^{8} ± 0.8	20.0 ± 0.2	$97.^{4}$ ± $4.^{0}$
3	0.440 ± 0.004	44.0 ± 0.4	19.8^{8} ± 0.8	20.0 ± 0.2	$99.^{4}$ ± $4.^{0}$
4	0.467 ± 0.004	46.7 ± 0.4	22.5^{8} ± 0.8	20.0 ± 0.2	$112.^{9}$ ± $4.^{0}$
5	0.431 ± 0.004	43.1 ± 0.4	18.9^{8} ± 0.8	20.0 ± 0.2	$94.^{9}$ ± $4.^{0}$
D-1	0.538 ± 0.008	53.8 ± 0.8	29.9^{5} ± 0.8	30.0 ± 0.3	$98.^{5}$ ± $2.^{7}$
2	0.587 ± 0.008	58.7 ± 0.8	34.4^{7} ± 0.8	30.0 ± 0.3	$114.^{9}$ ± $2.^{7}$
3	0.525 ± 0.004	52.5 ± 0.4	28.3^{8} ± 0.8	30.0 ± 0.3	$94.^{6}$ ± $2.^{7}$
4	0.538 ± 0.004	53.8 ± 0.4	29.6^{8} ± 0.8	30.0 ± 0.3	$98.^{9}$ ± $2.^{7}$
5	0.574 ± 0.004	57.4 ± 0.4	33.3 ± 0.8	30.0 ± 0.3	$111.^{0}$ ± $2.^{7}$
E-1	0.747 ± 0.008	74.7 ± 0.8	50.5^{3} ± 0.8	50.0 ± 0.3	$101.^{1}$ ± $1.^{6}$
2	0.729 ± 0.004	72.9 ± 0.4	48.7^{8} ± 0.8	50.0 ± 0.3	$97.^{6}$ ± $1.^{6}$
3	0.702 ± 0.008	70.2 ± 0.8	46.0^{8} ± 0.8	50.0 ± 0.3	$92.^{2}$ ± $1.^{6}$
4	0.734 ± 0.004	73.4 ± 0.4	49.2^{8} ± 0.8	50.0 ± 0.3	$98.^{6}$ ± $1.^{6}$
5	0.747 ± 0.008	74.7 ± 0.8	50.5^{8} ± 0.8	50.0 ± 0.3	$101.^{2}$ ± $1.^{6}$
F-1	0.827 ± 0.004	82.7 ± 0.4	58.5^{8} ± 0.8	60.0 ± 0.3	$97.^{6}$ ± $1.^{3}$
2	0.836 ± 0.008	83.6 ± 0.8	59.4^{8} ± 0.8	60.0 ± 0.3	$99.^{1}$ ± $1.^{3}$
3	0.845 ± 0.004	84.5 ± 0.4	60.3^{8} ± 0.8	60.0 ± 0.3	$100.^{6}$ ± $1.^{3}$
4	0.849 ± 0.004	84.9 ± 0.4	60.7^{8} ± 0.8	60.0 ± 0.3	$101.^{3}$ ± $1.^{3}$
5	0.822 ± 0.008	82.2 ± 0.8	58.0^{8} ± 0.8	60.0 ± 0.3	$96.^{8}$ ± $1.^{3}$
G-1	0.925 ± 0.008	92.5 ± 0.8	68.2^{9} ± 0.8	70.0 ± 0.3	$97.^{6}$ ± $1.^{1}$
2	0.916 ± 0.004	91.6 ± 0.4	67.5 ± 0.8	70.0 ± 0.3	$96.^{4}$ ± $1.^{1}$
3	0.952 ± 0.004	95.2 ± 0.4	71.0^{8} ± 0.8	70.0 ± 0.3	$101.^{5}$ ± $1.^{1}$
4	1.009 ± 0.008	100.9 ± 0.8	76.7^{8} ± 0.8	70.0 ± 0.3	$109.^{7}$ ± $1.^{1}$
5	1.036 ± 0.008	103.6 ± 0.8	79.4^{5} ± 0.8	70.0 ± 0.3	$113.^{5}$ ± $1.^{1}$

*corrected fish base

Based on the higher of the two uncertainties:- fish base or total zinc in 100 ml.

**Using the linear equation from Page 198.

If the average value for each of the Series B to G is taken with respect to the "Total Zn (ug/100 ml)" column data we have,

Series	B	$34.4^{0} \pm 0.9^{4}$
	C	$44.^{2} \pm 1.^{4}$
	D	$55.^{2} \pm 2.^{6}$
	E	$73.^{2} \pm 1.^{8}$
	F	$83.^{6} \pm 1.^{1}$
	G	$96.^{8} \pm 5.^{3}$

Linear regression analysis yields:- $r = 0.9986$, $b = 24.0$, $m = 1.014^{3}$

Thus the zero intercept on the ordinate yields a value of 24.0 ug Zn/100 ml of solution or, for sample weights approximating 0.20 g of dried fish base, 120.0 ug Zn/g fish tissue. Carrying out reverse calculations indicates an uncertainty for these values respectively of $\pm$ 0.8 ug Zn/100 ml and $\pm$ 4.0 ug Zn/g, so that we have, from the method of additions, a calculated value of dried rainbow trout base content of zinc of $120.^{0} \pm 4.^{0}$ ug/g fish base. This compares quite well, within the respective tolerance limits, with the value of $120.^{6} \pm 3.^{8}$ ug Zn/ g dried fish base reported on the previous page.

Using the value of $120.^{0} \pm 4.0$ ug Zn/g dried fish base as the correction value to the "Total Zn (ug/100 ml)" data column yields an average recovery percent for spikes up to 70.0 ug on 0.2 g of dried fish base of $101.^{7} \pm 6.^{8}$. This agrees well, within the respective tolerance limits, with the value of $101.^{3} \pm 6.^{7}$ % reported on the previous page.

By including the average result of Series A to the average for each of the Series B to G with respect to the "Total Zn (ug/100 ml)" data column from Table C-12 in a graphical determination of zinc in dried rainbow trout by the method of additions, the plot on Figure C-3 was obtained. The zinc content in dried whole base tissue can be extrapolated to the abscissa to 24.0 $\pm$ 0.2 ug Zn/0.2 grams of dried whole tissue. The $\pm$ 0.2 value represents the reading uncertainty on the graph. The extrapolated value corresponds to $120.^{0} \pm 1.^{0}$ ug Zn/g dried rainbow trout base. This compares quite well, within the respective tolerance limits, with the value of $120.^{6} \pm 3.^{8}$ ug Zn/g dried fish base reported on Page 199.

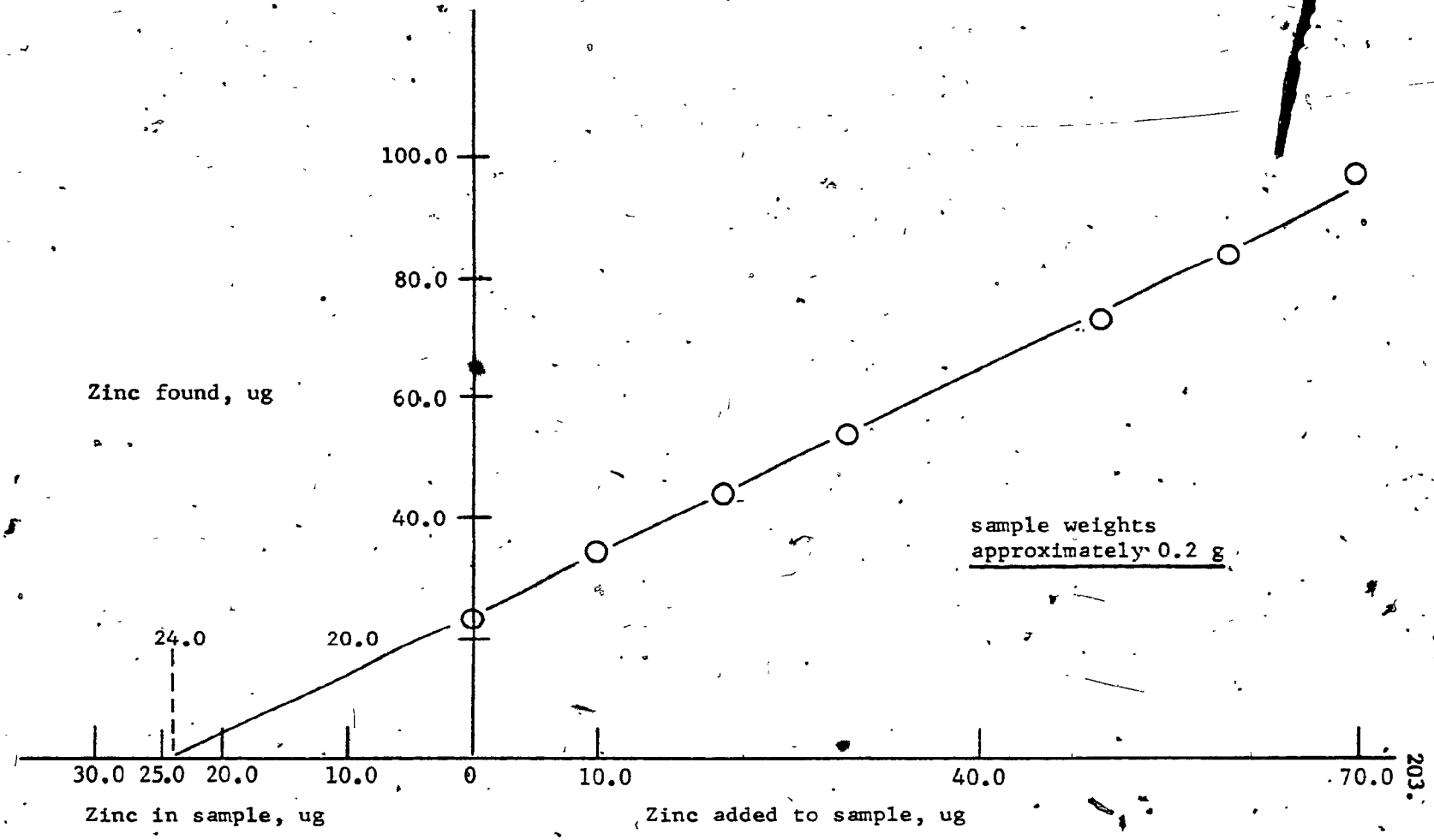


FIGURE C-3 GRAPHICAL METHOD OF ADDITIONS - ZINC DETERMINATION IN RAINBOW TROUT - DRY ASHING

4. Recovery of Zinc from Aliquoted Fish Base Samples

Two sets of macerated and dried rainbow trout tissue were weighed out in duplicate in 50-ml beakers. One set (A) consisted of 0.4999 ± 0.0002 g, while set (B) consisted of 1.0007 ± 0.0002 g of fish tissue.

All samples were ashed and extracted according to the details of Sub-section 2.2.3 - (b). A blank was carried through simultaneously. The amount of HCl was 2.00 ± 0.02 ml and each sample was made up to the mark in a 100.00 ± 0.04 ml volumetric flask.

Set (A) - tested as-is
- diluted 5 ml to 10 ml with 2% HCl
- diluted 2 ml to 12 ml with 2% HCl

Set (B) - tested as-is
- diluted 5 ml to 15 ml with 2% HCl
- diluted 5 ml to 25 ml with 2% HCl

The results obtained are given in Table C-13.

The recovery percent values showed limited but reasonable agreement, within the expected tolerance ranges for the determination of zinc. Therefore, aliquoting of tissue sample solutions, suspected to contain zinc in ppm beyond the linear range maximum, should present no difficulty.

TABLE C-13

RECOVERY OF ZINC FROM ALIQUOTED FISH BASE SAMPLES

Set	Average absorbance corrected for blank			Total Zn* (ug/100 ml)			Zn (ug/g) fish base			Recovery (%)**		
	as-is	aliq.x2	aliq.x6	as-is	aliq.x2	aliq.x6	as-is	aliq.x2	aliq.x6	as-is	aliq.x2	aliq.x6
A	0.138	0.069	0.023	62.2	63.1	66.7	124.4	126.2	133.0	103	104	110

Set	Average absorbance corrected for blank			Total Zn* (ug/100 ml)			Zn (ug/g) fish base			Recovery (%)**		
	as-is	aliq.x3	aliq.x5	as-is	aliq.x3	aliq.x5	as-is	aliq.x3	aliq.x5	as-is	aliq.x3	aliq.x5
B	0.251	0.084	0.049	112.4	114.7	113.3	112.3	114.6	113.2	93	95	94

* Using the linear equation from Page 198.

** Based on $120.^{6} \pm 3.^{8}$ ug Zn/g dried rainbow trout base value from Page 199.

APPENDIX C-4

Determination of Lead in Rainbow Trout Tissue by the Dry-Ashing/ Graphite Furnace Technique

1. General

The following sections outline the experimental approach applied to the testing, recovery from spiking and recovery from aliquoting of lead in rainbow trout base tissue.

The graphite furnace/atomic absorption method of analysis was applied because of the relatively low lead content found for rainbow trout unexposed to experimentally high lead levels in the water and/or food.

General operating conditions

Certain general operating conditions were maintained throughout the analytical procedures. These are outlined in Appendix B-3. The only differences involved were:-

Instrument:-	Perkin-Elmer Model 503 Atomic Absorption Spectrophotometer with HGA 2000 Graphite Furnace
Flush system:-	none

Preparation of aqueous-base standard solutions

Appendix B-3 outlines the preparation of the aqueous-base standard solutions. Additional working standard solutions were prepared as follows:-

Solution preparation*	Lead (ppm)
2.00 ± 0.02 ml of 0.100 ± 0.001 ppm Pb	0.0020 ± 0.0001
3.00 ± 0.02 ml " " " "	0.0030 ± 0.0001

* All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Preparation of fish tissue

The preparation of rainbow trout base tissue is described in Sub-section 2.2.3 - (a).

2. Testing of Dry Ashed Rainbow Trout Base Tissue

The general procedure is outlined in Sub-section 2.2.3 - (b). The specific details for the determination of lead are given below.

The sample weights were as follows:-

Sample No.	Fish weight (g)
1.	0.0000
2.	0.5141 ± 0.0002
3.	0.5013 ± 0.0002
4.	0.5211 ± 0.0002
5.	1.0031 ± 0.0002
6.	1.0039 ± 0.0002
7.	1.0006 ± 0.0002
8.	1.5038 ± 0.0002
9.	1.5107 ± 0.0002
10.	1.5016 ± 0.0002
11.	2.0028 ± 0.0002
12.	2.0138 ± 0.0002
13.	1.9963 ± 0.0002

The amount of HCl pipetted was,

$$1.50 \pm 0.02 \text{ ml}$$

Each sample was made up to the mark in a 100.00 ± 0.04 ml volumetric flask.

Testing of working standards by graphite furnace atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Lead (ppm)	Average absorbance*	Corr. ave. abs.
0.000	0.025 ± 0.000	0.000 ± 0.000
0.0030 ± 0.0001	0.045 ± 0.001	0.020 ± 0.001
0.0050 ± 0.0001	0.080 ± 0.001	0.055 ± 0.001
0.0100 ± 0.0001	0.117 ± 0.003	0.092 ± 0.003
0.0200 ± 0.0004	0.206 ± 0.005	0.181 ± 0.005
0.0300 ± 0.0005	$0.28^0 \pm 0.01^0$	$0.25^5 \pm 0.01^0$
0.0400 ± 0.0006	0.365 ± 0.008	0.340 ± 0.008

* Average and standard deviation of five peak integration readings.

Linear regression analysis

0.000 - 0.0400 ppm Pb

$r = 0.9986$
$b = 0.004$
$m = 8.463^6$

Sensitivity (1% absorption) = $0.0044/8.463^6$ = 0.001 ppm Pb

Reverse calculations for lead

Actual lead (ppm)	Calculated lead (ppm)	Absolute devn.
0.0000	0.0004	0.0004
0.0030 ± 0.0001	0.0020	0.0010
0.0050 ± 0.0001	0.0060	0.0010
0.0100 ± 0.0001	0.0100	0.0000
0.0200 ± 0.0004	0.0210	0.0010
0.0300 ± 0.0005	0.0300	0.0000
0.0400 ± 0.0006	0.0400	0.0000
	Average absolute deviation	0.0004

Values of lead (ppm) from the above equation subject to ± 0.0004 ppm.

Results of lead determination in dry ashed fish base tissue

The results of the blank and fish base samples are tabulated in Table C-14.

Samples with fish weight (dry) of up to 4.00 g were prepared and analyzed but the results were discarded due to large interfering effects. After injection of the sample in the graphite furnace, at the charring step, the D_2 beam corrector energy would drop below that of the hollow cathode lead lamp resulting in erratic results. This may be due to the large quantities of interfering material not fully ashed.

From the 12 values recorded in the last column of Table C-14 an average of,

0.84 ± 0.08 ppm Pb

or,

0.84 ± 0.08 ug Pb/g dried rainbow trout base

TABLE C-14

DETERMINATION OF LEAD IN RAINBOW TROUT BASE TISSUE
BY DRY ASHING/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

Sample No.	Average* absorbance	Corrected average absorbance	**Pb in 100 ml soln. (ppm)	Lead ug/100 ml	Lead in fish base (ppm)
1.	0.040 ± 0.000	0.000 ± 0.000	---	---	---
2.	0.076 ± 0.000	0.036 ± 0.000	0.0038 ± 0.0004	0.38 ± 0.04	0.7^{6} ± 0.08
3.	0.077 ± 0.001	0.037 ± 0.001	0.0039 ± 0.0008	0.39 ± 0.08	0.7^{8} ± 0.1^{6}
4.	0.084 ± 0.000	0.044 ± 0.000	0.0047 ± 0.0004	0.47 ± 0.04	0.90 ± 0.08
5.	0.129 ± 0.002	0.089 ± 0.002	0.010 ± 0.001	1.0 ± 0.1	1.0 ± 0.1
6.	0.108 ± 0.001	0.068 ± 0.001	0.0076 ± 0.0008	0.76 ± 0.08	0.76 ± 0.08
7.	0.120 ± 0.001	0.080 ± 0.001	0.0090 ± 0.0008	0.90 ± 0.08	0.90 ± 0.08
8.	0.133 ± 0.004	0.093 ± 0.004	0.010^{5} ± 0.002^{0}	1.0^{5} ± 0.2^{0}	0.7 ± 0.1
9.	0.150 ± 0.005	0.110 ± 0.005	0.012^{5} ± 0.002^{4}	1.2^{5} ± 0.2^{4}	0.8^{3} ± 0.1^{3}
10.	0.158 ± 0.007	0.118 ± 0.007	0.013^{5} ± 0.003^{2}	1.3^{5} ± 0.3^{2}	0.9 ± 0.2
11.	0.19^{6} ± 0.01^{0}	0.15^{6} ± 0.01^{0}	0.018 ± 0.004	1.8 ± 0.4	0.9 ± 0.2
12.	0.184 ± 0.009	0.144 ± 0.009	0.016^{5} ± 0.004^{0}	1.6^{5} ± 0.4^{0}	0.8^{2} ± 0.2^{0}
13.	0.188 ± 0.005	0.148 ± 0.005	0.017 ± 0.002	1.7 ± 0.2	0.8^{5} ± 0.1^{0}

* Average and standard deviation of five peak integration readings

** Using the linear equation from Page 209.

3. Recovery of Lead from Spiked Fish Base Samples

Dry ashing of fish tissue

The weights of macerated and dried fish tissue weighed into 50-ml beakers are given in Table C-15.

These fish tissue samples were spiked with 1.00 ± 0.01 ppm Pb solution as follows:-

Series	Spike	ug lead added
A	none	0.0
B	0.50 ± 0.02 ml of 1.00 ppm Pb	0.50 ± 0.02
C	1.00 ± 0.02 ml " " " "	1.00 ± 0.03
D	1.50 ± 0.02 ml " " " "	1.50 ± 0.04
E	2.00 ± 0.02 ml " " " "	2.00 ± 0.04
F	2.50 ± 0.02 ml " " " "	2.50 ± 0.04

Subsequent to spiking each sample was placed into the oven and heated at just under 100 °C until the liquid spike volume had dried out. The subsequent ashing procedure duplicated the details of Sub-section 2.2.3 - (b). A blank with no spike was carried through the same procedure.

The amount of HCl pipetted was,

1.50 ± 0.02 ml

The samples were carefully transfered to 100.00 ± 0.04 ml volumetric flasks and bulked to the mark.

Testing of working standards by graphite furnace atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Lead (ppm)	Average absorbance*	Corr. ave. abs.
0.000	0.020 ± 0.000	0.000 ± 0.000
0.0020 ± 0.0001	0.035 ± 0.000	0.015 ± 0.000
0.0050 ± 0.0001	0.070 ± 0.001	0.050 ± 0.001
0.0100 ± 0.0001	0.110 ± 0.000	0.090 ± 0.000
0.0150 ± 0.0004	0.160 ± 0.002	0.140 ± 0.002
0.0200 ± 0.0004	0.210 ± 0.001	0.190 ± 0.001
0.0300 ± 0.0005	0.290 ± 0.002	0.270 ± 0.002
0.0400 ± 0.0006	0.380 ± 0.004	0.360 ± 0.004

* Average and standard deviation of five peak integration readings.

TABLE C-15

SAMPLE WEIGHTS OF BASE FISH TISSUE FOR LEAD DRY ASHING SPIKING PROCEDURE

No.	Series A	Series B	Series C
1	1.0038 ± 0.0002	1.0044 ± 0.0002	1.0060 ± 0.0002
2	1.0411 ± 0.0002	1.0028 ± 0.0002	1.0029 ± 0.0002
3	1.0021 ± 0.0002	1.0024 ± 0.0002	1.0000 ± 0.0002
4	1.0013 ± 0.0002	1.0047 ± 0.0002	1.0111 ± 0.0002
5	1.0028 ± 0.0002	1.0076 ± 0.0002	1.0006 ± 0.0002

No.	Series D	Series E	Series F
1	1.0023 ± 0.0002	1.0116 ± 0.0002	1.0041 ± 0.0002
2	1.0023 ± 0.0002	1.0013 ± 0.0002	1.0024 ± 0.0002
3	1.0028 ± 0.0002	1.0021 ± 0.0002	1.0012 ± 0.0002
4	1.0004 ± 0.0002	1.0066 ± 0.0002	0.9995 ± 0.0002
5	1.0070 ± 0.0002	1.0055 ± 0.0002	1.0063 ± 0.0002

Linear regression analysis

0.000 - 0.0400 ppm Pb

r = 0.9995
b = 0.0017
m = 9.025

Sensitivity (1% absorption) = 0.0044/9.025 = 0.001 ppm Pb

Reverse calculations for lead

Actual lead (ppm)	Calculated lead (ppm)	Absolute devn.
0.000	-0.0001	0.0001
0.0020 ± 0.0001	0.0017	0.0003
0.0050 ± 0.0001	0.0055	0.0005
0.0100 ± 0.0001	0.0100	0.0000
0.0150 ± 0.0004	0.0155	0.0005
0.0200 ± 0.0004	0.0210	0.0010
0.0300 ± 0.0005	0.0299	0.0001
0.0400 ± 0.0006	0.0399	0.0001
	Average absolute deviation	0.0003

Values of lead (ppm) from the above equation subject to ± 0.0003 ppm.

Results of lead determination of spiked dry ashed fish base tissue

The absorbance results of the blank and fish base samples spiked with lead (ug) are tabulated on Table C-16.

The calculated lead recoveries for Series B to F are tabulated on Table C-17.

The values for the 100 ml each Series A samples yield the following results:-

Series and No.	*Pb in 100 ml soln. (ppm)	Pb (ug/g) in fish base
A-1	$0.0074^{6} \pm 0.0003^{0}$	$0.74^{3} \pm 0.03^{0}$
2	$0.0086^{8} \pm 0.0003^{0}$	$0.83^{4} \pm 0.03^{0}$
3	$0.0081^{2} \pm 0.0003^{0}$	0.81 ± 0.03
4	$0.0086^{8} \pm 0.0006^{0}$	$0.86^{7} \pm 0.06^{0}$
5	$0.0080^{1} \pm 0.0006^{0}$	0.80 ± 0.06

* Using the linear equation from Page 213.

TABLE C-16

DRY ASHING/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

RESULTS OF LEAD DETERMINATION
ON SPIKED FISH BASE SAMPLES

Series and No.	Average* absorbance	Corrected average absorbance
blank	0.030 ± 0.000	0.000 ± 0.000
A-1	0.099 ± 0.000	0.069 ± 0.000
2	0.110 ± 0.000	0.080 ± 0.000
3	0.105 ± 0.000	0.075 ± 0.000
4	0.110 ± 0.001	0.080 ± 0.001
5	0.104 ± 0.001	0.074 ± 0.001
B-1	0.153 ± 0.002	0.123 ± 0.002
2	0.156 ± 0.001	0.126 ± 0.001
3	0.147 ± 0.001	0.117 ± 0.001
4	0.161 ± 0.000	0.131 ± 0.000
5	0.160 ± 0.002	0.130 ± 0.002
C-1	0.199 ± 0.000	0.169 ± 0.000
2	0.188 ± 0.003	0.158 ± 0.003
3	0.214 ± 0.001	0.184 ± 0.001
4	0.189 ± 0.002	0.159 ± 0.002
5	0.188 ± 0.002	0.158 ± 0.002
D-1	0.252 ± 0.003	0.222 ± 0.003
2	0.240 ± 0.002	0.210 ± 0.002
3	0.262 ± 0.003	0.232 ± 0.003
4	0.256 ± 0.003	0.226 ± 0.003
5	0.234 ± 0.002	0.204 ± 0.002
E-1	0.279 ± 0.002	0.249 ± 0.002
2	lost	
3	0.281 ± 0.004	0.251 ± 0.004
4	0.290 ± 0.002	0.260 ± 0.002
5	0.285 ± 0.001	0.255 ± 0.001
F-1	lost	
2	0.314 ± 0.008	0.284 ± 0.008
3	0.305 ± 0.003	0.275 ± 0.003
4	0.301 ± 0.004	0.271 ± 0.004
5	0.324 ± 0.003	0.294 ± 0.003

* Average and standard deviation of five peak integration readings.

TABLE C-17

DRY ASHING/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

RECOVERY OF LEAD IN SPIKED FISH BASE SAMPLES

Series and No.	**Pb in 100 ml soln, (ppm)	Total Pb (ug/100 ml)	*Pb (ug/100 ml) corr. fish base	Actual Pb spike (ug)	Recovery of Pb (%)
B-1	0.0134 ± 0.0009	1.34 ± 0.09	0.51 ± 0.09	0.50 ± 0.02	102 ± 18
2	0.0138 ± 0.0006	1.38 ± 0.06	0.55 ± 0.08	0.50 ± 0.02	110 ± 16
3	0.0128 ± 0.0006	1.28 ± 0.06	0.45 ± 0.08	0.50 ± 0.02	90 ± 16
4	0.0143 ± 0.0003	1.43 ± 0.03	0.60 ± 0.08	0.50 ± 0.02	120 ± 16
5	0.0142 ± 0.0009	1.42 ± 0.09	0.58^{4} ± 0.09	0.50 ± 0.02	$116.^{7}$ ± 18.0
C-1	0.0185 ± 0.0003	1.85 ± 0.03	1.01^{5} ± 0.08	1.00 ± 0.03	$101.^{5}$ ± 8.0
2	0.017^{3} ± 0.001^{2}	1.7^{3} ± 0.1^{2}	0.9^{0} ± 0.1^{2}	1.00 ± 0.03	90 ± 12
3	0.0202 ± 0.0003	2.02 ± 0.03	1.19 ± 0.08	1.00 ± 0.03	119 ± 8
4	0.0174 ± 0.0009	1.74 ± 0.09	0.90^{1} ± 0.09	1.00 ± 0.03	$90.^{1}$ ± 9.0
5	0.0173 ± 0.0009	1.73 ± 0.09	0.90 ± 0.09	1.00 ± 0.03	90 ± 9
D-1	0.024^{4} ± 0.001^{2}	2.4^{4} ± 0.1^{2}	1.6^{1} ± 0.1^{2}	1.50 ± 0.04	$107.^{3}$ ± 8.0
2	0.0231 ± 0.0009	2.31 ± 0.09	1.48 ± 0.09	1.50 ± 0.04	$98.^{7}$ ± 6.0
3	0.025^{5} ± 0.001^{2}	2.5^{5} ± 0.1^{2}	1.7^{2} ± 0.1^{2}	1.50 ± 0.04	$114.^{7}$ ± 8.0
4	0.024^{9} ± 0.001^{2}	2.4^{9} ± 0.1^{2}	1.6^{6} ± 0.1^{2}	1.50 ± 0.04	$110.^{7}$ ± 8.0
5	0.0224 ± 0.0009	2.24 ± 0.09	1.40 ± 0.09	1.50 ± 0.04	$93.^{3}$ ± 6.0
E-1	0.0274 ± 0.0009	2.74 ± 0.09	1.90 ± 0.09	2.00 ± 0.04	$95.^{0}$ ± 4.5
2	lost				
3	0.027^{6} ± 0.001^{5}	2.7^{6} ± 0.1^{5}	1.9^{3} ± 0.1^{5}	2.00 ± 0.04	$96.^{5}$ ± 7.5
4	0.0286 ± 0.0009	2.86 ± 0.09	2.02 ± 0.09	2.00 ± 0.04	$101.^{2}$ ± 4.5
5	0.0281 ± 0.0006	2.81 ± 0.06	1.98 ± 0.08	2.00 ± 0.04	99 ± 4
F-1	lost				
2	0.031^{3} ± 0.002^{7}	3.1^{3} ± 0.2^{7}	2.3^{0} ± 0.2^{7}	2.50 ± 0.04	$92.^{0}$ ± 10.8
3	0.030^{3} ± 0.001^{2}	3.0^{3} ± 0.1^{2}	2.2^{0} ± 0.1^{2}	2.50 ± 0.04	$88.^{0}$ ± 4.8
4	0.029^{8} ± 0.001^{5}	2.9^{8} ± 0.1^{5}	2.1^{5} ± 0.1^{5}	2.50 ± 0.04	$86.^{0}$ ± 6.1
5	0.032^{4} ± 0.001^{2}	3.2^{4} ± 0.1^{2}	2.4^{0} ± 0.1^{2}	2.50 ± 0.04	$96.^{0}$ ± 4.8

* Based on the higher of the two uncertainties:- fish base or total lead in 100 ml.

** Using the linear equation from Page 213.

These again represent fish base values and, combined with the 12 values obtained under Appendix C-4 - (2)Testing of dry ashed rainbow trout base tissue, provide a total of 17 results. These results yield a final value for the lead content of dried rainbow trout unexposed to experimentally high lead contents in the water and/or food of:-

0.83 ± 0.08 ppm of 0.83 ± 0.08 ug Pb/g dried rainbow trout base

An overall average recovery percent was obtained for lead spikes on rainbow trout base of up to 2.5 ug/g dried fish base of:-

$$100.^{3} \pm 10.^{5} \%$$

If the average for each of the Series B to F is taken with respect to the "Total Pb (ug/100 ml)" column data we have:-

Series	B	1.37 ± 0.06
	C	$1.8^{1} \pm 0.1^{2}$
	D	$2.4^{1} \pm 0.1^{2}$
	E	2.79 ± 0.05
	F	$3.1^{0} \pm 0.11$

Linear regression analysis yields:-

$r = 0.9935$
$b = 0.964$
$m = 0.888^{0}$

Thus the zero intercept on the ordinate yields a value of 0.964 ug Pb/100 ml of solution or, for the sample weights approximating 1.00 g of dried fish base, 0.96^{4} ug Pb/g dried fish base. Carrying out reverse calculations indicates an uncertainty for this value of $\pm$ 0.06 ug, so that we have, from the method of additions, a calculated value for dried rainbow trout base content of lead of 0.96 $\pm$ 0.06 ug/g fish base. This compares, within the respective tolerance limits, with the value of 0.83 ± 0.08 ug Pb/g dried fish base reported above.

Using the value of 0.96 ± 0.06 ug Pb/g dried fish base as the correction value to the "Total Pb (ug/100 ml)" data column yields an average recovery percent for the spikes up to 2.5 ug on 1 g of dried fish base of $89.^{1} \pm 8.^{8}$. This also agrees within the respective tolerance limits, with the value of $100.^{3} \pm 10.^{5}$ percent reported on the previous page.

By including the average result of Series A to the average for each of the Series B to F with respect to the "Total Pb (ug/100 ml)" data column from Table C-17 in a graphical determination of lead in dried rainbow trout by the method of additions, the plot on Figure C-4 was obtained. The lead content in dried rainbow trout base tissue can be extrapolated to the abscissa to 0.90 ± 0.02 ug Pb/g dried fish base. The ± 0.02 value represents the reading uncertainty on the graph. This compares, within the respective tolerance limits, with the value of 0.83 ± 0.08 ug Pb/g dried fish base reported on the previous page.

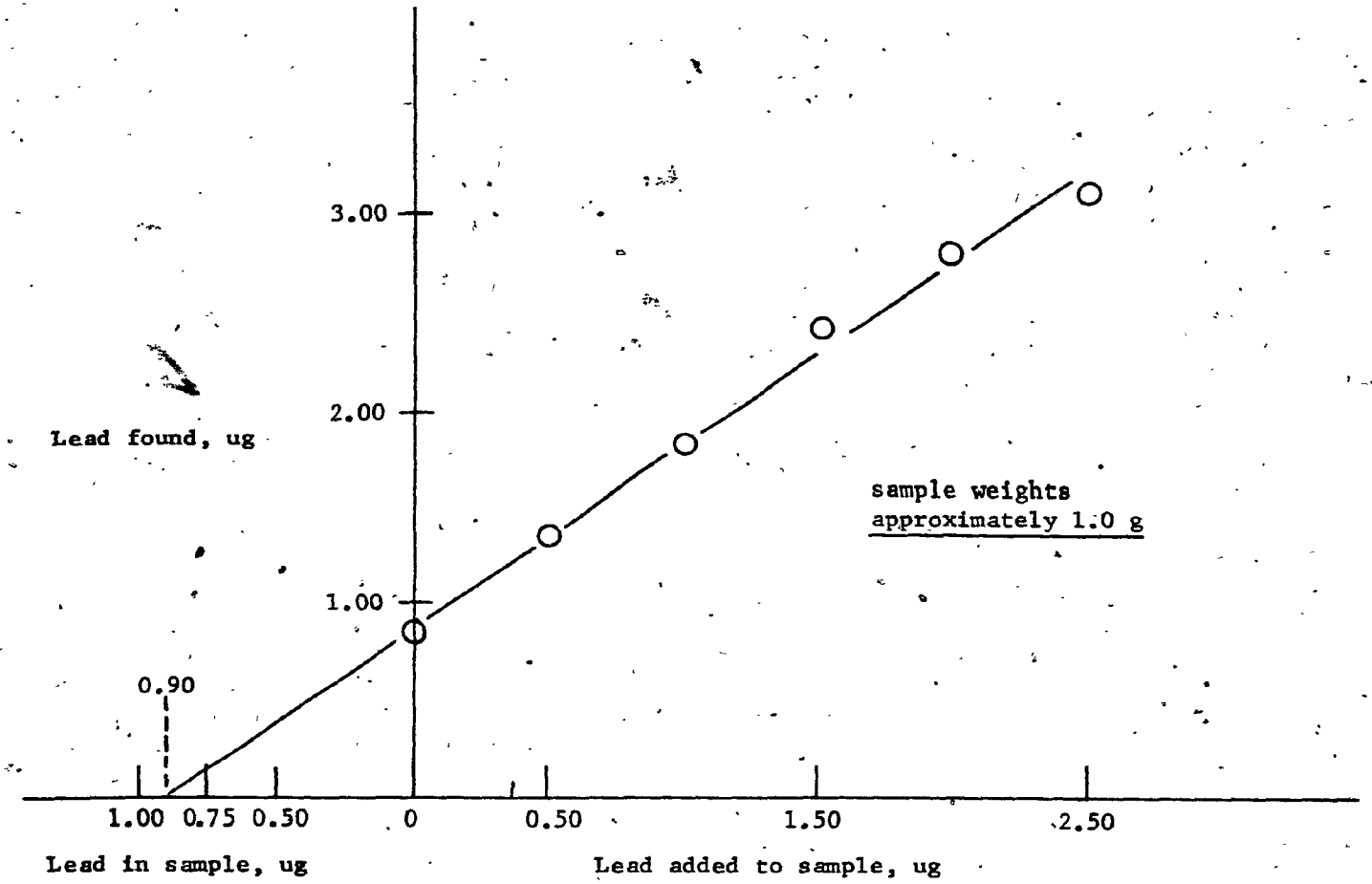


FIGURE C-4 GRAPHICAL METHOD OF ADDITIONS - LEAD DETERMINATION IN RAINBOW TROUT - DRY ASHING

4. Recovery of Lead from Aliquoted Fish Base Samples

Two sets of macerated and dried rainbow trout tissue were weighed out in duplicate. Each sample consisted of 1.0000 ± 0.0002 g and was placed in a 50-ml beaker.

One set (A) was spiked with 2.50 ± 0.04 ug of Pb each, while the second set (B) was spiked with 3.50 ± 0.05 ug of Pb each.

All samples were dried, ashed and extracted according to the details of Sub-section 2.2.3 - (b). A blank was carried through simultaneously. The amount of HCl pipetted was 1.50 ± 0.02 ml and each sample was made up to the mark in a 100.00 ± 0.04 ml volumetric flask.

For each set, 10 ml of the final solution was tested as-is, and 10 ml was diluted to 50 ml with 1.5 percent HCl and then tested. The following results were obtained:-

Set	Ave. absorbance corr. for blank		Total Pb* (ug/100 ml)		Pb corr. for** base fish (ug/g)		Actual spike	Recovery (%)	
	as-is	aliq.	as-is	aliqx5	as-is	aliqx5	(ug)	as-is	aliq.
A	0.300	0.065	3.30^5	3.50^7	2.48	2.68	2.50	$99.^2$	$107.^2$
B	0.380	0.086	4.19	4.67	3.36	3.84	3.50	$96.^0$	$109.^7$

* Using the linear equation from Page 213.

** Using the correction factor of 0.83 ug Pb/g dried rainbow trout base from Page 216

The recovery percent values agree within the expected tolerance limits for lead recovery on spiked samples, so that aliquoting of tissue sample solutions, suspected to contain lead in ppm beyond the linear range maximum, should present no difficulty.

APPENDIX C-5

Determination of Cadmium in Rainbow Trout Tissue by the Dry-Ashing/ Graphite Furnace Technique

1. General

The following sections outline the experimental approach applied to the testing, recovery from spiking and recovery from aliquoting of cadmium in rainbow trout base tissue.

The graphite furnace method of analysis was applied because of the relatively low cadmium content found for rainbow trout unexposed to experimentally high cadmium levels in the water and/or food.

General operating conditions

Certain general operating conditions were maintained throughout the analytical procedures. These are outlined in Appendix B-4. The only differences involved were:-

Instrument:-	Perkin-Elmer Model 503 Atomic Absorption Spectrophotometer with HGA 2000 Graphite Furnace
Flush system:-	none

Preparation of aqueous-base standard solutions

Appendix B-4 outlines the preparation of the aqueous-base standard solutions. Additional working standards were prepared as follows:-

Solution preparation*	Cadmium (ppm)
2.00 ± 0.02 ml of 0.0100 ± 0.0001 ppm Cd	0.00020 ± 0.00001

* All dilutions were made up in 100.00 ± 0.04 ml volumetric flasks.

Preparation of fish tissue

The preparation of rainbow trout base tissue is described in Sub-section 2.2.3 - (a).

2. Testing of Dry Ashed Rainbow Trout Base Tissue

The general procedure is outlined in Sub-section 2.2.3 - (b). The specific details for the determination of cadmium are given below.

The sample weights were as follows:-

Sample No.	Fish weight (g)
1.	0.0000
2.	0.5141 ± 0.0002
3.	0.5013 ± 0.0002
4.	0.5211 ± 0.0002
5.	1.0031 ± 0.0002
6.	1.0039 ± 0.0002
7.	1.0006 ± 0.0002
8.	1.5038 ± 0.0002
9.	1.5107 ± 0.0002
10.	1.5016 ± 0.0002
11.	2.0028 ± 0.0002
12.	2.0138 ± 0.0002
13.	1.9963 ± 0.0002
14.	2.5050 ± 0.0002
15.	2.5121 ± 0.0002
16.	2.5035 ± 0.0002

The amount of HCl pipetted was,

$$1.50 \pm 0.02 \text{ ml}$$

Each sample was diluted to the mark in a 100.00 ± 0.04 ml volumetric flask.

Testing of working standards by graphite furnace atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Cadmium (ppm)	Average absorbance*	Corr. ave. abs.
0.00000	0.012 ± 0.000	0.000 ± 0.000
0.00050 ± 0.00001	0.058 ± 0.001	0.046 ± 0.001
0.00100 ± 0.00001	0.089 ± 0.000	0.077 ± 0.000
0.00200 ± 0.00004	0.172 ± 0.002	0.160 ± 0.002
0.00300 ± 0.00005	0.247 ± 0.003	0.235 ± 0.003
0.00400 ± 0.00006	0.339 ± 0.003	0.327 ± 0.003

* Average and standard deviation of five peak integration readings.

Linear regression analysis

0.00000 - 0.00400 ppm Cd

$r = 0.9992^6$
$b = 0.000^2$
$m = 80.35^8$

Sensitivity (1% absorption) = $0.0044/80.35^8$ = 0.00005 ppm Cd

Reverse calculations for cadmium

Actual cadmium (ppm)	Calculated cadmium (ppm)	Absolute devn.
0.00000	0.00000	0.00000
0.00050 ± 0.00001	0.00056	0.00006
0.00100 ± 0.00001	0.00095	0.00005
0.00200 ± 0.00004	0.00200	0.00000
0.00300 ± 0.00005	0.00292	0.00008
0.00400 ± 0.00006	0.00406	0.00006
	Average absolute deviation	0.00004

Values of cadmium (ppm) from the above equation subject to ± 0.00004 ppm.

Results of cadmium determination in dry ashed fish base tissue

The results of the blank and fish base tissue samples are tabulated in Table C-18.

From the 15 values recorded in the last column the following average was obtained:-

$0.033^1 \pm 0.002^1$ ppm Cd

or,

$0.033^1 \pm 0.002^1$ ug Cd/g dried rainbow trout base

TABLE C-18

DETERMINATION OF CADMIUM IN RAINBOW TROUT BASE TISSUE
BY DRY ASHING/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

Sample No.	Average* absorbance	Corrected average absorbance	** Cd in 100 ml soln. (ppm)	Cadmium ug/100 ml	Cadmium in fish base (ppm)
1.	0.030 ± 0.000	0.000 ± 0.000	---	---	---
2.	0.043 ± 0.000	0.013 ± 0.000	$0.00015^{9} \pm 0.00004^{0}$	$0.015^{9} \pm 0.004^{0}$	0.031 ± 0.008
3.	0.042 ± 0.001	0.012 ± 0.001	$0.00014^{7} \pm 0.00008^{0}$	$0.014^{7} \pm 0.008^{0}$	$0.02^{9} \pm 0.015$
4.	0.045 ± 0.001	0.015 ± 0.001	$0.00018^{4} \pm 0.00008^{0}$	$0.018^{4} \pm 0.008^{0}$	$0.03^{5} \pm 0.015$
5.	0.055 ± 0.000	0.025 ± 0.000	$0.00030^{8} \pm 0.00004^{0}$	$0.030^{8} \pm 0.004^{0}$	0.031 ± 0.004
6.	0.058 ± 0.001	0.028 ± 0.001	$0.00034^{6} \pm 0.00008^{0}$	$0.034^{6} \pm 0.008^{0}$	$0.034^{5} \pm 0.008^{0}$
7.	0.057 ± 0.002	0.027 ± 0.002	$0.0003^{3} \pm 0.0001^{2}$	$0.03^{3} \pm 0.01^{2}$	$0.03^{3} \pm 0.01^{2}$
8.	0.075 ± 0.000	0.045 ± 0.000	$0.00055^{7} \pm 0.00004^{0}$	$0.055^{7} \pm 0.004^{0}$	0.037 ± 0.003
9.	0.072 ± 0.002	0.042 ± 0.002	$0.0005^{2} \pm 0.0001^{2}$	$0.05^{2} \pm 0.01^{2}$	0.034 ± 0.008
10.	0.070 ± 0.000	0.040 ± 0.000	$0.00049^{5} \pm 0.00004^{0}$	$0.049^{5} \pm 0.004^{0}$	0.033 ± 0.003
11.	0.084 ± 0.001	0.054 ± 0.001	$0.00066^{9} \pm 0.00008^{0}$	$0.066^{9} \pm 0.008^{0}$	0.033 ± 0.004
12.	0.085 ± 0.001	0.055 ± 0.001	$0.00068^{2} \pm 0.00008^{0}$	$0.068^{2} \pm 0.008^{0}$	0.034 ± 0.004
13.	0.088 ± 0.001	0.058 ± 0.001	$0.00071^{9} \pm 0.00008^{0}$	$0.071^{9} \pm 0.008^{0}$	0.036 ± 0.004
14.	0.092 ± 0.000	0.062 ± 0.000	$0.00076^{9} \pm 0.00004^{0}$	$0.076^{9} \pm 0.004^{0}$	0.031 ± 0.002
15.	0.095 ± 0.001	0.065 ± 0.001	$0.00080^{6} \pm 0.00008^{0}$	$0.080^{6} \pm 0.008^{0}$	0.032 ± 0.003
16.	0.096 ± 0.002	0.066 ± 0.002	$0.0008^{2} \pm 0.0001^{2}$	$0.08^{2} \pm 0.01^{2}$	0.033 ± 0.005

* Average and standard deviation of five peak integration readings

** Using the linear equation from Page 223.

3. Recovery of Cadmium from Spiked Fish Base Samples

Dry ashing of fish tissue

The weights of macerated and dried fish tissue weighed into 50-ml beakers are given in Table C-19.

These tissue samples were spiked with 0.100 ± 0.001 ppm Cd solution as follows:-

Series	Spike	ug cadmium added
A	none	0.00
B	0.50 ± 0.02 ml of 0.100 ppm Cd	0.050 ± 0.002
C	1.00 ± 0.02 ml " " " "	0.100 ± 0.003
D	1.50 ± 0.02 ml " " " "	0.150 ± 0.004
E	2.00 ± 0.02 ml " " " "	0.200 ± 0.004
F	2.50 ± 0.02 ml " " " "	0.250 ± 0.004

Subsequent to spiking each sample was placed in the oven and heated at just under 100 °C until the liquid spike volume had dried out. The subsequent ashing procedure duplicated the details of Sub-section 2.2.3 - (b). A blank with no spike was carried through the same procedure.

The amount of HCl pipetted was,

1.50 ± 0.02 ml

The samples were carefully transfered to 100.00 ± 0.04 ml volumetric flasks and bulked to the mark.

Testing of working standards by graphite furnace atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Cadmium (ppm)	Average absorbance*	Corr. ave. abs.
0.00000	0.015 ± 0.000	0.000 ± 0.000
0.00020 ± 0.00001	0.027 ± 0.000	0.012 ± 0.000
0.00050 ± 0.00001	0.059 ± 0.001	0.044 ± 0.001
0.00100 ± 0.00001	0.097 ± 0.000	0.082 ± 0.000
0.00200 ± 0.00004	0.170 ± 0.001	0.155 ± 0.001
0.00300 ± 0.00005	0.250 ± 0.002	0.235 ± 0.002
0.00400 ± 0.00006	0.325 ± 0.002	0.310 ± 0.002

* Average of five peak mode integration readings.

TABLE C-19

SAMPLE WEIGHTS OF BASE FISH TISSUE FOR CADMIUM DRY ASHING SPIKING PROCEDURE

No.	Series A	Series B	Series C
1	1.0038 ± 0.0002	1.0044 ± 0.0002	1.0060 ± 0.0002
2	1.0411 ± 0.0002	1.0028 ± 0.0002	1.0029 ± 0.0002
3	1.0021 ± 0.0002	1.0024 ± 0.0002	1.0000 ± 0.0002
4	1.0013 ± 0.0002	1.0047 ± 0.0002	1.0111 ± 0.0002
5	1.0028 ± 0.0002	1.0076 ± 0.0002	1.0006 ± 0.0002

No.	Series D	Series E	Series F
1	1.0023 ± 0.0002	1.0116 ± 0.0002	1.0041 ± 0.0002
2	1.0023 ± 0.0002	1.0013 ± 0.0002	1.0024 ± 0.0002
3	1.0028 ± 0.0002	1.0021 ± 0.0002	1.0012 ± 0.0002
4	1.0004 ± 0.0002	1.0066 ± 0.0002	0.9995 ± 0.0002
5	1.0070 ± 0.0002	1.0055 ± 0.0002	1.0063 ± 0.0002

Linear regression analysis

0.00000 - 0.00400 ppm Cd

$r = 0.9996^7$
$b = 0.001$
$m = 77.539$

Sensitivity (1% absorption) = 0.0044/77.539 = 0.00006 ppm Cd

Reverse calculations for cadmium

Actual cadmium (ppm)	Calculated cadmium (ppm)	Absolute devn.
0.00000	-0.00001	0.00001
0.00020 ± 0.00001	0.00014	0.00006
0.00050 ± 0.00001	0.00055	0.00005
0.00100 ± 0.00001	0.00104	0.00004
0.00200 ± 0.00004	0.00198	0.00002
0.00300 ± 0.00005	0.00301	0.00001
0.00400 ± 0.00006	0.00398	0.00002
	Average absolute deviation	0.00003

Values of cadmium (ppm) from the above equation subject to ± 0.00003 ppm.

Results of cadmium determination of spiked dry ashed fish base tissue

The absorbance results of the blank and fish base samples spiked with cadmium (ug) are tabulated on Table C-20.

The calculated cadmium recoveries for Series B to F are tabulated on Table C-21.

The values for the 100 ml each Series A yield the following results:

Series and No.	Cd in 100 ml soln. (ppm)	Cd (ug/g) in fish base
A-1	0.000346 ± 0.00003^0	$0.034^5 \pm 0.003^0$
2	0.00032 ± 0.00006	0.030 ± 0.006
3	$0.00037^2 \pm 0.00003^0$	$0.037^1 \pm 0.003^0$
4	$0.00029^4 \pm 0.00003^0$	$0.029^4 \pm 0.003^0$
5	$0.00030^7 \pm 0.00003^0$	$0.030^6 \pm 0.003^0$

TABLE C-20

DRY ASHING/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

RESULTS OF CADMIUM DETERMINATION
ON SPIKED FISH BASE SAMPLES

Series and No.	Average* absorbance	Corrected average absorbance
blank	0.020 ± 0.000	0.000 ± 0.000
A-1	0.048 ± 0.000	0.028 ± 0.000
2	0.046 ± 0.001	0.026 ± 0.001
3	0.050 ± 0.000	0.030 ± 0.000
4	0.044 ± 0.000	0.024 ± 0.000
5	0.045 ± 0.000	0.025 ± 0.000
B-1	0.085 ± 0.001	0.065 ± 0.001
2	0.089 ± 0.001	0.069 ± 0.001
3	0.073 ± 0.000	0.053 ± 0.000
4	0.087 ± 0.000	0.067 ± 0.000
5	0.084 ± 0.000	0.064 ± 0.000
C-1	0.119 ± 0.002	0.099 ± 0.002
2	0.117 ± 0.001	0.097 ± 0.001
3	0.114 ± 0.000	0.094 ± 0.000
4	0.115 ± 0.001	0.095 ± 0.001
5	0.124 ± 0.000	0.104 ± 0.000
D-1	0.157 ± 0.000	0.137 ± 0.000
2	0.161 ± 0.001	0.141 ± 0.001
3	0.150 ± 0.002	0.130 ± 0.002
4	0.145 ± 0.003	0.125 ± 0.003
5	0.147 ± 0.000	0.127 ± 0.000
E-1	0.191 ± 0.001	0.171 ± 0.001
2	0.192 ± 0.000	0.172 ± 0.000
3	0.179 ± 0.001	0.159 ± 0.001
4	0.180 ± 0.002	0.160 ± 0.002
5	0.189 ± 0.000	0.169 ± 0.000
F-1	0.206 ± 0.001	0.186 ± 0.001
2	0.220 ± 0.001	0.200 ± 0.001
3	0.213 ± 0.003	0.193 ± 0.003
4	0.211 ± 0.000	0.191 ± 0.000
5	0.208 ± 0.001	0.188 ± 0.001

* average and standard deviation of five peak integration readings.

TABLE C-21

DRY ASHING/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

RECOVERY OF CADMIUM IN SPIKED FISH BASE SAMPLES

Series and No.	**Cd in 100 ml soln. (ppm)	Total Cd (ug/100 ml)	*Cd (ug/100 ml) corr. fish base	Actual Cd spike (ug)	Recovery of Cd (%)
B-1	$0.000082^{5} \pm 0.00006^{0}$	$0.082^{5} \pm 0.006^{0}$	$0.049^{4} \pm 0.006^{0}$	0.050 ± 0.002	99 ± 12
2	$0.000087^{7} \pm 0.00006^{0}$	$0.087^{7} \pm 0.006^{0}$	$0.054^{7} \pm 0.006^{0}$	0.050 ± 0.002	109 ± 12
3	0.000671 ± 0.00003^{0}	$0.067^{1} \pm 0.003^{0}$	$0.034^{1} \pm 0.003^{0}$	0.050 ± 0.002	$68.^{2} \pm 6.^{0}$
4	0.000851 ± 0.00003^{0}	$0.085^{1} \pm 0.003^{0}$	0.052 ± 0.003	0.050 ± 0.002	$104.^{1} \pm 6.^{0}$
5	0.000812 ± 0.00003^{0}	$0.081^{2} \pm 0.003^{0}$	0.048 ± 0.003	0.050 ± 0.002	$96.^{0} \pm 6.^{0}$
C-1	0.001264 ± 0.00009^{0}	$0.126^{4} \pm 0.009^{0}$	$0.093^{3} \pm 0.009^{0}$	0.100 ± 0.003	$93.^{3} \pm 9.^{0}$
2	0.001238 ± 0.00006^{0}	$0.123^{8} \pm 0.006^{0}$	$0.090^{8} \pm 0.006^{0}$	0.100 ± 0.003	$90.^{8} \pm 6.^{0}$
3	0.00120 ± 0.00003	0.120 ± 0.003	$0.087^{1} \pm 0.003^{0}$	0.100 ± 0.003	$87.^{1} \pm 3.^{0}$
4	0.001212 ± 0.00006^{0}	$0.121^{2} \pm 0.006^{0}$	$0.087^{9} \pm 0.006^{0}$	0.100 ± 0.003	$87.^{9} \pm 6.^{0}$
5	0.001328 ± 0.00003^{0}	$0.132^{8} \pm 0.003^{0}$	$0.099^{9} \pm 0.003^{0}$	0.100 ± 0.003	$99.^{9} \pm 3.^{0}$
D-1	0.001754 ± 0.00003^{0}	$0.175^{4} \pm 0.003^{0}$	0.142 ± 0.003	0.150 ± 0.004	$94.^{7} \pm 2.^{0}$
2	0.001806 ± 0.00006^{0}	$0.180^{6} \pm 0.006^{0}$	0.148 ± 0.006	0.150 ± 0.004	$98.^{4} \pm 4.^{0}$
3	0.001664 ± 0.00009^{0}	$0.166^{4} \pm 0.009^{0}$	0.133 ± 0.009	0.150 ± 0.004	$88.^{7} \pm 6.^{0}$
4	$0.0016^{0} \pm 0.0001^{2}$	$0.16^{0} \pm 0.01^{2}$	0.127 ± 0.01^{2}	0.150 ± 0.004	$84.^{7} \pm 8.^{0}$
5	0.001625 ± 0.00003^{0}	$0.162^{5} \pm 0.003^{0}$	0.129 ± 0.003	0.150 ± 0.004	$86.^{2} \pm 2.^{0}$
E-1	0.002192 ± 0.00006^{0}	$0.219^{2} \pm 0.006^{0}$	0.186 ± 0.006	0.200 ± 0.004	93 ± 3
2	0.002205 ± 0.00003^{0}	$0.220^{5} \pm 0.003^{0}$	0.188 ± 0.003	0.200 ± 0.004	$94.^{0} \pm 1.^{5}$
3	0.00204 ± 0.00006	0.204 ± 0.006	0.171 ± 0.006	0.200 ± 0.004	$85.^{5} \pm 3.^{0}$
4	0.00205 ± 0.00009	0.205 ± 0.009	0.172 ± 0.009	0.200 ± 0.004	$86.^{0} \pm 4.^{5}$
5	0.00217 ± 0.00003	0.217 ± 0.003	0.184 ± 0.003	0.200 ± 0.004	$92.^{0} \pm 1.^{5}$
F-1	0.002386 ± 0.00006^{0}	$0.238^{6} \pm 0.006^{0}$	0.206 ± 0.006	0.250 ± 0.004	$82.^{4} \pm 2.^{4}$
2	0.002566 ± 0.00006^{0}	$0.256^{6} \pm 0.006^{0}$	0.224 ± 0.006	0.250 ± 0.004	$89.^{6} \pm 2.^{4}$
3	$0.0024^{8} \pm 0.0001^{2}$	$0.24^{8} \pm 0.01^{2}$	$0.21^{5} \pm 0.01^{2}$	0.250 ± 0.004	$86.^{0} \pm 4.^{8}$
4	0.00245 ± 0.00003	0.245 ± 0.003	0.212 ± 0.003	0.250 ± 0.004	$84.^{8} \pm 1.^{2}$
5	0.002412 ± 0.00006^{0}	$0.241^{2} \pm 0.006^{0}$	0.208 ± 0.006	0.250 ± 0.004	$83.^{2} \pm 2.^{4}$

*Based on the higher of the two uncertainties:- fish base or total cadmium in 100 ml.
**Using the linear equation from Page 227.

These again represent fish base values and combined with the 15 values obtained under Appendix C-5 - (2) Testing of Dry Ashed Rainbow Trout Base Tissue, provide a total of 20 results. These results yield a final value for the cadmium content of dried rainbow trout unexposed to experimentally high cadmium contents in the water and/or food of:-

$0.032^{9} \pm 0.002^{3}$ ppm or $0.032^{9} \pm 0.002^{3}$ ug Cd/g dried rainbow trout

Using the 2s rejection criterion to examine the recovery percent data, it was noted that rejection of the $68.^{2}$ % value of sample B-3 was required. The remaining values yield an overall recovery percent for cadmium spiked on rainbow trout dried base of up to 0.25 ug/g dried fish base of:-

$$91.^{5} \pm 6.^{9} \%$$

If the average for each of the Series B to F is taken with respect to the "Total Cd (ug/100 ml)" column data (Table C-21) we have, omitting the B-3 value:-

Series	B	$0.084^{1} \pm 0.002^{8}$
	C	$0.124^{8} \pm 0.005^{0}$
	D	$0.169^{0} \pm 0.008^{7}$
	E	$0.209^{1} \pm 0.008^{4}$
	F	$0.245^{9} \pm 0.006^{9}$

Linear regression analysis yields:-

$r = 0.9995^{3}$
$b = 0.0441$
$m = 0.815^{8}$

Thus the zero intercept on the ordinate yields a value of 0.0441 ug Cd/100 ml of solution or, for the sample weights approximating 1.00 g of dried fish base, 0.0441 ug Cd/g dried fish base. Carrying out reverse calculations indicated an uncertainty for this value of $\pm 0.001^{6}$ ug, so that we have, from the method of additions, a calculated value for dried rainbow trout content of cadmium of $0.044^{1} \pm 0.001^{6}$ ug/g fish base. This does not fall within the respective tolerance limits with the value of $0.032^{9} \pm 0.002^{3}$ ug Cd/g dried fish base reported above. This difference, however significant, can be attributed mostly to the very small amounts of cadmium we are determining in dried rainbow trout base.

Using the value of $0.044^{1} \pm 0.001^{6}$ ug Cd/g dried fish base as the correction factor for the "Total Cd (ug/100 ml)" data column yields, omitting the B-3 value, an average recovery for spikes up to 0.25 ug on 1 g of dried fish base of $81.^{6} \pm 4.^{6}$ %. This result agrees, within the respective tolerance limits, with the value of $91.^{5} \pm 6.^{9}$ percent reported on the previous page, only because of the relatively high standard deviations appearing after each average.

By including the average result of Series A to the average for each of the Series B to F taken with respect to the "Total Cd (ug/100 ml)" data column from Table C-21 (omitting the B-3 result) in a graphical determination of cadmium in dried rainbow trout by the method of additions, the plot on Figure C-5 was obtained. The cadmium content in dried rainbow trout base tissue can be extrapolated to the abscissa to 0.039 ± 0.001 ug Cd/g fish base. The $\pm$ 0.001 represents the reading uncertainty on the graph (1/2 the smallest division). This does not quite compare, within the respective tolerance limits, with the value $0.032^{9} \pm 0.002^{3}$ ug Cd/g dried fish base reported on the previous page. However, in view of the very small quantities of cadmium in question, the differences may not be due to possible errors in the method.

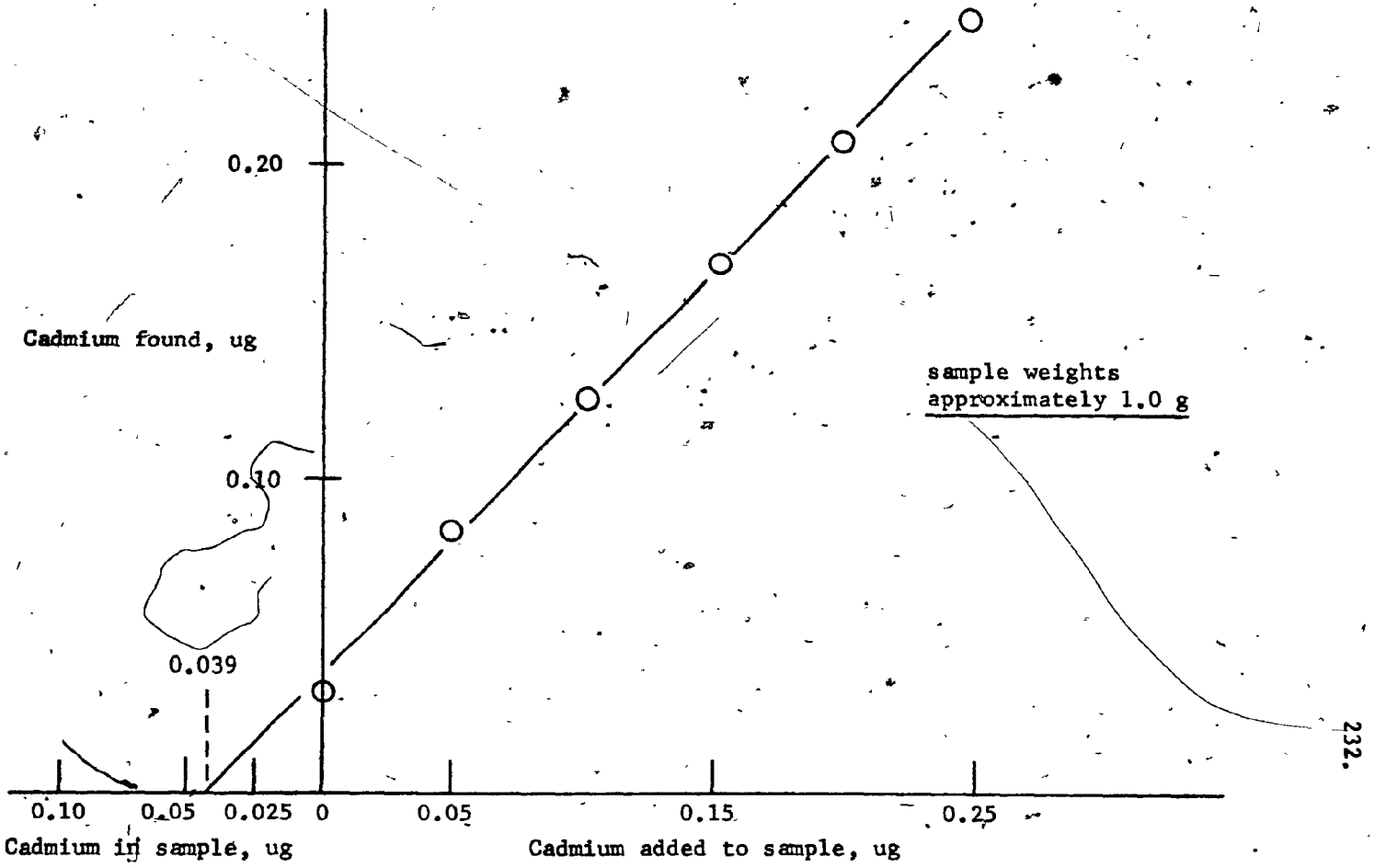


FIGURE C-5 GRAPHICAL METHOD OF ADDITIONS - CADMIUM DETERMINATION IN RAINBOW TROUT - DRY ASHING

4. Recovery of Cadmium from Aliquoted Fish Base Samples

Two sets of macerated and dried (65 ± 5 °C) rainbow trout tissue were weighed out in duplicate. Each sample consisted of 1.0000 ± 0.0002 g and was placed in a 50-ml beaker.

One set (A) was spiked with 0.250 ± 0.004 ug of cadmium each, while the second set (B) was spiked with 0.300 ± 0.005 ug of cadmium each.

All samples were dried, ashed and extracted according to the details of Sub-section 2.2.3 - (b). A blank was carried through simultaneously. The amount of HCl pipetted was 1.50 ± 0.02 ml and each sample was made up to the mark in a 100.00 ± 0.04 ml volumetric flask.

For each set, 10 ml of the final solution was tested as-is, and 10 ml was diluted to 50 ml with 1.5 percent HCl and then tested. The following results were obtained:-

Set	Ave. absorbance corr. for blank		Total Cd* (ug/100 ml)		Cd corr. for** base fish (ug/g)		Actual spike	Recovery (%)	
	as-is	aliq.	as-is	aliqx5	as-is	aliqx5	(ug)	as-is	aliq.
A	0.215	0.041	0.276	0.257^9	0.243^1	0.225	0.250	97.2	90.0
B	0.240	0.047	0.308^2	0.296^6	0.275^3	0.263^7	0.300	91.8	87.9

* Using the linear equation from Page 227.

** Using the correction factor of 0.032^9 ug Cd/g dried rainbow trout base from Page 230.

The recovery percent values agree within the expected tolerance limits for cadmium recovery on spiked samples, so that aliquoting of tissue sample solutions, suspected to contain cadmium in ppm beyond the linear range maximum, should present no difficulty.

APPENDIX C-6

Determination of Vanadium in Rainbow Trout Tissue by the Dry-Ashing/ Graphite Furnace Technique

1. General

The following sections outline the experimental approach applied to the testing, recovery from spiking and recovery from aliquoting of vanadium in dried rainbow trout base tissue.

The graphite furnace method of analysis was applied because of the relatively low vanadium content found for rainbow trout unexposed to experimentally high vanadium levels in the water and/or food.

General operating conditions

Certain general operating conditions were maintained throughout the analytical procedures. These are outlined in Appendix B-5.

Preparation of aqueous-base standard solutions

Appendix B-5 outlines the preparation of the aqueous-base standard solutions. Additional working standards of vanadium were prepared as follows:-

Solution preparation*	Vanadium (ppm)
0.25 ± 0.02 ml of 10.00 ± 0.02 ppm V	0.025 ± 0.002

* All dilutions were made up in 100.00 ± 0.04 ml volumetric flasks.

Preparation of fish tissue

The preparation of rainbow trout base tissue is described in Sub-section 2.2.3 - (a).

2. Testing of Dry Ashed Rainbow Trout Base Tissue

The general procedure is outlined in Sub-section 2.2.3 - (b). The specific details for the determination of vanadium are given below.

The sample weights were as follows:-

Sample No.	Fish weight (g)
1.	0.0000
2.	0.2055 ± 0.0002
3.	0.2073 ± 0.0002
4.	0.2043 ± 0.0002
5.	0.5006 ± 0.0002
6.	0.5083 ± 0.0002
7.	0.5060 ± 0.0002
8.	1.0000 ± 0.0002
9.	1.0033 ± 0.0002
10.	1.0005 ± 0.0002
11.	1.4972 ± 0.0002
12.	1.5813 ± 0.0002
13.	1.4757 ± 0.0002
14.	1.9881 ± 0.0002
15.	2.0113 ± 0.0002
16.	1.9805 ± 0.0002
17.	2.9533 ± 0.0002
18.	2.9937 ± 0.0002
19.	2.9737 ± 0.0002

The amount of HCl pipetted was,

$$0.25 \pm 0.01 \text{ ml}$$

Each sample was made up to 10 ml by the pipetting of 9.75 ± 0.02 ml of glass-distilled water.

Testing of working standards by graphite furnace atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Vanadium (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.025 ± 0.002	0.008 ± 0.002
0.050 ± 0.002	0.015 ± 0.002
0.100 ± 0.002	0.028 ± 0.000
0.250 ± 0.002	0.066 ± 0.001
0.500 ± 0.003	0.130 ± 0.003
0.750 + 0.004	0.201 ± 0.005

* Average and standard deviation of five peak integration readings.

Linear regression analysis

0.000 - 0.750 ppm V

r = 0.99977
b = 0.0007
m = 0.2643

Sensitivity (1% absorption) = 0.0044/0.2643 = 0.016 ppm V

Reverse calculations for vanadium

Actual vanadium (ppm)	Calculated vanadium (ppm)	Absolute devn.
0.000	-0.002	0.002
0.025 ± 0.002	0.027	0.002
0.050 ± 0.002	0.054	0.004
0.100 ± 0.002	0.103	0.003
0.250 ± 0.002	0.247	0.003
0.500 ± 0.003	0.489	0.011
0.750 ± 0.004	0.758	0.008
	Average absolute deviation	0.0047

Values of vanadium (ppm) from the above equation subject to ± 0.0047 ppm.

Results of vanadium determination in dry ashed fish base tissue

The results of the blank and fish base tissue samples are tabulated in Table C-22.

From the 18 values recorded in the last column the following average was obtained:-

TABLE C-22

DETERMINATION OF VANADIUM IN RAINBOW TROUT BASE TISSUE
BY DRY ASHING/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

Sample No.	Average* absorbance	V in 10 ml** soln. (ppm)	Vanadium ug/10 ml	Vanadium in fish base (ppm)
1.	0.000	---	---	---
2.	0.002 ± 0.000	$0.004^{7} \pm 0.004^{7}$	$0.04^{7} \pm 0.04^{7}$	$0.2^{3} \pm 0.2^{3}$
3.	0.002 ± 0.001	$0.004^{7} \pm 0.009^{4}$	$0.04^{7} \pm 0.09^{4}$	$0.2^{3} \pm 0.4^{5}$
4.	0.002 ± 0.000	$0.004^{7} \pm 0.004^{7}$	$0.04^{7} \pm 0.04^{7}$	$0.2^{3} \pm 0.2^{3}$
5.	0.004 ± 0.001	$0.012^{3} \pm 0.009^{4}$	$0.12^{3} \pm 0.09^{4}$	$0.2^{5} \pm 0.1^{9}$
6.	0.005 ± 0.000	$0.016^{0} \pm 0.004^{7}$	$0.16^{0} \pm 0.04^{7}$	$0.31^{5} \pm 0.09^{2}$
7.	0.006 ± 0.001	$0.019^{8} \pm 0.009^{4}$	$0.19^{8} \pm 0.09^{4}$	$0.3^{9} \pm 0.1^{8}$
8.	0.009 ± 0.000	$0.031^{2} \pm 0.004^{7}$	$0.31^{2} \pm 0.04^{7}$	$0.31^{2} \pm 0.04^{7}$
9.	0.009 ± 0.001	$0.031^{2} \pm 0.009^{4}$	$0.31^{2} \pm 0.09^{4}$	$0.31^{1} \pm 0.09^{4}$
10.	0.009 ± 0.001	$0.031^{2} \pm 0.009^{4}$	$0.31^{2} \pm 0.09^{4}$	$0.31^{2} \pm 0.09^{4}$
11.	0.012 ± 0.001	$0.042^{5} \pm 0.009^{4}$	$0.42^{5} \pm 0.09^{4}$	$0.2^{8} \pm 0.06^{2}$
12.	0.013 ± 0.002	$0.04^{6} \pm 0.01^{4}$	$0.4^{6} \pm 0.1^{4}$	$0.29^{1} \pm 0.08^{8}$
13.	0.010 ± 0.002	$0.03^{5} \pm 0.01^{4}$	$0.3^{5} \pm 0.1^{4}$	$0.23^{7} \pm 0.09^{5}$
14.	0.014 ± 0.001	$0.050^{1} \pm 0.009^{4}$	$0.50^{1} \pm 0.09^{4}$	$0.25^{2} \pm 0.04^{7}$
15.	0.014 ± 0.001	$0.050^{1} \pm 0.009^{4}$	$0.50^{1} \pm 0.09^{4}$	$0.24^{9} \pm 0.04^{7}$
16.	0.013 ± 0.001	$0.046^{3} \pm 0.009^{4}$	$0.46^{3} \pm 0.09^{4}$	$0.23^{4} \pm 0.04^{7}$
17.	0.015 ± 0.002	$0.05^{4} \pm 0.01^{4}$	$0.5^{4} \pm 0.1^{4}$	$0.18^{3} \pm 0.04^{7}$
18.	0.016 ± 0.002	$0.05^{4} \pm 0.01^{4}$	$0.5^{4} \pm 0.1^{4}$	$0.18^{0} \pm 0.04^{7}$
19.	0.016 ± 0.001	$0.057^{7} \pm 0.009^{4}$	$0.57^{7} \pm 0.09^{4}$	$0.19^{4} \pm 0.03^{2}$

* Average and standard deviation of three peak integration readings

** Using the linear equation from Page 237.

$0.26^{0} \pm 0.05^{4}$ ppm V

or,

$0.26^{0} \pm 0.05^{4}$ ug V/g dried rainbow trout base

3. Recovery of Vanadium from Spiked Fish Base Samples

Dry ashing of fish tissue

The weights of macerated and dried fish tissue weighed into 50-ml beakers are given in Table C-23.

These tissue samples (dried at 65 ± 5 °C) were spiked with 1.000 ± 0.004 ppm vanadium solution as follows:-

Series	Spike	ug vanadium added
A	none	0.0
B	1.50 ± 0.02 ml of 1.000 ppm V	1.50 ± 0.03
C	3.00 ± 0.02 ml " " " "	3.00 ± 0.03
D	4.50 ± 0.02 ml " " " "	4.50 ± 0.04
E	6.00 ± 0.02 ml " " " "	6.00 ± 0.04
F	7.50 ± 0.02 ml " " " "	7.50 ± 0.05

Subsequent to spiking each sample was placed in the oven and heated at just under 100 °C until the liquid spike volume had dried out. The subsequent ashing procedure duplicated the details of Sub-section 2.2.3 - (b). A blank with no spike was carried through the same procedure.

The amount of HCl pipetted was,

0.25 ± 0.02 ml

The amount of glass-distilled water pipetted was,

9.75 ± 0.02 ml

Testing of working standards by graphite furnace atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

TABLE C-23

SAMPLE WEIGHTS OF BASE FISH TISSUE FOR VANADIUM DRY ASHING SPIKING PROCEDURE

No.	Series A	Series B	Series C
1	0.9986 ± 0.0002	1.0019 ± 0.0002	0.9848 ± 0.0002
2	1.0023 ± 0.0002	1.0126 ± 0.0002	1.0135 ± 0.0002
3	0.9993 ± 0.0002	1.0177 ± 0.0002	1.0057 ± 0.0002
4	1.0140 ± 0.0002	0.9953 ± 0.0002	0.9896 ± 0.0002
5	1.0042 ± 0.0002	0.9856 ± 0.0002	1.0083 ± 0.0002

No.	Series D	Series E	Series F
1	1.0140 ± 0.0002	1.0100 ± 0.0002	0.9986 ± 0.0002
2	1.0021 ± 0.0002	0.9161 ± 0.0002	1.0059 ± 0.0002
3	1.0006 ± 0.0002	1.0212 ± 0.0002	1.0061 ± 0.0002
4	1.0622 ± 0.0002	0.9992 ± 0.0002	1.0016 ± 0.0002
5	1.0023 ± 0.0002	1.0004 ± 0.0002	1.0008 ± 0.0002

Vanadium (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.050 ± 0.002	0.013 ± 0.000
0.100 ± 0.002	0.030 ± 0.001
0.250 ± 0.002	0.068 ± 0.001
0.500 ± 0.003	0.134 ± 0.003
0.750 ± 0.004	0.205 ± 0.002
1.000 ± 0.004	0.270 ± 0.003

* Average and standard deviation of five peak integration readings.

Linear regression analysis

0.000 - 1.000 ppm V

$r = 0.9999$
$b = 0.000^6$
$m = 0.2700^8$

Sensitivity (1% absorption) = $0.0044/0.2700^8 = 0.01^6$ ppm V

Reverse calculations for vanadium

Actual vanadium (ppm)	Calculated vanadium (ppm)	Absolute devn.
0.000	-0.002	0.002
0.050 ± 0.002	0.046	0.004
0.100 ± 0.002	0.109	0.009
0.250 ± 0.002	0.250	0.000
0.500 ± 0.003	0.494	0.006
0.750 ± 0.004	0.757	0.007
1.000 ± 0.004	0.997	0.003
	Average absolute deviation	0.004

Values of vanadium (ppm) from the above equation subject to ± 0.004 ppm.

Results of vanadium determination of spiked dry ashed fish base tissue

The absorbance results for the blank and fish base samples spiked with vanadium (ug) are tabulated on Table C-24.

The calculated vanadium recoveries for Series B to F are tabulated on Table C-25.

TABLE C-24

DRY ASHING/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

RESULTS OF VANADIUM DETERMINATION
ON SPIKED FISH BASE SAMPLES

Series and No.	Average* absorbance	Corrected average absorbance
blank	0.005 ± 0.000	0.000 ± 0.000
A-1	0.014 ± 0.000	0.009 ± 0.000
2	0.014 ± 0.000	0.009 ± 0.000
3	0.015 ± 0.000	0.010 ± 0.000
4	0.014 ± 0.000	0.009 ± 0.000
5	0.013 ± 0.000	0.008 ± 0.000
B-1	0.051 ± 0.000	0.046 ± 0.000
2	0.057 ± 0.001	0.052 ± 0.001
3	0.053 ± 0.000	0.048 ± 0.000
4	0.057 ± 0.002	0.052 ± 0.002
5	0.053 ± 0.000	0.048 ± 0.000
C-1	0.095 ± 0.001	0.090 ± 0.001
2	0.095 ± 0.002	0.090 ± 0.002
3	0.089 ± 0.001	0.084 ± 0.001
4	0.091 ± 0.000	0.086 ± 0.000
5	0.091 ± 0.000	0.086 ± 0.000
D-1	0.143 ± 0.002	0.138 ± 0.002
2	0.144 ± 0.000	0.139 ± 0.000
3	0.134 ± 0.001	0.129 ± 0.001
4	0.144 ± 0.000	0.139 ± 0.000
5	0.133 ± 0.000	0.128 ± 0.000
E-1	0.178 ± 0.002	0.173 ± 0.002
2	0.179 ± 0.001	0.174 ± 0.001
3	0.164 ± 0.000	0.159 ± 0.000
4	0.176 ± 0.001	0.171 ± 0.001
5	0.184 ± 0.003	0.179 ± 0.003
F-1	0.219 ± 0.002	0.214 ± 0.002
2	0.217 ± 0.003	0.212 ± 0.003
3	0.219 ± 0.002	0.214 ± 0.002
4	0.214 ± 0.001	0.209 ± 0.001
5	0.211 ± 0.003	0.206 ± 0.003

* Average and standard deviation of three peak integration readings.

TABLE C-25

DRY ASHING/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

RECOVERY OF VANADIUM IN SPIKED FISH BASE SAMPLES

Series and No.	V in 10 ml* soln. (ppm)	Total V (ug/10 ml)	**V (ug/10 ml) corr. fish base	Actual V spike (ug)	Recovery of V (%)
B-1	0.168 ± 0.004	1.68 ± 0.04	1.41 ± 0.05	1.50 ± 0.03	94.0 ± 3.3
2	0.190 ± 0.008	1.90 ± 0.08	1.63 ± 0.08	1.50 ± 0.03	108.7 ± 5.3
3	0.175 ± 0.004	1.75 ± 0.04	1.48 ± 0.05	1.50 ± 0.03	98.7 ± 3.3
4	0.190 ± 0.012	1.90 ± 0.12	1.63 ± 0.12	1.50 ± 0.03	108.7 ± 5.3
5	0.175 ± 0.004	1.75 ± 0.04	1.49 ± 0.05	1.50 ± 0.03	99.3 ± 3.3
C-1	0.331 ± 0.008	3.31 ± 0.08	3.04 ± 0.08	3.00 ± 0.03	101.3 ± 2.7
2	0.331 ± 0.012	3.31 ± 0.12	3.04 ± 0.12	3.00 ± 0.03	101.3 ± 4.0
3	0.309 ± 0.008	3.09 ± 0.08	2.82 ± 0.08	3.00 ± 0.03	94.0 ± 2.7
4	0.316 ± 0.004	3.16 ± 0.04	2.89 ± 0.05	3.00 ± 0.03	96.3 ± 1.7
5	0.316 ± 0.004	3.16 ± 0.04	2.89 ± 0.05	3.00 ± 0.03	96.3 ± 1.7
D-1	0.509 ± 0.012	5.09 ± 0.12	4.81 ± 0.12	4.50 ± 0.04	106.9 ± 2.7
2	0.512 ± 0.004	5.12 ± 0.04	4.85 ± 0.05	4.50 ± 0.04	107.8 ± 1.1
3	0.475 ± 0.008	4.75 ± 0.08	4.48 ± 0.08	4.50 ± 0.04	99.6 ± 1.8
4	0.512 ± 0.004	5.12 ± 0.04	4.84 ± 0.05	4.50 ± 0.04	107.6 ± 1.1
5	0.472 ± 0.004	4.72 ± 0.04	4.44 ± 0.05	4.50 ± 0.04	98.7 ± 1.1
E-1	0.638 ± 0.012	6.38 ± 0.12	6.11 ± 0.12	6.00 ± 0.04	101.8 ± 2.0
2	0.642 ± 0.008	6.42 ± 0.08	6.17 ± 0.08	6.00 ± 0.04	103.0 ± 1.3
3	0.586 ± 0.004	5.86 ± 0.04	5.59 ± 0.05	6.00 ± 0.04	93.2 ± 1.0
4	0.631 ± 0.008	6.31 ± 0.08	6.04 ± 0.08	6.00 ± 0.04	100.7 ± 1.3
5	0.660 ± 0.016	6.60 ± 0.16	6.33 ± 0.16	6.00 ± 0.04	105.5 ± 2.7
F-1	0.790 ± 0.012	7.90 ± 0.12	7.63 ± 0.12	7.50 ± 0.05	101.7 ± 1.6
2	0.783 ± 0.016	7.83 ± 0.16	7.55 ± 0.16	7.50 ± 0.05	100.7 ± 2.1
3	0.790 ± 0.012	7.90 ± 0.12	7.63 ± 0.12	7.50 ± 0.05	101.7 ± 1.6
4	0.772 ± 0.008	7.72 ± 0.08	7.44 ± 0.08	7.50 ± 0.05	99.2 ± 1.1
5	0.760 ± 0.016	7.60 ± 0.16	7.33 ± 0.16	7.50 ± 0.05	97.7 ± 2.1

* Using the linear equation from Page 241.

** Based on the higher of the two uncertainties:- fish base or total vanadium in 10 ml.

The values for the 10 ml each Series A yield the following results:-

Series and No.	V in 10 ml soln. (ppm)	V (ug/g) in fish base
A-1	0.031 ± 0.004	0.31 ± 0.04
2	0.031 ± 0.004	0.31 ± 0.04
3	0.035 ± 0.004	0.35 ± 0.04
4	0.031 ± 0.004	0.30 ± 0.04
5	0.027 ± 0.004	0.27 ± 0.04

These again represent fish base values and, combined with the 18 values obtained under Appendix C-6 - (2) Testing of Dry Ashed Rainbow Trout Base Tissue, provide a total of 23 values. The average vanadium content of dried rainbow trout unexposed to experimentally high vanadium contents in the water and/or food resulted in:-

$0.27^{1} \pm 0.05^{3}$ ppm or $0.27^{1} \pm 0.05^{3}$ ug V/g dried rainbow trout

The overall recovery percent for vanadium on rainbow trout dried base of up to 7.5 ug/g dried fish base resulted in:-

$$100.^{7} \pm 4.^{9} \%$$

If the average for each of the Series B to F is taken with respect to the "Total V (ug/10 ml)" column data (Table C-25) we have:-

Series	B	$1.79^{6} \pm 0.09^{9}$
	C	$3.20^{6} \pm 0.09^{9}$
	D	$4.9^{6} \pm 0.2^{1}$
	E	$6.3^{1} \pm 0.2^{7}$
	F	$7.7^{9} \pm 0.1^{3}$

Linear regression analysis yields:- $r = 0.9993$
$b = 0.28^{5}$
$m = 1.006^{1}$

Thus the zero intercept on the ordinate yields a value of 0.28^5 ug V/10 ml of solution or, for the sample weights approximating 1.00 grams of dried fish base, 0.28^5 ug V/g dried fish base. Carrying out reverse calculations indicated an uncertainty for this value of 0.05^9 ug, so that we have, from the method of additions, a calculated value for dried rainbow trout base content of vanadium of $0.28^5 \pm 0.05^9$ ug/g fish base. This compares very well, within the respective tolerance limits, with the value of $0.27^1 \pm 0.05^3$ ug V/g dried fish base reported on the previous page.

Using the value of $0.28^5 \pm 0.05^9$ ug V/g dried fish base as the correction value to the "Total V (ug/10 ml)" data column yields an average recovery percent for spikes up to 7.5 ug on 1 g dried fish base of $100.^4 \pm 4.^6$. This also agrees well, within the respective tolerance limits, with the recovery average of $100.^7 \pm 4.^9$ % reported on the previous page.

By including the average result of Series A with the average for each of the Series B to F taken with respect to the "Total V (ug/10 ml)" data column from Table C-25 in a graphical determination of vanadium in dried rainbow trout by the method of additions, the plot on Figure C-6 was obtained. The vanadium content in dried rainbow trout base tissue can be extrapolated to the abscissa to 0.29 ± 0.02 ug V/g fish base. The reading uncertainty is represented by the value $\pm$ 0.02. This extrapolated vanadium concentration compares very well, within the respective tolerance limits, with the value of $0.27^1 \pm 0.05^3$ ug V/g dried fish base reported on the previous page.

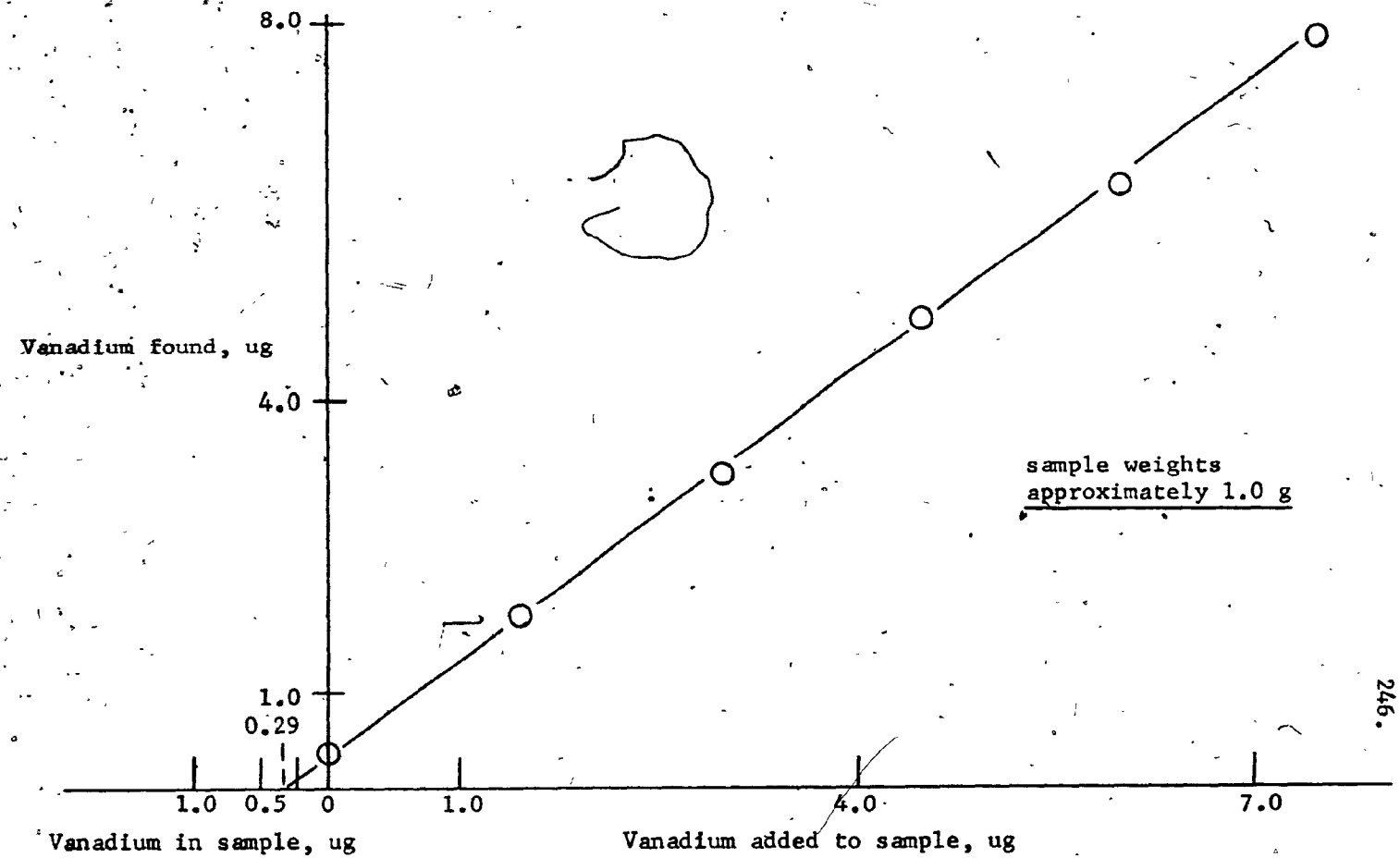


FIGURE C-6 GRAPHICAL METHOD OF ADDITIONS - VANADIUM DETERMINATION IN RAINBOW TROUT - DRY ASHING

4. Recovery of Vanadium from Aliquoted Fish Base Samples

Two sets of macerated and dried (65 ± 5 °C) rainbow trout tissue were weighed out in duplicate. Each sample consisted of 1.0000 ± 0.0002 g and was placed in a 50-ml beaker.

One set (A) was spiked with 6.00 ± 0.04 ug of V each, while the second set (B) was spiked with 7.50 ± 0.05 ug of V each.

All samples were dried, ashed and extracted according to the details of Sub-section 2.2.3 - (b). A blank was carried through simultaneously. The amount of HCl pipetted was 0.25 ± 0.02 ml and the amount of glass-distilled water was 9.75 ± 0.02 ml.

For each set, 5 ml of the final solution was tested as-is, and 4 ml was diluted with 10 ml of 2.5 percent HCl and then tested. The following results were obtained:-

Set	Ave. absorbance corr. for blank		Total V* (ug/10 ml)		**V corr. for base fish (ug/g)		Actual spike (ug)	Recovery (%)	
	as-is	aliq.	as-is	aliqx3.5	as-is	aliqx3.5		as-is	aliq.
A	0.175	0.048	6.46	6.14	6.19	5.87	6.00	103	98
B	0.217	0.060	8.05	7.70	7.78	7.43	7.50	104	99

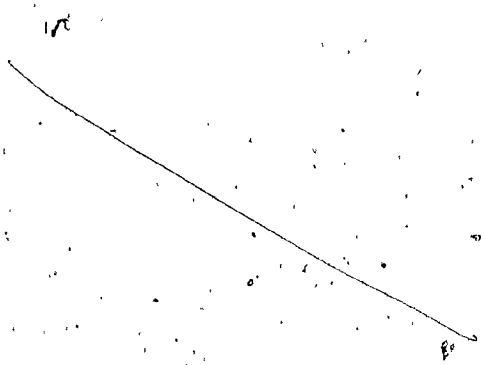
* Using the linear equation from Page 241.

** Using the correction factor of 0.27[1] ug V/g dried rainbow trout base.

The recovery percent values agree within the expected tolerance limits for vanadium recovery on spiked samples, so that aliquoting of tissue sample solutions, suspected to contain vanadium in ppm beyond the linear range maximum, should present no difficulties.

APPENDIX D

APPENDIX D-1



Determination of Copper in Rainbow Trout by the Wet Pressure-Digestion/Flame Atomic Absorption Technique

1. General

The following sections outline the experimental approach applied to the testing, recovery from spiking and recovery from aliquoting of copper in dried rainbow trout base tissue.

The flame method of atomic absorption analysis was applied, rather than the graphite furnace technique, because of the relatively high copper content found for rainbow trout unexposed to experimentally high copper levels in the water and/or food.

General operating conditions

Certain general operating conditions were maintained throughout the analytical procedures. These are outlined in Appendix A-1.

Preparation of aqueous-base standard solutions

Appendix A-1 outlines the preparation of the aqueous-base standard solutions. Additional working standard solutions were prepared as follows:-

Solution preparation*	Copper (ppm)
1.00 ± 0.02 ml of 10.00 ± 0.02 ppm Cu	0.100 ± 0.002
3.00 ± 0.02 ml of 10.00 ± 0.02 ppm Cu	0.300 ± 0.003

* All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Preparation of fish tissue

The preparation of rainbow trout base tissue is described in Subsection 2.2.3 - (a).

2. Testing of Wet Pressure-digested Rainbow Trout Base Tissue

Wet ashing (pressure/digestion) of fish tissue

The general procedure is outlined in Sub-section 2.2.3 - (c). The specific details for the determination of copper are given below./

The sample weights were as follows:-

Sample No.	Fish weight (g)
1.	0.0000
2.	0.2521 ± 0.0002
3.	0.2858 ± 0.0002
4.	0.2986 ± 0.0002
5.	0.3589 ± 0.0002
6.	0.3694 ± 0.0002
7.	0.3750 ± 0.0002
8.	0.4729 ± 0.0002
9.	0.5804 ± 0.0002
10.	0.7419 ± 0.0002
11.	0.7633 ± 0.0002
12.	0.8189 ± 0.0002
13.	1.1071 ± 0.0002
14.	1.5013 ± 0.0002
15.	1.6415 ± 0.0002

The amount of HCl pipetted was,

0.50 ± 0.01 ml

The amount of water pipetted was,

14.50 ± 0.04 ml

Testing of working standards by flame atomic absorption

The set of working standard solutions prepared for this series of tests gave the following results:-

Copper (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.100 ± 0.002	0.006 ± 0.000
0.300 ± 0.003	0.016 ± 0.000
0.500 ± 0.003	0.026 ± 0.000
1.000 ± 0.004	0.051 ± 0.001
2.00 ± 0.02	0.103 ± 0.001
5.00 ± 0.02	0.256 ± 0.001

* Average and standard deviation of five 3-seconds integration readings.

Linear regression analysis

0.000 - 5.00 ppm Cu

r = 0.9999
b = 0.000
m = 0.05119

Sensitivity (1% absorption) = 0.0044/0.05119 = 0.09 ppm Cu

Reverse calculations for copper

Actual copper (ppm)	Calculated copper (ppm)	Absolute devn.
0.000	0.000	0.000
0.100 ± 0.002	0.117	0.017
0.300 ± 0.003	0.312	0.012
0.500 ± 0.003	0.508	0.008
1.000 ± 0.004	0.996	0.004
2.00 ± 0.02	2.01[2]	0.01[2]
5.00 ± 0.02	5.00	0.00
	Average absolute deviation	0.008

Values of copper (ppm) from the above equation subject to ± 0.008 ppm.

Results of copper determination in wet pressure-digested fish base tissue

The results of the blank and fish base samples are tabulated in Table D-1.

TABLE D-1

DETERMINATION OF COPPER IN RAINBOW TROUT BASE TISSUE BY WET PRESSURE-DIGESTION/FLAME ATOMIC ABSORPTION TECHNIQUE

Sample No.	Average* absorbance	Corrected average absorbance	**Cu in 15 ml soln. (ppm)	Copper ug/15 ml	Copper in fish base (ppm)
1.	0.001 ± 0.000	0.000 ± 0.000	---	---	---
2.	0.005 ± 0.000	0.004 ± 0.000	0.078 ± 0.008	$1.1^{7} \pm 0.1^{2}$	$4.6^{4} \pm 0.4^{8}$
3.	0.005 ± 0.000	0.004 ± 0.000	0.078 ± 0.008	$1.1^{7} \pm 0.1^{2}$	$4.0^{9} \pm 0.4^{2}$
4.	0.005 ± 0.000	0.004 ± 0.000	0.078 ± 0.008	$1.1^{7} \pm 0.1^{2}$	$3.9^{2} \pm 0.4^{0}$
5.	0.006 ± 0.000	0.005 ± 0.000	0.098 ± 0.008	$1.4^{7} \pm 0.1^{2}$	$4.1^{0} \pm 0.3^{3}$
6.	0.006 ± 0.000	0.005 ± 0.000	0.098 ± 0.008	$1.4^{7} \pm 0.1^{2}$	$3.9^{8} \pm 0.3^{2}$
7.	0.006 ± 0.000	0.005 ± 0.000	0.098 ± 0.008	$1.4^{7} \pm 0.1^{2}$	$3.9^{2} \pm 0.3^{2}$
8.	0.007 ± 0.000	0.006 ± 0.000	0.117 ± 0.008	$1.7^{6} \pm 0.1^{2}$	$3.7^{2} \pm 0.2^{5}$
9.	0.009 ± 0.000	0.008 ± 0.000	0.156 ± 0.008	$2.3^{4} \pm 0.1^{2}$	$4.0^{3} \pm 0.2^{1}$
10.	0.012 ± 0.000	0.011 ± 0.000	0.215 ± 0.008	$3.2^{2} \pm 0.1^{2}$	$4.3^{4} \pm 0.1^{6}$
11.	0.013 ± 0.000	0.012 ± 0.000	0.234 ± 0.008	$3.5^{1} \pm 0.1^{2}$	$4.6^{0} \pm 0.1^{6}$
12.	0.013 ± 0.000	0.012 ± 0.000	0.234 ± 0.008	$3.5^{1} \pm 0.1^{2}$	$4.2^{9} \pm 0.1^{5}$
13.	0.016 ± 0.000	0.015 ± 0.000	0.293 ± 0.008	$4.4^{0} \pm 0.1^{2}$	$3.9^{7} \pm 0.1^{1}$
14.	0.021 ± 0.000	0.020 ± 0.000	0.391 ± 0.008	$5.8^{6} \pm 0.1^{2}$	3.90 ± 0.08
15.	0.025 ± 0.000	0.024 ± 0.000	0.469 ± 0.008	$7.0^{3} \pm 0.1^{2}$	4.28 ± 0.07

* Each absorbance value represents the average of five 3-seconds integration readings. ± values are standard deviations.

** Using the linear equation from Page 252.

The 14 values of copper in fish tissue resulted in an average of,

$$4.1^3 \pm 0.2^7 \text{ ppm}$$

or,

$$4.1^3 \pm 0.2^7 \text{ ug Cu/g dried rainbow trout base}$$

3. Recovery of Copper from Spiked Fish Base Samples

Wet ashing (pressure/digestion) of fish tissue

The weights of macerated and dried fish tissue weighed into 12-cm test tubes are tabulated in Table D-2.

These fish tissue samples were spiked with 10.00 ± 0.02 ppm Cu solution as follows:-

Series	Spike	ug copper added
A	none	0.0
B	1.00 ± 0.02 ml of 10.00 ppm Cu	10.0 ± 0.2
C	2.50 ± 0.02 ml " " " "	25.0 ± 0.2
D	4.00 ± 0.02 ml " " " "	40.0 ± 0.3
E	5.50 ± 0.02 ml " " " "	55.0 ± 0.3
F	7.00 ± 0.02 ml " " " "	70.0 ± 0.3

Subsequent to spiking each sample was placed in the oven and heated at just under 100 ^{o}C until the liquid spike volume had just dried out. The subsequent acid-digestion procedure duplicated the details of Sub-section 2.2.3 - (c). A blank with no spike was carried through the same procedure.

The amount of HCl pipetted was,

$$0.50 \pm 0.01 \text{ ml}$$

The amount of water pipetted was,

$$14.50 \pm 0.04 \text{ ml}$$

TABLE D-2

SAMPLE WEIGHTS OF BASE FISH TISSUE FOR WET PRESSURE/DIGESTION SPIKING TECHNIQUE

No.	Series A	Series B	Series C
1	1.0226 ± 0.0002	0.9998 ± 0.0002	1.1120 ± 0.0002
2	1.0141 ± 0.0002	0.9734 ± 0.0002	1.0329 ± 0.0002
3	1.1519 ± 0.0002	0.9894 ± 0.0002	0.9758 ± 0.0002
4	0.9930 ± 0.0002	1.0134 ± 0.0002	1.0195 ± 0.0002
5	0.9893 ± 0.0002	0.9900 ± 0.0002	0.9597 ± 0.0002

No.	Series D	Series E	Series F
1	1.0215 ± 0.0002	1.0410 ± 0.0002	0.9924 ± 0.0002
2	1.0199 ± 0.0002	1.2006 ± 0.0002	1.0408 ± 0.0002
3	1.0561 ± 0.0002	0.9097 ± 0.0002	0.9782 ± 0.0002
4	1.0400 ± 0.0002	0.9925 ± 0.0002	0.9729 ± 0.0002
5	1.1067 ± 0.0002	0.9883 ± 0.0002	0.9944 ± 0.0002

Testing of working standards by flame atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Copper (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.500 ± 0.003	0.026 ± 0.000
1.000 ± 0.004	0.050 ± 0.000
2.00 ± 0.02	0.102 ± 0.000
3.00 ± 0.02	0.150 ± 0.001
5.00 ± 0.02	0.251 ± 0.001

* Average and standard deviation of five 3-seconds integration readings.

Linear regression analysis

0.000 - 5.00 ppm Cu

$r = 0.9999^6$

$b = 0.000^4$

$m = 0.05010^6$

Sensitivity (1% absorption) $= 0.0044/0.05010^6 = 0.08^7$ ppm Cu

Reverse calculations for copper

Actual copper (ppm)	Calculated copper (ppm)	Absolute devn.
0.000	-0.008	0.008
0.500 ± 0.003	0.511	0.011
1.000 ± 0.004	0.990	0.010
2.00 ± 0.02	2.028	0.028
3.00 ± 0.02	2.986	0.014
5.00 ± 0.02	5.001	0.001
	Average absolute deviation	0.01^2

Values of copper (ppm) from the above equation subject to $\pm 0.01^2$ ppm.

Results of copper determination of spiked wet pressure-digested fish base tissue

The absorbance results of the blank and fish base samples spiked with copper (ug) are tabulated on Table D-3.

The calculated copper recoveries are shown on Table D-4.

The values for the 15 ml each Series A samples yield the following results:-

Series and No.	Cu in 15 ml soln. (ppm)	Cu (ug/g) in fish base
A-1	$0.29^{1} \pm 0.01^{2}$	$4.2^{6} \pm 0.1^{7}$
2	$0.29^{1} \pm 0.01^{2}$	$4.3^{0} \pm 0.1^{7}$
3	$0.31^{1} \pm 0.01^{2}$	$4.0^{5} \pm 0.1^{5}$
4	$0.27^{1} \pm 0.01^{2}$	$4.1^{0} \pm 0.1^{8}$
5	$0.27^{1} \pm 0.01^{2}$	$4.1^{1} \pm 0.1^{8}$

These again represent fish base values and combined with the 14 values obtained under Appendix D-1 (2) Testing of Wet Pressure-digested Rainbow Trout Base Tissue, provide a total of 19 values. These yield a final value for the copper content of dried rainbow trout unexposed to experimentally high copper contents in the water and/or food of:-

$4.1^{4} \pm 0.2^{4}$ ppm or $4.1^{4} \pm 0.2^{4}$ ug Cu/g dried rainbow trout base

Using the 2s rejection criterion to examine the recovery percent data of the last column (Table D-4), it was noted that rejection of sample C-2 was required. The remaining values yield an overall recovery percent for copper spikes on rainbow trout dried base of up to 70 ug/g dried fish base of:-

$$96.^{1} \pm 8.^{8} \%$$

TABLE D-3

WET PRESSURE-DIGESTION/FLAME ATOMIC ABSORPTION TECHNIQUE

RESULTS OF COPPER DETERMINATION
ON SPIKED FISH BASE SAMPLES

Series and No.	Average* absorbance	Corrected average absorbance
blank	0.005 ± 0.000	0.000 ± 0.000
A-1	0.020 ± 0.000	0.015 ± 0.000
2	0.020 ± 0.000	0.015 ± 0.000
3	0.021 ± 0.000	0.016 ± 0.000
4	0.019 ± 0.000	0.014 ± 0.000
5	0.019 ± 0.000	0.014 ± 0.000
B-1	0.054 ± 0.000	0.049 ± 0.000
2	0.050 ± 0.001	0.045 ± 0.001
3	0.051 ± 0.000	0.046 ± 0.000
4	0.058 ± 0.000	0.053 ± 0.000
5	0.057 ± 0.000	0.052 ± 0.000
C-1	0.104 ± 0.000	0.099 ± 0.000
2	0.135 ± 0.001	0.130 ± 0.001
3	0.094 ± 0.001	0.089 ± 0.001
4	0.091 ± 0.000	0.086 ± 0.000
5	0.092 ± 0.000	0.087 ± 0.000
D-1	0.150 ± 0.000	0.145 ± 0.000
2	0.152 ± 0.001	0.147 ± 0.001
3	0.145 ± 0.001	0.140 ± 0.001
4	0.149 ± 0.001	0.144 ± 0.001
5	0.157 ± 0.001	0.152 ± 0.001
E-1	0.219 ± 0.000	0.214 ± 0.000
2	0.208 ± 0.000	0.203 ± 0.000
3	0.204 ± 0.000	0.199 ± 0.000
4	0.201 ± 0.000	0.196 ± 0.000
5	0.183 ± 0.001	0.178 ± 0.001
F-1	0.217 ± 0.000	0.212 ± 0.000
2	0.222 ± 0.000	0.217 ± 0.000
3	0.233 ± 0.001	0.228 ± 0.001
4	0.224 ± 0.000	0.219 ± 0.000
5	0.238 ± 0.001	0.233 ± 0.001

* Average and standard deviation of five 3-seconds integration readings.

TABLE D-4

WET PRESSURE-DIGESTION/FLAME ATOMIC ABSORPTION TECHNIQUE

RECOVERY OF COPPER IN SPIKED FISH BASE SAMPLES

Series and No.	*Cu in 15 ml soln. (ppm)	Total Cu (ug/15 ml)	**Cu (ug/15 ml) corr. fish base	Actual Cu spike (ug)	Recovery of Cu (%)
B-1	$0.97^{0} \pm 0.01^{2}$	14.55 ± 0.18	$10.4^{1} \pm 0.2^{4}$	10.0 ± 0.2	104.1 ± 2.4
2	$0.89^{0} \pm 0.02^{4}$	13.35 ± 0.36	$9.3^{2} \pm 0.3^{6}$	10.0 ± 0.2	93.2 ± 3.6
3	$0.91^{0} \pm 0.01^{2}$	13.65 ± 0.18	$9.5^{5} \pm 0.2^{4}$	10.0 ± 0.2	95.5 ± 2.4
4	$1.05^{0} \pm 0.01^{2}$	15.75 ± 0.18	$11.5^{5} \pm 0.2^{4}$	10.0 ± 0.2	115.5 ± 2.4
5	$1.03^{0} \pm 0.01^{2}$	15.45 ± 0.18	$11.3^{5} \pm 0.2^{4}$	10.0 ± 0.2	113.5 ± 2.4
C-1	$1.96^{7} \pm 0.01^{2}$	29.50 ± 0.18	24.90 ± 0.2^{4}	25.0 ± 0.2	99.6 ± 1.0
2	$2.58^{6} \pm 0.02^{4}$	38.79 ± 0.36	$34.5^{1} \pm 0.3^{6}$	25.0 ± 0.2	138.0 ± 1.4
3	$1.76^{8} \pm 0.02^{4}$	26.52 ± 0.36	$22.4^{8} \pm 0.3^{6}$	25.0 ± 0.2	89.9 ± 1.4
4	$1.70^{8} \pm 0.01^{2}$	25.62 ± 0.18	$21.4^{0} \pm 0.2^{4}$	25.0 ± 0.2	85.6 ± 1.0
5	$1.72^{8} \pm 0.01^{2}$	25.92 ± 0.18	21.95 ± 0.2^{4}	25.0 ± 0.2	87.8 ± 1.0
D-1	$2.88^{5} \pm 0.01^{2}$	43.28 ± 0.18	$39.0^{5} \pm 0.2^{4}$	40.0 ± 0.3	97.6 ± 0.6
2	$2.92^{6} \pm 0.02^{4}$	$43.8^{9} \pm 0.36$	$39.6^{7} \pm 0.3^{6}$	40.0 ± 0.3	99.2 ± 0.9
3	$2.78^{6} \pm 0.02^{4}$	$41.7^{9} \pm 0.$	$37.4^{8} \pm 0.3^{6}$	40.0 ± 0.3	83.7 ± 0.8
4	$2.86^{6} \pm 0.02^{4}$	$42.9^{9} \pm 0.36$	$38.6^{8} \pm 0.3^{6}$	40.0 ± 0.3	96.7 ± 0.9
5	$3.02^{5} \pm 0.02^{4}$	$45.3^{8} \pm 0.36$	$40.8^{0} \pm 0.36$	40.0 ± 0.3	102.0 ± 0.9
E-1	$4.26^{3} \pm 0.01^{2}$	$63.9^{4} \pm 0.18$	$59.6^{3} \pm 0.2^{4}$	55.0 ± 0.3	108.4 ± 0.4
2	$4.04^{3} \pm 0.01^{2}$	60.64 ± 0.18	$55.6^{7} \pm 0.2^{4}$	55.0 ± 0.3	101.2 ± 0.4
3	$3.96^{3} \pm 0.01^{2}$	$59.4^{4} \pm 0.18$	$55.6^{7} \pm 0.2^{4}$	55.0 ± 0.3	101.2 ± 0.4
4	$3.90^{4} \pm 0.01^{2}$	$58.5^{6} \pm 0.18$	54.45 ± 0.24	55.0 ± 0.3	99.0 ± 0.4
5	$3.54^{4} \pm 0.02^{4}$	$53.1^{6} \pm 0.36$	$49.0^{7} \pm 0.3^{6}$	55.0 ± 0.3	89.2 ± 0.6
F-1	$4.22^{3} \pm 0.01^{2}$	$63.3^{4} \pm 0.18$	$59.2^{3} \pm 0.2^{4}$	70.0 ± 0.3	84.6 ± 0.3
2	$4.32^{3} \pm 0.01^{2}$	$64.8^{4} \pm 0.18$	$60.5^{3} \pm 0.2^{4}$	70.0 ± 0.3	86.5 ± 0.3
3	$4.54^{2} \pm 0.02^{4}$	$68.1^{3} \pm 0.36$	$64.0^{8} \pm 0.36$	70.0 ± 0.3	91.5 ± 0.5
4	$4.36^{3} \pm 0.01^{2}$	$65.4^{4} \pm 0.18$	$61.4^{1} \pm 0.2^{4}$	70.0 ± 0.3	87.7 ± 0.3
5	$4.64^{2} \pm 0.02^{4}$	$69.6^{3} \pm 0.36$	$65.5^{1} \pm 0.36$	70.0 ± 0.3	93.6 ± 0.5

* Using the linear equation from Page 256.

** Based on the higher of the two uncertainties:- fish base copper or total copper in 15 ml.

If the average for each of the Series B to F is taken with respect to the "Total Cu (ug/15 ml)" column data we have, omitting the C-2 value:-

Series	B	14.6 ± 0.9
	C	26.5 ± 0.9
	D	$41.^{4} \pm 1.^{3}$
	E	$58.^{0} \pm 5.^{9}$
	F	$66.^{6} \pm 3.^{4}$

Linear regression analysis yields:- $r = 0.9960$, $b = 5.29$, $m = 0.9033$

Thus the zero intercept on the ordinate yields a value of 5.29 ug Cu/15 ml of solution or, for the sample weights approximating an average of 1.0 g of dried fish base, 5.29 ug Cu/g dried fish base. Carrying out reverse calculations indicates an uncertainty for this value of $\pm 1.^{3}$ ug, so that we have, from the method of additions, a calculated value of dried rainbow trout base content of copper of $5.^{3} \pm 1.^{3}$ ug Cu/g fish base. This compares, within the respective tolerance limits, with the value of $4.1^{4} \pm 0.2^{4}$ ug Cu/g dried fish base reported on the previous page, only because of the very large uncertainty ($\pm 1.^{3}$ ug) found in the method of additions. This large uncertainty is due to the wide variation in sample weights used for spiking (Table D-2).

Using the value of $5.^{3} \pm 1.^{3}$ ug Cu/g dried fish base as a correction value to the "Total Cu (ug/15 ml)" data column yields, omitting the C-2 result, an average recovery percent for spikes up to 70 ug on one gram of dried fish base of $91.^{9} \pm 7.^{4}$. This agrees well, within the respective tolerance limits, with the value of $96.^{1} \pm 8.^{8}$ % reported on the previous page.

By including the average result of Series A with the average for each of the Series B to F taken with respect to the "Total Cu (ug/15 ml)" data column from Table D-4 in a graphical determination of copper in dried rainbow trout by the method of additions, the plot on Figure D-1 was obtained. The copper content in dried rainbow trout base tissue can be extrapolated to the abscissa to $4.5^{0} \pm 0.2^{5}$ ug Cu/g fish base. The value of $\pm 0.2^{5}$ represents the reading uncertainty on the graph. This extrapolated copper concentration compares, within the respective tolerance limits, with the value of $4.1^{4} \pm 0.2^{4}$ ug Cu/g dried fish base reported on the previous page.

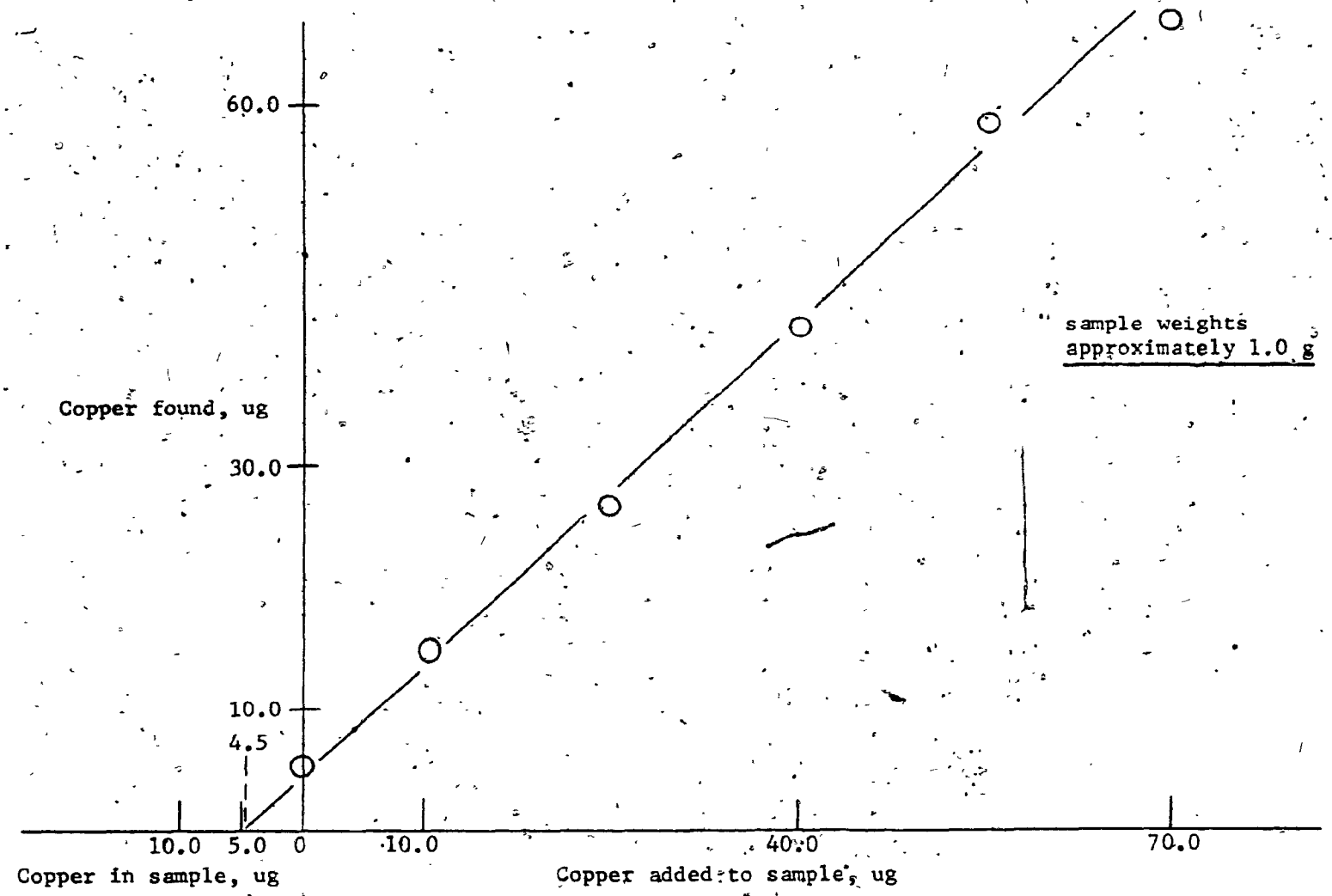


FIGURE D-1 GRAPHICAL METHOD OF ADDITIONS - COPPER DETERMINATION IN RAINBOW TROUT - WET PRESSURE-DIGESTION

4. Recovery of Copper from Aliquoted Spiked Fish Base Samples

Two sets of macerated and dried (65 ± 5 °C) rainbow trout tissue were weighed out in duplicate. Each sample consisted of 1.0000 ± 0.0002 g and was placed in a Kimax screwcap 12-cm test tube.

One set (A) was spiked with 55.0 ± 0.3 ug of Cu each, while the other set (B) was spiked with 70.0 ± 0.3 ug of Cu each.

All samples were dried, digested and extracted according to the details of Sub-section 2.2.3 - (c). A blank was carried through simultaneously. The amount of HCl pipetted was 0.50 ± 0.01 ml while the amount of water pipetted was 14.50 ± 0.04 ml.

For each set, 5 ml of the final solution was tested as-is, and 10 ml was diluted to 20 ml with 2.5 percent HCl and then tested. The results were:-

Set	Ave. absorbance corr. for blank		Total Cu* (ug/15 ml)		Cu corr. for** base fish (ug/g)		Actual spike	Recovery (%)	
	as-is	aliq.	as-is	aliqx2	as-is	aliqx2	(ug)	as-is	aliq.
A	0.205	0.095	61.2	56.6	57.1	52.5	55.0	103.8	95.4
B	0.230	0.120	68.7	71.6	64.6	67.5	70.0	92.3	96.4

* Using the linear equation from Page 256.

** Using the correction value of $4.1^4 \pm 0.2^4$ ug Cu/g dried rainbow trout base.

The recovery percent values agree within the expected tolerance limits for copper recovery on spiked samples. Thus, aliquoting of tissue sample solution, suspected to contain copper in ppm beyond the linear range maximum, should present no difficulty.

APPENDIX D-2

Determination of Nickel in Rainbow Trout by the Wet Pressure-Digestion/Flame Atomic Absorption Technique

1. General

The following sections outline the experimental approach applied to the testing, recovery from spiking and recovery from aliquoting of nickel in dried rainbow trout base tissue.

The flame method of atomic absorption analysis was applied, rather than the graphite furnace technique, because of the relatively high nickel content found for rainbow trout unexposed to experimentally high nickel levels in the water and/or food.

General operating conditions

Certain general operating conditions were maintained throughout the analytical procedures. These are outlined in Appendix A-2.

Glassware was washed several times with detergent soap solution, soaked overnight in a 1:1 HCl/HNO_3 solution and rinsed several times with glass-distilled water.

Preparation of aqueous-base standard solutions

Appendix A-2 outlines the preparation of the aqueous-base standard solutions. Additional working standard solutions were prepared as follows:-

Solution preparation*	Nickel (ppm)
3.00 ± 0.02 ml of 10.00 ± 0.02 ppm Ni	0.300 ± 0.003

* All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Preparation of fish tissue

The preparation of rainbow trout base tissue is described in Subsection 2.2.3 - (a).

2. Testing of Wet Pressure-Digested Rainbow Trout Base Tissue

Wet ashing (pressure/digestion) of fish tissue

The general procedure is outlined in Sub-section 2.2.3 - (c). The specific details for the determination of nickel are given below.

The sample weights were as follows:-

Sample No.	Fish weight (g)
1.	0.0000
2.	0.5171 ± 0,0002
3.	0.5741 ± 0.0002
4.	0.5730 ± 0.0002
5.	0.6214 ± 0.0002
6.	0.7573 ± 0.0002
7.	0.8416 ± 0.0002
8.	1.2448 ± 0.0002
9.	1.1713 ± 0.0002
10.	1.5795 ± 0.0002
11.	1.5608 ± 0.0002
12.	1.7320 ± 0.0002
13.	1.7409 ± 0.0002
14.	1.7500 ± 0.0002
15.	2.0742 ± 0.0002
16.	2.6518 ± 0.0002
17.	2.7465 ± 0.0002
18.	3.0311 ± 0.0002
19.	3.5000 ± 0.0002
20.	3.8530 ± 0.0002

The amount of HCl pipetted was,

0.50 ± 0.01 ml

The amount of glass-distilled water was,

14.50 ± 0.04 ml

Testing of working standards by flame atomic absorption

The set of working standard solutions prepared for this series of tests gave the following results:-

Nickel (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.100 ± 0.002	0.005 ± 0.000
0.300 ± 0.003	0.012 ± 0.000
0.500 ± 0.003	0.019 ± 0.000
1.000 ± 0.004	0.037 ± 0.000
2.00 ± 0.02	0.073 ± 0.000

* Average and standard deviation of five 3-seconds integration readings.

Linear regression analysis

0.000 - 2.00 ppm Ni

$r = 0.9998^5$

$b = 0.000^9$

$m = 0.0361^3$

Sensitivity (1% absorption) = $0.0044/0.0361^3 = 0.1^2$ ppm Ni

Reverse calculations for nickel

Actual nickel (ppm)	Calculated nickel (ppm)	Absolute devn.
0.000	-0.023	0.023
0.100 ± 0.002	0.115	0.015
0.300 ± 0.003	0.309	0.009
0.500 ± 0.003	0.502	0.002
1.000 ± 0.004	1.001	0.001
2.00 ± 0.02	1.997	0.003
	Average absolute deviation	0.008^8

Values of nickel (ppm) from the above linear equation subject to ± 0.008^8 ppm.

Results of nickel determination in wet pressure-digested fish base tissue

The results of the blank and fish base samples are given in Table D-5.

The 19 values of nickel in fish tissue resulted in an average of,

$$1.8^3 \pm 0.2^7 \text{ ppm Ni}$$

or,

$$1.8^3 \pm 0.2^7 \text{ ug Ni/g dried rainbow trout base}$$

3. Recovery of Nickel from Spiked Fish Base Samples

Wet ashing (pressure/digestion) of fish tissue

The weights of macerated and dried fish tissue weighed into 12-cm test tubes are listed in Table D-6.

These fish tissue samples were spiked with 10.00 ± 0.02 ppm Ni solution as follows:-

Series	Spike	ug Ni added
A	none	0.00
B	1.00 ± 0.02 ml of 10.00 ppm Ni	10.0 ± 0.2
C	2.50 ± 0.02 ml " " " "	25.0 ± 0.2
D	4.00 ± 0.02 ml " " " "	40.0 ± 0.3
E	5.50 ± 0.02 ml " " " "	55.0 ± 0.3
F	7.00 ± 0.02 ml " " " "	70.0 ± 0.3

Subsequent to spiking each sample was placed in the oven and heated at just under 100 °C until the liquid spike volume had dried out. The subsequent digestion procedure duplicated the details of Subsection 2.2.3 - (c). A blank with no spike was carried through the same procedure.

The amount of HCl pipetted was,

0.50 ± 0.01 ml

The amount of glass-distilled water was,

14.50 ± 0.04 ml

TABLE D-5

DETERMINATION OF NICKEL IN RAINBOW TROUT BASE TISSUE BY WET PRESSURE-DIGESTION/FLAME ATOMIC ABSORPTION TECHNIQUE

Sample No.	Average* absorbance	Corrected average absorbance	**Ni in 15 ml soln. (ppm)	Nickel ug/15 ml	Nickel in fish base (ppm)
1.	0.003 ± 0.000	0.000 ± 0.000	---	---	---
2.	0.006 ± 0.000	0.003 ± 0.000	$0.058^{1} \pm 0.008^{8}$	$0.8^{7} \pm 0.1^{3}$	$1.6^{8} \pm 0.2^{6}$
3.	0.006 ± 0.000	0.003 ± 0.000	$0.058^{1} \pm 0.008^{8}$	$0.8^{7} \pm 0.1^{3}$	$1.5^{2} \pm 0.2^{3}$
4.	0.006 ± 0.000	0.003 ± 0.000	$0.058^{1} \pm 0.008^{8}$	$0.8^{7} \pm 0.1^{3}$	$1.5^{2} \pm 0.2^{3}$
5.	0.006 ± 0.000	0.003 ± 0.000	$0.058^{1} \pm 0.008^{8}$	$0.8^{7} \pm 0.1^{3}$	$1.4^{0} \pm 0.2^{1}$
6.	0.006 ± 0.000	0.003 ± 0.000	$0.058^{1} \pm 0.008^{8}$	$0.8^{7} \pm 0.1^{3}$	$1.1^{5} \pm 0.1^{7}$
7.	0.008 ± 0.000	0.005 ± 0.000	$0.113^{5} \pm 0.008^{8}$	$1.7^{0} \pm 0.1^{3}$	$2.0^{2} \pm 0.1^{5}$
8.	0.010 ± 0.000	0.007 ± 0.000	$0.168^{8} \pm 0.008^{8}$	$2.5^{3} \pm 0.1^{3}$	$2.0^{3} \pm 0.1^{0}$
9.	0.010 ± 0.000	0.007 ± 0.000	$0.168^{8} \pm 0.008^{8}$	$2.5^{3} \pm 0.1^{3}$	$2.1^{6} \pm 0.1^{1}$
10.	0.010 ± 0.000	0.007 ± 0.000	$0.168^{8} \pm 0.008^{8}$	$2.5^{3} \pm 0.1^{3}$	1.60 ± 0.08
11.	0.012 ± 0.000	0.009 ± 0.000	$0.224^{2} \pm 0.008^{8}$	$3.3^{6} \pm 0.1^{3}$	2.15 ± 0.08
12.	0.012 ± 0.000	0.009 ± 0.000	$0.224^{2} \pm 0.008^{8}$	$3.3^{6} \pm 0.1^{3}$	1.94 ± 0.08
13.	0.012 ± 0.000	0.009 ± 0.000	$0.224^{2} \pm 0.008^{8}$	$3.3^{6} \pm 0.1^{3}$	1.93 ± 0.07
14.	0.012 ± 0.000	0.009 ± 0.000	$0.224^{2} \pm 0.008^{8}$	$3.3^{6} \pm 0.1^{3}$	1.92 ± 0.07
15.	0.014 ± 0.000	0.011 ± 0.000	$0.279^{5} \pm 0.008^{8}$	$4.1^{9} \pm 0.1^{3}$	2.02 ± 0.06
16.	0.016 ± 0.000	0.013 ± 0.000	$0.334^{9} \pm 0.008^{8}$	$5.0^{2} \pm 0.1^{3}$	1.89 ± 0.05
17.	0.018 ± 0.000	0.015 ± 0.000	$0.390^{2} \pm 0.008^{8}$	$5.8^{5} \pm 0.1^{3}$	2.13 ± 0.05
18.	0.018 ± 0.000	0.015 ± 0.000	$0.390^{2} \pm 0.008^{8}$	$5.8^{5} \pm 0.1^{3}$	1.93 ± 0.04
19.	0.020 ± 0.000	0.017 ± 0.000	$0.445^{6} \pm 0.008^{8}$	$6.6^{8} \pm 0.1^{3}$	1.91 ± 0.04
20.	0.022 ± 0.000	0.019 ± 0.000	$0.501^{0} \pm 0.008^{8}$	$7.5^{2} \pm 0.1^{3}$	1.95 ± 0.03

* Average and standard deviation of five 3-seconds integration readings.

** Using the linear equation from Page 266.

TABLE D-6

SAMPLE WEIGHTS OF BASE FISH TISSUE FOR WET PRESSURE/DIGESTION SPIKING TECHNIQUE

No.	Series A	Series B	Series C
1	1.0226 ± 0.0002	0.9998 ± 0.0002	1.1120 ± 0.0002
2	1.0141 ± 0.0002	0.9734 ± 0.0002	1.0329 ± 0.0002
3	1.1519 ± 0.0002	0.9894 ± 0.0002	0.9758 ± 0.0002
4	1.0730 ± 0.0002	1.0134 ± 0.0002	1.0195 ± 0.0002
5	0.9893 ± 0.0002	0.9900 ± 0.0002	0.9597 ± 0.0002

No.	Series D	Series E	Series F
1	1.0215 ± 0.0002	1.0410 ± 0.0002	0.9924 ± 0.0002
2	1.0199 ± 0.0002	1.2006 ± 0.0002	1.0408 ± 0.0002
3	1.0561 ± 0.0002	0.9097 ± 0.0002	0.9782 ± 0.0002
4	1.0400 ± 0.0002	0.9925 ± 0.0002	0.9729 ± 0.0002
5	1.1067 ± 0.0002	0.9883 ± 0.0002	0.9944 ± 0.0002

Testing of working standards by flame atomic absorption

The set of working standard solutions prepared for this series of tests gave the following results:-

Nickel (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.500 ± 0.003	0.016 ± 0.000
1.000 ± 0.004	0.031 ± 0.000
2.00 ± 0.02	0.061 ± 0.000
3.00 ± 0.02	0.091 ± 0.000
5.00 ± 0.02	0.151 ± 0.000

* Average and standard deviation of five 3-seconds integration readings.

Linear regression analysis

0.000 - 5.00 ppm Ni

$r = 0.999^8$
$b = 0.000^6$
$m = 0.03011$

Sensitivity (1% absorption) = 0.0044/0.03011 = 0.1^4 ppm Ni

Reverse calculations for nickel

Actual nickel (ppm)	Calculated nickel (ppm)	Absolute devn.
0.000	0.020	0.020
0.500 ± 0.003	0.511	0.011
1.000 ± 0.004	1.010	0.010
2.00 ± 0.02	2.006	0.006
3.00 ± 0.02	3.002	0.002
5.00 ± 0.02	4.995	0.005
	Average absolute deviation	0.009

Values of nickel from the above linear equation subject to ± 0.009 ppm.

Results of nickel determination of spiked wet pressure-digested fish base tissue

The absorbance results of the blank and fish base samples spiked with nickel (ug) are listed on Table D-7.

The calculated nickel recoveries for Series B to F are tabulated in Table D-8.

The values for the 15 ml each Series A samples yield the following results:-

Series and No.	*Ni in 15 ml soln. (ppm)	Ni (ug/g) in fish base
A-1	0.146 ± 0.009	$2.1^4 \pm 0.1^4$
2	0.113 ± 0.009	$1.6^8 \pm 0.1^4$
3	0.146 ± 0.009	$1.9^0 \pm 0.1^2$
4	0.113 ± 0.009	$1.5^8 \pm 0.1^3$
5	0.113 ± 0.009	$1.7^2 \pm 0.1^4$

* Using the linear equation from Page 270.

These again represent fish base values and, combined with the 19 values obtained under Appendix D-2 (2) Testing of Wet Pressure-Digested Rainbow Trout Base Tissue, provide a total of 24 values. These results yield a final value for the nickel content of dried (65 ± 5 °C) rainbow trout unexposed to experimentally high contents of nickel in the water and/or food of:-

$1.8^2 \pm 0.2^6$ ppm or $1.8^2 \pm 0.2^6$ ug Ni/g dried rainbow trout base

Using the 2s rejection criterion to examine the recovery percent data of the last column (Table D-8), it was noted that rejection of sample B-4 was required. The remaining values yield an overall recovery percent for nickel spikes on rainbow trout dried base of up to 70 ug/g dried fish base of:-

$97.^2 \pm 4.^0$ %

TABLE D-7

WET PRESSURE-DIGESTION/FLAME ATOMIC ABSORPTION TECHNIQUE

RESULTS OF NICKEL DETERMINATION
ON SPIKED FISH BASE SAMPLES

Series and No.	Average* absorbance	Corrected average absorbance
blank	0.002 ± 0.000	0.000 ± 0.000
A-1	0.007 ± 0.000	0.005 ± 0.000
2	0.006 ± 0.000	0.004 ± 0.000
3	0.007 ± 0.000	0.005 ± 0.000
4	0.006 ± 0.000	0.004 ± 0.000
5	0.006 ± 0.000	0.004 ± 0.000
B-1	0.027 ± 0.000	0.025 ± 0.000
2	0.025 ± 0.000	0.023 ± 0.000
3	0.026 ± 0.000	0.024 ± 0.000
4	0.020 ± 0.000	0.018 ± 0.000
5	0.026 ± 0.000	0.024 ± 0.000
C-1	0.054 ± 0.000	0.052 ± 0.000
2	0.056 ± 0.000	0.054 ± 0.000
3	0.055 ± 0.000	0.053 ± 0.000
4	0.050 ± 0.000	0.048 ± 0.000
5	0.055 ± 0.001	0.053 ± 0.001
D-1	0.092 ± 0.000	0.090 ± 0.000
2	0.083 ± 0.000	0.081 ± 0.000
3	0.084 ± 0.001	0.082 ± 0.001
4	0.089 ± 0.001	0.087 ± 0.001
5	0.086 ± 0.000	0.084 ± 0.000
E-1	0.115 ± 0.000	0.113 ± 0.000
2	0.112 ± 0.001	0.110 ± 0.001
3	0.115 ± 0.001	0.113 ± 0.001
4	0.115 ± 0.001	0.113 ± 0.001
5	0.118 ± 0.001	0.116 ± 0.001
F-1	0.135 ± 0.001	0.133 ± 0.001
2	0.140 ± 0.001	0.138 ± 0.001
3	0.140 ± 0.000	0.138 ± 0.000
4	0.138 ± 0.000	0.136 ± 0.000
5	0.140 ± 0.001	0.138 ± 0.001

* Average and standard deviation of five 3-seconds integration readings.

TABLE D-8

WET PRESSURE-DIGESTION/FLAME ATOMIC ABSORPTION TECHNIQUE

RECOVERY OF NICKEL IN SPIKED FISH BASE SAMPLES

Series and No.	*Ni in 15 ml soln. (ppm)	Total Ni (ug/15 ml)	**Ni (ug/15 ml) corr. fish base	Actual Ni spike (ug)	Recovery of Ni (%)
B-1	0.810 ± 0.009	12.15 ± 0.1^4	$10.3^3 \pm 0.2^6$	10.0 ± 0.2	103.3 ± 2.6
2	0.744 ± 0.009	$11.1^6 \pm 0.14$	$9.3^9 \pm 0.2^6$	10.0 ± 0.2	93.9 ± 2.6
3	0.777 ± 0.009	$11.6^6 \pm 0.14$	$9.8^6 \pm 0.2^6$	10.0 ± 0.2	98.6 ± 2.6
4	0.578 ± 0.009	$8.6^7 \pm 0.14$	$6.8^2 \pm 0.2^6$	10.0 ± 0.2	68.2 ± 2.6
5	0.777 ± 0.009	$11.6^6 \pm 0.14$	$9.8^6 \pm 0.2^6$	10.0 ± 0.2	98.6 ± 2.6
C-1	1.707 ± 0.009	$25.6^0 \pm 0.1^4$	$23.5^8 \pm 0.2^6$	25.0 ± 0.2	94.3 ± 1.0
2	1.773 ± 0.009	$26.6^0 \pm 0.14$	$24.7^2 \pm 0.2^6$	25.0 ± 0.2	98.9 ± 1.0
3	1.740 ± 0.009	$26.1^0 \pm 0.1^4$	$24.3^2 \pm 0.2^6$	25.0 ± 0.2	97.3 ± 1.0
4	1.574 ± 0.009	$23.6^1 \pm 0.14$	$21.7^5 \pm 0.2^6$	25.0 ± 0.2	87.0 ± 1.0
5	$1.74^0 \pm 0.01^8$	26.10 ± 0.2^7	$24.3^5 \pm 0.2^7$	25.0 ± 0.2	97.4 ± 1.1
D-1	2.969 ± 0.009	$44.5^4 \pm 0.14$	$42.6^8 \pm 0.2^6$	40.0 ± 0.3	106.7 ± 0.6
2	2.670 ± 0.009	$40.0^5 \pm 0.14$	$38.1^9 \pm 0.2^6$	40.0 ± 0.3	95.5 ± 0.6
3	2.703 ± 0.01^8	$40.5^4 \pm 0.2^7$	$38.6^2 \pm 0.2^7$	40.0 ± 0.3	96.6 ± 0.7
4	$2.86^9 \pm 0.01^8$	$43.0^4 \pm 0.2^7$	$41.1^5 \pm 0.2^7$	40.0 ± 0.3	102.9 ± 0.7
5	2.770 ± 0.009	$41.5^5 \pm 0.14$	$39.5^4 \pm 0.2^6$	40.0 ± 0.3	98.8 ± 0.6
E-1	3.733 ± 0.009	$56.0^0 \pm 0.14$	$54.1^0 \pm 0.2^6$	55.0 ± 0.3	98.4 ± 0.5
2	$3.63^3 \pm 0.01^8$	$54.5^0 \pm 0.2^7$	$52.3^1 \pm 0.2^7$	55.0 ± 0.3	95.1 ± 0.5
3	$3.73^3 \pm 0.01^8$	$56.0^0 \pm 0.2^7$	$54.3^4 \pm 0.2^7$	55.0 ± 0.3	98.8 ± 0.5
4	$3.73^3 \pm 0.01^8$	$56.0^0 \pm 0.2^7$	$54.1^9 \pm 0.2^7$	55.0 ± 0.3	98.5 ± 0.5
5	$3.83^3 \pm 0.01^8$	$57.5^0 \pm 0.2^7$	$55.7^0 \pm 0.2^7$	55.0 ± 0.3	101.3 ± 0.5
F-1	$4.39^7 \pm 0.01^8$	$65.9^6 \pm 0.27$	$64.1^5 \pm 0.2^7$	70.0 ± 0.3	91.6 ± 0.4
2	$4.56^3 \pm 0.01^8$	$68.4^4 \pm 0.2^7$	$66.5^4 \pm 0.2^7$	70.0 ± 0.3	95.0 ± 0.4
3	$4.56^3 \pm 0.009$	68.44 ± 0.1^4	$66.6^6 \pm 0.2^6$	70.0 ± 0.3	95.2 ± 0.4
4	4.497 ± 0.009	$67.4^6 \pm 0.14$	$65.6^9 \pm 0.2^6$	70.0 ± 0.3	93.8 ± 0.4
5	$4.56^3 \pm 0.01^8$	$68.4^4 \pm 0.2^7$	$66.6^3 \pm 0.2^7$	70.0 ± 0.3	95.2 ± 0.4

* Using the linear equation from Page 270.

** Based on the higher of the two uncertainties:- fish base nickel or total nickel in 15 ml.

If the average for each of the Series B to F is taken with respect to the "Total Ni (ug/15 ml)" column data (Table D-8) we have, omitting the B-4 value:-

Series	B	$11.6^{6} \pm 0.4^{0}$
	C	25.6 ± 1.2
	D	41.9 ± 1.8
	E	56.0 ± 1.1
	F	67.7 ± 1.1

Linear regression analysis yields:-

$r = 0.9986$
$b = 2.57$
$m = 0.9499$

Thus the zero intercept on the ordinate axis yields a value of 2.57 ug Ni/15 ml of solution or, for the sample weights approximating 1.0 g of dried fish base, 2.57 ug Ni/g dried fish base. Carrying out reverse calculations indicates an uncertainty for this value of ± 1.0 ug, so that we have, from the method of additions, a dried rainbow trout base calculated content of nickel of 2.6 ± 1.0 ug/g fish base. The relatively high uncertainty value is the result of highly variable sample weights used for the spiking determination. However, this compares, within the respective tolerance limits, with the value of $1.8^{2} \pm 0.26$ ug Ni/g dried fish base reported on the previous page.

Using the value of 2.6 ± 1.0 ug Ni/g dried fish base as the correction value to the "Total Ni (ug/15 ml)" column data yields, omitting the B-4 value, an average recovery percent for spikes up to 70 ug on 1 g of dried fish base of 94.4 ± 4.4. This also agrees well, within the respective tolerance limits, with the value of 97.2 ± 4.0 reported on the previous page.

By including the average result of Series A with the average for each of the Series B to F taken with respect to the "Total Ni (ug/15 ml)" data column (Table D-8) in a graphical determination of nickel in dried rainbow trout by the method of additions, the plot on Figure D-2 was obtained. The nickel content in dried rainbow trout base tissue can be extrapolated to the abscissa to 2.0 ± 0.2 ug Ni/g fish base. The ± 0.2 value represents the reading uncertainty on the graph. This extrapolated nickel concentration compares, within the respective tolerance limits, with the value of $1.8^{2} \pm 0.26$ ug Ni/g dried fish base reported on the previous page.

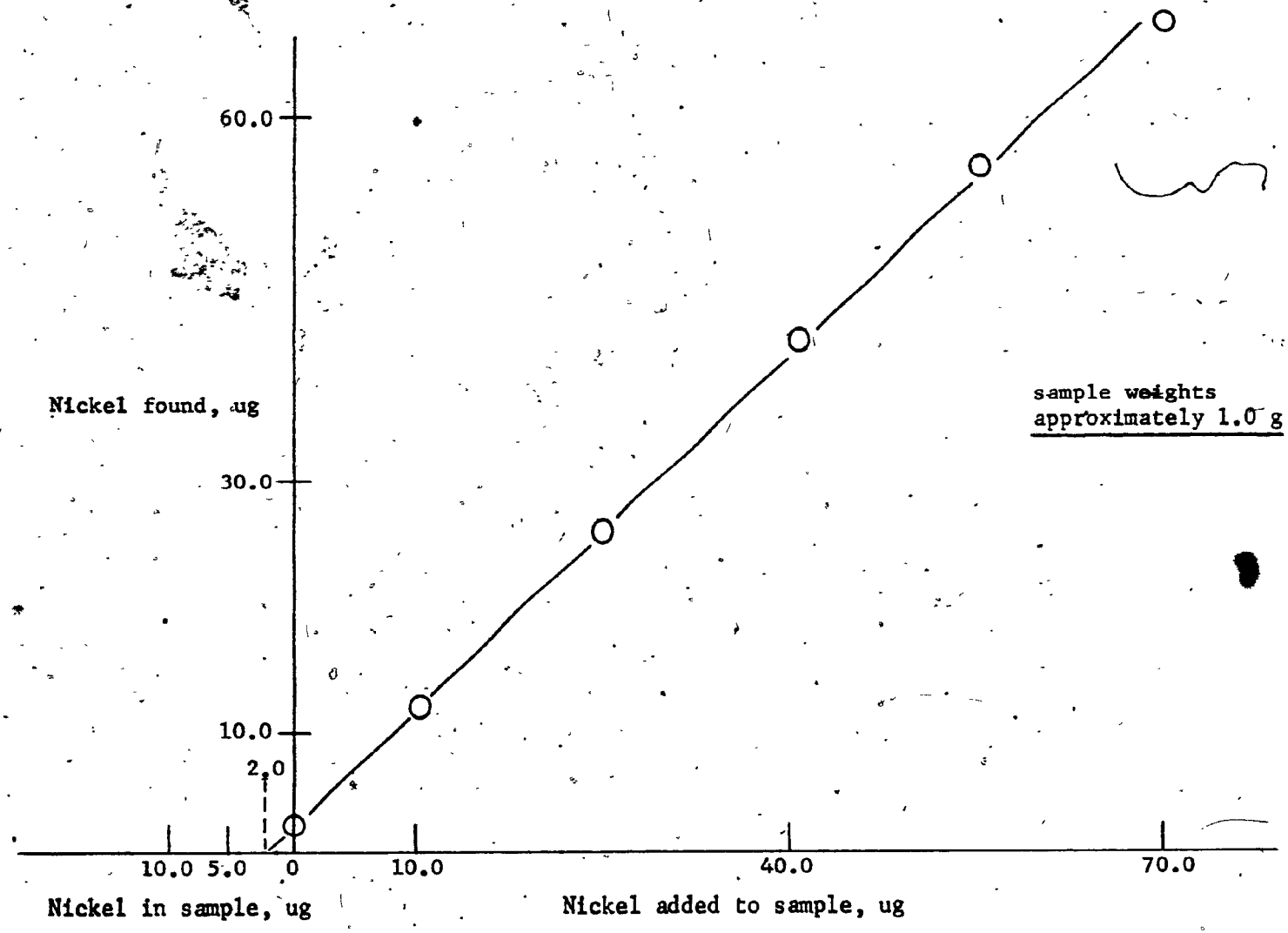


FIGURE D-2 GRAPHICAL METHOD OF ADDITIONS - NICKEL DETERMINATION IN RAINBOW TROUT - WET PRESSURE-DIGESTION

4. Recovery of Nickel from Aliquoted Spiked Fish Base Samples

Two sets of macerated and dried rainbow trout tissue were weighed out in duplicate. Each sample consisted of 1.0000 ± 0.0002 g and was placed in a Kimax screwcap 12-cm test tube.

One set (A) was spiked with 55.0 ± 0.3 ug of nickel each, while the second set (B) was spiked with 70.0 ± 0.3 ug of nickel each.

All samples were dried, digested and extracted according to the details of Sub-section 2.2.3 - (c). A blank was carried through simultaneously. The amount of HCl pipetted was 0.50 ± 0.01 ml while the amount of glass-distilled water pipetted was 14.50 ± 0.04 ml.

For each set, 5 ml of the final solution was tested as-is, and 10 ml was diluted to 20 ml with 2.5 percent HCl and then tested. The results were:-

Set	Ave. absorbance corr. for blank		Total Ni* (ug/15 ml)		Ni corr. for** base fish (ug/g)		Actual spike (ug)	Recovery (%)	
	as-is	aliq.	as-is	aliqx2	as-is	aliqx2		as-is	aliq.
A	0.110	0.058	54.5	57.2	52.7	55.4	55.0	96	101
B	0.135	0.070	67.0	69.1	65.1	67.3	70.0	93	96

* Using the linear equation from Page 270.

** Using the correction value of 1.8^2 ug Ni/g dried rainbow trout base.

The recovery percent values agree within the expected tolerance limits for nickel recovery on spiked samples, so that aliquoting of tissue sample solutions, suspected to contain nickel in ppm beyond the linear range maximum, should present no difficulty.

APPENDIX D-3

Determination of Zinc in Rainbow Trout by the Wet Pressure-Digestion/Flame Atomic Absorption Technique

1. General

The following sections outline the experimental approach applied to the testing, recovery from spiking and recovery from aliquoting on zinc in dried rainbow trout base tissue.

The flame method of atomic absorption analysis was applied, rather than the graphite furnace technique, because of the relatively high zinc content found for rainbow trout unexposed to experimentally high zinc levels in the water and/or food.

General operating conditions

Certain general operating conditions were maintained throughout the analytical procedures. These are outlined in Appendix A-3.

Glassware was washed several times with detergent soap solution, soaked overnight in a 1:1 HCl/HNO_3 solution and rinsed several times with glass-distilled water.

Preparation of aqueous-base standard solutions

Appendix A-3 outlines the preparation of aqueous-base standard solutions. Additional working standards were prepared as follows:-

Solution preparation*		Zinc (ppm)
2.00 ± 0.02 ml of	1.000 ± 0.004 ppm Zn	0.0200 ± 0.0003
6.00 ± 0.02 ml "	" "	0.0600 ± 0.0004
2.00 ± 0.02 ml "	10.00 ± 0.02 " "	0.200 ± 0.002
4.00 ± 0.02 ml "	" " "	0.400 ± 0.003
5.00 ± 0.02 ml "	" " "	0.500 ± 0.003
6.00 ± 0.02 ml "	" " "	0.600 ± 0.003

* All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Preparation of fish tissue

The preparation of rainbow trout base tissue is described in Sub-section 2.2.3 - (a).

2. Testing of Wet Pressure-Digested Rainbow Trout Base Tissue

Wet ashing (pressure/digestion) of fish tissue

The general procedure is outlined in Sub-section 2.2.3 - (c). The specific details for the determination of zinc are given below.

The sample weights were as follows:-

Sample No.	Fish weight (g)
1.	0.0000
2.	0.0514 ± 0.0002
3.	0.1004 ± 0.0002
4.	0.1258 ± 0.0002
5.	0.1240 ± 0.0002
6.	0.1613 ± 0.0002
7.	0.1655 ± 0.0002
8.	0.1790 ± 0.0002
9.	0.1800 ± 0.0002
10.	0.1847 ± 0.0002
11.	0.2064 ± 0.0002
12.	0.2364 ± 0.0002
13.	0.2736 ± 0.0002
14.	0.3709 ± 0.0002
15.	0.3815 ± 0.0002
16.	0.3892 ± 0.0002
17.	0.4952 ± 0.0002
18.	0.5036 ± 0.0002
19.	0.5200 ± 0.0002

The amount of HCl pipetted was,

$$2.00 \pm 0.02 \text{ ml}$$

Each sample was made up to 100.00 ± 0.04 ml in a volumetric flask.

Testing of working standards by flame atomic absorption

The set of working standard solutions prepared for this series of tests gave the following results:-

Zinc (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.0200 ± 0.0003	0.007 ± 0.000
0.0600 ± 0.0004	0.019 ± 0.000
0.100 ± 0.002	0.029 ± 0.000
0.200 ± 0.002	0.059 ± 0.000
0.400 ± 0.003	0.109 ± 0.000
0.600 ± 0.003	0.165 ± 0.001

* Average and standard deviation of five 3-seconds integration readings.

Linear regression analysis

0.000 - 0.600 ppm Zn

$r = 0.9991^3$
$b = 0.001^8$
$m = 0.2716^7$

Sensitivity (1% absorption) = $0.0044/0.2716^7 = 0.01^6$ ppm Zn

Reverse calculations for zinc

Actual zinc (ppm)	Calculated zinc (ppm)	Absolute devn.
0.000	-0.0066	0.0066
0.0200 ± 0.0003	0.0191	0.0009
0.0600 ± 0.0004	0.0633	0.0033
0.100 ± 0.002	0.1001	0.0001
0.200 ± 0.002	0.2105	0.0105
0.400 ± 0.003	0.394	0.006
0.600 ± 0.003	0.601	0.001
	Average absolute deviation	0.004

Values of zinc (ppm) from the above linear equation subject to ± 0.004 ppm.

Results of zinc determination in wet pressure-digested fish base tissue

The results of the blank and fish base samples are given in Table D-9.

The 2s criterion rejects the 83.6 value of sample 19. The remaining 17 values recorded in the last column yield an average of,

$$114.^{9} \pm 9.^{6} \text{ ppm Zn}$$

or,

$$114.^{9} \pm 9.^{6} \text{ ug Zn/g dried rainbow trout base}$$

3. Recovery of Zinc from Spiked Fish Base Samples

Wet ashing (pressure/digestion) of fish tissue

The weights of macerated and dried fish tissue weighed into 12-cm test tubes are listed in Table D-10.

These fish tissue samples were spiked with 10.00 ± 0.02 ppm Zinc solution as follows:-

Series	Spike	ug Zn added
A		0.0
B	1.00 ± 0.02 ml of 10.00 ppm Zn	10.0 ± 0.2
C	2.00 ± 0.02 ml " " " "	20.0 ± 0.2
D	3.00 ± 0.02 ml " " " "	30.0 ± 0.3
E	4.00 ± 0.02 ml " " " "	40.0 ± 0.3
F	5.00 ± 0.02 ml " " " "	50.0 ± 0.3

Subsequent to spiking each sample was placed in the oven and heated at just under 100 °C until the liquid spike volume had dried out. The subsequent ashing procedure duplicated the details of Subsection 2.2.3 - (c). A blank with no spike was carried through the same procedure.

The amount of HCl pipetted was,

$$2.00 \pm 0.02 \text{ ml}$$

The samples were made up to 100.00 ± 0.04 ml in a volumetric flask.

TABLE D-9

DETERMINATION OF ZINC IN RAINBOW TROUT BASE TISSUE BY WET PRESSURE-DIGESTION/FLAME ATOMIC ABSORPTION TECHNIQUE

Sample No.	Average* absorbance	Corrected average absorbance	**Zn in 100 ml soln. (ppm)	Zinc ug/100 ml	Zinc in fish base (ppm)
1.	0.004 ± 0.000	0.000 ± 0.000	---	---	---
2.	0.024 ± 0.000	0.020 ± 0.000	0.067 ± 0.004	6.7 ± 0.4	130.4 ± 7.7
3.	0.034 ± 0.000	0.030 ± 0.000	0.104 ± 0.004	10.4 ± 0.4	103.6 ± 3.9
4.	0.046 ± 0.000	0.042 ± 0.000	0.148 ± 0.004	14.8 ± 0.4	117.6 ± 3.1
5.	0.044 ± 0.000	0.040 ± 0.000	0.141 ± 0.004	14.1 ± 0.4	113.7 ± 3.2
6.	0.052 ± 0.000	0.048 ± 0.000	0.170 ± 0.004	17.0 ± 0.4	105.4 ± 2.4
7.	0.053 ± 0.000	0.049 ± 0.000	0.174 ± 0.004	17.4 ± 0.4	105.1 ± 2.4
8.	0.063 ± 0.000	0.059 ± 0.000	0.211 ± 0.004	21.1 ± 0.4	117.9 ± 2.2
9.	0.068 ± 0.000	0.064 ± 0.000	0.229 ± 0.004	22.9 ± 0.4	127.2 ± 2.2
10.	0.062 ± 0.000	0.058 ± 0.000	0.207 ± 0.004	20.7 ± 0.4	112.1 ± 2.1
11.	0.077 ± 0.000	0.073 ± 0.000	0.262 ± 0.004	26.2 ± 0.4	126.9 ± 1.9
12.	0.080 ± 0.000	0.076 ± 0.000	0.273 ± 0.004	27.3 ± 0.4	115.5 ± 1.7
13.	0.097 ± 0.000	0.093 ± 0.000	0.336 ± 0.004	33.6 ± 0.4	122.8 ± 1.4
14.	0.135 ± 0.000	0.131 ± 0.000	0.476 ± 0.004	47.6 ± 0.4	128.3 ± 1.1
15.	0.118 ± 0.000	0.114 ± 0.000	0.376 ± 0.004	37.6 ± 0.4	108.2 ± 1.0
16.	0.116 ± 0.000	0.112 ± 0.000	0.406 ± 0.004	40.6 ± 0.4	104.3 ± 1.0
17.	0.159 ± 0.000	0.155 ± 0.000	0.564 ± 0.004	56.4 ± 0.4	113.9 ± 0.8
18.	0.144 ± 0.000	0.140 ± 0.000	0.509 ± 0.004	50.9 ± 0.4	101.1 ± 0.8
19.	0.124 ± 0.000	0.120 ± 0.000	0.435 ± 0.004	43.5 ± 0.4	83.6 ± 0.7

* Average and standard deviation of five 3-seconds integration readings.

** Using the linear equation from Page 280.

TABLE D-10.

SAMPLE WEIGHTS OF BASE FISH TISSUE FOR WET PRESSURE/DIGESTION SPIKING TECHNIQUE

No.	Series A	Series B	Series C
1	0.1999 ± 0.0002	0.2013 ± 0.0002	0.1989 ± 0.0002
2	0.2000 ± 0.0002	0.2039 ± 0.0002	0.2000 ± 0.0002
3	0.2000 ± 0.0002	0.2004 ± 0.0002	0.1992 ± 0.0002
4	0.2027 ± 0.0002	0.1990 ± 0.0002	0.2021 ± 0.0002
5	0.2019 ± 0.0002	0.2000 ± 0.0002	0.2021 ± 0.0002

No.	Series D	Series E	Series F
1	0.1994 ± 0.0002	0.2008 ± 0.0002	0.2141 ± 0.0002
2	0.2000 ± 0.0002	0.2018 ± 0.0002	0.2005 ± 0.0002
3	0.2037 ± 0.0002	0.2063 ± 0.0002	0.2009 ± 0.0002
4	0.2038 ± 0.0002	0.2000 ± 0.0002	0.1992 ± 0.0002
5	0.2023 ± 0.0002	0.1990 ± 0.0002	0.2037 ± 0.0002

Testing of working standards by flame atomic absorption

The set of working standard solutions prepared for this series of tests gave the following results:-

Zinc (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.100 ± 0.002	0.027 ± 0.000
0.200 ± 0.002	0.053 ± 0.000
0.500 ± 0.003	0.135 ± 0.000
0.600 ± 0.003	0.163 ± 0.000
0.800 ± 0.004	0.215 ± 0.002

* Average and standard deviation of five 3-seconds integration readings.

Linear regression analysis

0.000 - 0.800 ppm Zn

$r = 0.9999^6$
$b = 0.000$
$m = 0.2699^3$

Sensitivity (1% absorption) = $0.0044/0.2699^3 = 0.01^6$ ppm Zn

Reverse calculations for zinc

Actual zinc (ppm)	Calculated zinc (ppm)	Absolute devn.
0.000	0.000	0.000
0.100 ± 0.002	0.100	0.000
0.200 ± 0.002	0.196^3	0.003^7
0.500 ± 0.003	0.500^1	0.000^1
0.600 ± 0.003	0.603^8	0.003^8
0.800 ± 0.004	0.796^5	0.003^5
	Average absolute deviation	0.002

Values of zinc (ppm) from the above linear equation subject to ± 0.002 ppm.

Result of zinc determination of spiked wet pressure-digested fish base tissue

The absorbance results of the blank and fish base samples spiked with zinc (ug) are listed on Table D-11.

The calculated zinc recoveries for Series B to F are tabulated on Table D-12.

The values for the 100 ml each Series A samples yield the following results:-

Series and No.	*Zn in 100 ml soln. (ppm)	Zn (ug/g) in fish base
A-1	0.259 ± 0.002	$129.^{5} \pm 1.^{0}$
2	0.226 ± 0.002	$113.^{0} \pm 1.^{0}$
3	0.219 ± 0.002	$109.^{5} \pm 1.^{0}$
4	0.237 ± 0.002	$116.^{9} \pm 1.^{0}$
5	0.222 ± 0.002	$109.^{9} \pm 1.^{0}$

* Using the linear equation from Page 284.

These again represent fish base values and, combined with the 17 values obtained under Appendix D-3 (2) Testing of Wet Pressure-Digested Rainbow Trout Base Tissue, provide a total of 22 values. These results yield a final value for the zinc content of dried (65 ± 5 °C) rainbow trout unexposed to experimentally high contents of zinc in the water and/or food of:-

$115.^{1} \pm 9.^{1}$ ppm or $115.^{1} \pm 9.^{1}$ ug Zn/g dried rainbow trout base

An overall recovery percent was obtained for zinc spikes on rainbow trout dried base of up to 50 ug/0.2 g of tissue of:-

$89.^{0} \pm 6.^{4}$ %

TABLE D-11

WET PRESSURE-DIGESTION/FLAME ATOMIC ABSORPTION TECHNIQUE

RESULTS OF ZINC DETERMINATION
ON SPIKED FISH BASE SAMPLES

Series and No.	Average* absorbance	Corrected average absorbance
blank	0.006 ± 0.000	0.000 ± 0.000
A-1	0.076 ± 0.000	0.070 ± 0.000
2	0.067 ± 0.000	0.061 ± 0.000
3	0.065 ± 0.000	0.059 ± 0.000
4	0.070 ± 0.000	0.064 ± 0.000
5	0.066 ± 0.000	0.060 ± 0.000
B-1	0.090 ± 0.000	0.084 ± 0.000
2	0.093 ± 0.000	0.087 ± 0.000
3	0.093 ± 0.000	0.087 ± 0.000
4	lost	
5	0.095 ± 0.000	0.089 ± 0.000
C-1	0.112 ± 0.000	0.106 ± 0.000
2	0.114 ± 0.000	0.108 ± 0.000
3	lost	
4	0.119 ± 0.000	0.113 ± 0.000
5	0.125 ± 0.001	0.119 ± 0.001
D-1	0.138 ± 0.000	0.130 ± 0.000
2	0.132 ± 0.000	0.126 ± 0.000
3	0.139 ± 0.000	0.133 ± 0.000
4	0.142 ± 0.001	0.136 ± 0.001
5	0.141 ± 0.001	0.135 ± 0.001
E-1	0.172 ± 0.001	0.166 ± 0.001
2	0.159 ± 0.002	0.153 ± 0.002
3	0.164 ± 0.001	0.158 ± 0.001
4	0.162 ± 0.001	0.156 ± 0.001
5	0.162 ± 0.000	0.156 ± 0.000
F-1	0.191 ± 0.000	0.185 ± 0.000
2	0.178 ± 0.001	0.172 ± 0.001
3	0.195 ± 0.000	0.189 ± 0.000
4	0.193 ± 0.002	0.187 ± 0.002
5	0.201 ± 0.001	0.195 ± 0.001

* Average and standard deviation of five 3-seconds integration readings.

TABLE D-12

WET PRESSURE-DIGESTION/FLAME ATOMIC ABSORPTION TECHNIQUE

RECOVERY OF ZINC IN SPIKED FISH BASE SAMPLES

Series and No.	*Zn in 100 ml soln. (ppm)	Total Zn (ug/100 ml)	**Zn (ug/100 ml) corr. fish base	Actual Zn spike (ug)	Recovery of Zn (%)
B-1	0.311 ± 0.002	31.1 ± 0.2	8.0 ± 1.8	10.0 ± 0.2	80 ± 18
2	0.322 ± 0.002	32.2 ± 0.2	8.8 ± 1.8	10.0 ± 0.2	88 ± 18
3	0.322 ± 0.002	32.2 ± 0.2	9.2 ± 1.8	10.0 ± 0.2	92 ± 18
4	lost				
5	0.330 ± 0.002	33.0 ± 0.2	10.0 ± 1.8	10.0 ± 0.2	100 ± 18
C-1	0.393 ± 0.002	39.3 ± 0.2	16.4 ± 1.8	20.0 ± 0.2	82.0 ± 9.0
2	0.400 ± 0.002	40.0 ± 0.2	17.0 ± 1.8	20.0 ± 0.2	85.0 ± 9.0
3	lost				
4	0.419 ± 0.002	41.9 ± 0.2	18.7 ± 1.8	20.0 ± 0.2	93.5 ± 9.0
5	0.441 ± 0.002	44.1 ± 0.2	20.8 ± 1.8	20.0 ± 0.2	104.0 ± 9.0
D-1	0.482 ± 0.002	48.2 ± 0.2	25.2 ± 1.8	30.0 ± 0.3	84.0 ± 6.0
2	0.467 ± 0.002	46.7 ± 0.2	23.7 ± 1.8	30.0 ± 0.3	79.0 ± 6.0
3	0.493 ± 0.002	49.3 ± 0.2	25.8 ± 1.8	30.0 ± 0.3	86.0 ± 6.0
4	0.504 ± 0.004	50.4 ± 0.4	27.0 ± 1.8	30.0 ± 0.3	90.0 ± 6.0
5	0.500 ± 0.004	50.0 ± 0.4	26.7 ± 1.8	30.0 ± 0.3	89.0 ± 6.0
E-1	0.615 ± 0.004	61.5 ± 0.4	38.4 ± 1.8	40.0 ± 0.3	96.0 ± 4.5
2	0.567 ± 0.006	56.7 ± 0.6	33.5 ± 1.8	40.0 ± 0.3	83.8 ± 4.5
3	0.585 ± 0.004	58.5 ± 0.4	34.8 ± 1.8	40.0 ± 0.3	87.0 ± 4.5
4	0.578 ± 0.004	57.8 ± 0.4	34.8 ± 1.8	40.0 ± 0.3	87.0 ± 4.5
5	0.578 ± 0.002	57.8 ± 0.2	34.9 ± 1.8	40.0 ± 0.3	87.2 ± 4.6
F-1	0.685 ± 0.002	68.5 ± 0.2	43.9 ± 1.8	50.0 ± 0.3	87.8 ± 3.6
2	0.637 ± 0.004	63.7 ± 0.4	40.6 ± 1.8	50.0 ± 0.3	81.2 ± 3.6
3	0.700 ± 0.002	70.0 ± 0.2	46.9 ± 1.8	50.0 ± 0.3	93.8 ± 3.6
4	0.693 ± 0.006	69.3 ± 0.6	46.4 ± 1.8	50.0 ± 0.3	92.8 ± 3.6
5	0.722 ± 0.004	72.2 ± 0.4	48.8 ± 1.8	50.0 ± 0.3	97.6 ± 3.6

* Using the linear equation from Page 284.

** Based on the higher of the two uncertainties:- fish base zinc or total zinc in 100 ml

If the average for each of the Series B to F is taken with respect to the "Total Zn (ug/100 ml)" column data (Table D-12) we have:-

Series	B	$32.1^{2} \pm 0.7^{8}$
	C	$41.^{3} \pm 2.^{1}$
	D	$48.^{9} \pm 1.^{5}$
	E	$58.^{4} \pm 1.^{8}$
	F	$68.^{7} \pm 3.^{1}$

Linear regression analysis yields:- $r = 0.9987$
$b = 22.8$
$m = 0.9026$

Thus the zero intercept on the ordinate axis yields a value of 22.8 ug Zn/100 ml of solution or, for the sample weights approximating 0.20 g of dried fish base, 114.0 ug Zn/g dried fish base. Carrying out reverse calculations indicates an uncertainty for this value of $\pm 3.^{0}$ ug, so that we have, from the method of additions, a calculated value of dried rainbow trout base content of zinc of $114.^{0} \pm 3.^{0}$ ug/g fish base. This compares quite well, within the respective tolerance limits, with the value of $115.^{1} \pm 9.^{1}$ ug Zn/g dried fish base reported on the previous page.

Using the value of $114.^{0} \pm 3.^{0}$ ug Zn/g dried fish base as the correction value to the "Total Zn (ug/100 ml)" data column (Table D-12) yields an average recovery percent for spikes up to 50 ug on 0.2 g of dried fish base of $89.^{9} \pm 6.^{5}$. This also agrees well, within the respective tolerance limits, with the value of $89.^{0} \pm 6.^{4}$ percent reported on the previous page.

By including the average result of Series A with the average for each of the Series B to F taken with respect to the "Total Zn (ug/100 ml)" data column (Table D-12) in a graphical determination of zinc in dried rainbow trout by the method of additions, the plot on Figure D-3 was obtained. The zinc content in dried rainbow trout base tissue can be extrapolated to the abscissa to 23.8 ± 0.2 ug Zn/0.2 g fish base which represents 119 ± 1 ug Zn/g fish base. Both ± 0.2 and ± 1 represent the reading uncertainty on the graph. This extrapolated zinc concentration compares, within the respective tolerance limits, with the value of $115.^{1} \pm 9.^{1}$ ug Zn/g dried fish base reported on the previous page.

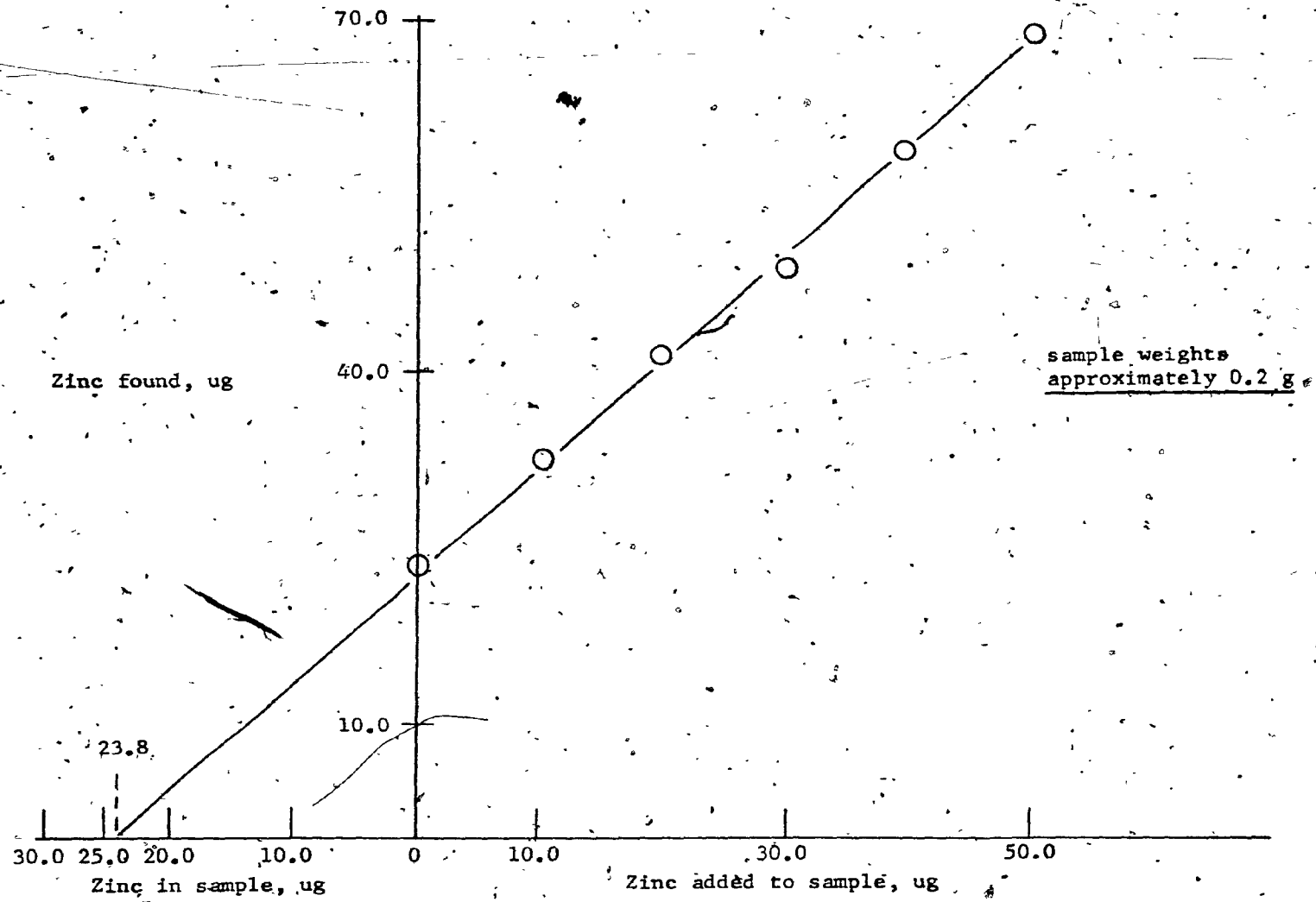


FIGURE D-3 GRAPHICAL METHOD OF ADDITIONS - ZINC DETERMINATION IN RAINBOW TROUT - WET PRESSURE-DIGESTION

4. Recovery of Zinc from Aliquoted Spiked Fish Base Samples

Two sets of macerated and dried rainbow trout tissue were weighed out in duplicate. Each sample consisted of 0.2000 ± 0.0002 g and was placed in a Kimax screwcap 12-cm test tube.

One set (A) was spiked with 50.0 ± 0.4 ug of zinc each, while the second set (B) was spiked with 75.0 ± 0.4 ug of zinc each.

All samples were dried, digested and extracted according to the details of Sub-section 2.2.3 - (c). A blank was carried through simultaneously. The amount of HCl pipetted was 2.00 ± 0.02 ml and each sample was made up to 100.00 ± 0.04 ml in a volumetric flask.

For each set, 10 ml of the final solution was tested as-is, and 10 ml was diluted to 50 ml with 2.0 percent HCl and then tested. The following results were obtained:-

Set.	Ave. absorbance corr. for blank		Total Zn* (ug/100 ml)		Zn corr. for** base fish (ug/g)		Actual spike (ug)	Recovery (%)	
	as-is	aliq.	as-is	aliqx5	as-is	aliqx5		as-is	aliq.
A	0.190	0.035	70.4	64.8	47.4	41.8	50.0	95	84
B	0.256	0.050	94.8	92.6	71.8	69.6	75.0	96	93

* Using the linear equation from Page 284.

** Using the correction value of 115.1 ug Zn/g dried rainbow trout base.

The recovery percent values agree within the expected tolerance limits for zinc recovery on spiked samples, so that aliquoting of tissue sample solutions, suspected to contain zinc in ppm beyond the linear range maximum, should present no difficulty.

APPENDIX D-4

Determination of Lead in Rainbow Trout Tissue by the Wet Pressure-Digestion/Graphite Furnace Atomic Absorption Technique

1. General

The following sections outline the experimental approach applied to the testing, recovery from spiking and recovery from aliquoting on lead in dried rainbow trout base tissue.

The graphite furnace method was applied because of the relatively low lead content found for rainbow trout unexposed to experimentally high lead levels in the water and/or food.

General operating conditions

Certain general conditions were maintained throughout the analytical procedures. These are outlined in Appendix B-3.

Preparation of aqueous-base standard solutions

Appendix B-3 outlines the preparation of the aqueous-base standard solutions. Additional working standard solutions were prepared as follows:-

Solution preparation*	Lead (ppm)
7.50 ± 0.02 ml of 0.100 ± 0.001 ppm Pb	0.0075 ± 0.0001

*All dilutions were made to 100.00 ± 0.04 ml in a volumetric flask.

Preparation of fish tissue

The preparation of rainbow trout base tissue is described in Sub-section 2.2.3 - (a).

2. Testing of Wet Pressure-Digested Rainbow Trout Base Tissue

Wet ashing (pressure/digestion) of fish tissue

The general procedure is outlined in Sub-section 2.2.3 - (c). The specific details for the determination of lead are given below:-

The sample weights were as follows:-

Sample No.	Fish weight (g)
1.	0.0000
2.	0.1211 ± 0.0002
3.	0.1615 ± 0.0002
4.	0.1795 ± 0.0002
5.	0.2259 ± 0.0002
6.	0.2557 ± 0.0002
7.	0.3062 ± 0.0002
8.	0.3506 ± 0.0002
9.	0.4621 ± 0.0002
10.	0.4807 ± 0.0002
11.	0.5687 ± 0.0002
12.	0.7058 ± 0.0002
13.	0.7374 ± 0.0002
14.	0.8843 ± 0.0002
15.	0.9462 ± 0.0002

The amount of HCl pipetted was,

1.00 ± 0.01 ml

The amount of glass-distilled water pipetted was,

24.00 ± 0.06 ml

Testing of working standards by graphite furnace atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Lead (ppm)	Average absorbance*	Corr. ave. abs.
0.0000	0.004 ± 0.000	0.000 ± 0.000
0.0010 ± 0.0001	0.011 ± 0.000	0.007 ± 0.000
0.0050 ± 0.0001	0.043 ± 0.001	0.039 ± 0.001
0.0100 ± 0.0001	0.078 ± 0.002	0.074 ± 0.002
0.0150 ± 0.0004	0.106 ± 0.002	0.102 ± 0.002
0.0200 ± 0.0004	0.148 ± 0.004	0.144 ± 0.004
0.0300 ± 0.0005	0.224 ± 0.004	0.220 ± 0.004

* Average and standard deviation of three peak integration reading.

Linear regression analysis

0.000 - 0.0300 ppm Pb

r = 0.9992
b = -0.0001
m = 7.2454

Sensitivity (1% absorption) = 0.0044/7.2454 = 0.0006 ppm Pb

Reverse calculations for lead

Actual lead (ppm)	Calculated lead (ppm)	Absolute devn.
0.0000	0.0000	0.0000
0.0010 ± 0.0001	0.0010	0.0000
0.0050 ± 0.0001	0.0054	0.0004
0.0100 ± 0.0001	0.0102	0.0002
0.0150 ± 0.0004	0.0141	0.0009
0.0200 ± 0.0004	0.0199	0.0001
0.0300 ± 0.0005	0.0304	0.0004
	Average absolute deviation	0.0003

Values of lead (ppm) from the above linear equation subject to ± 0.0003 ppm

Results of lead determination in wet pressure-digested fish base tissue

The results of the blank and fish base samples are tabulated in Table D-13.

The 14 values recorded in the last column yield an average of,

$$0.7^7 \pm 0.1^0 \text{ ppm Pb}$$

or,

$$0.7^7 \pm 0.1^0 \text{ ug Pb/g dried rainbow trout base}$$

3. Recovery of Lead from Spiked Fish Base Samples

Wet ashing (pressure-digestion) of fish tissue

The weights of macerated and dried fish tissue were weighed into 12-cm test tubes. These are listed in Table D-14.

These fish tissue samples were spiked with 1.000 ± 0.004 ppm lead solution as follows:-

Series	Spike	ug lead added
A	none	0.00
B	0.50 ± 0.02 ml of 1.000 ppm Pb	0.50 ± 0.02
C	1.00 ± 0.02 ml " " " "	1.00 ± 0.02
D	1.50 ± 0.02 ml " " " "	1.50 ± 0.03
E	2.00 ± 0.02 ml " " " "	2.00 ± 0.03
F	2.50 ± 0.02 ml " " " "	2.50 ± 0.04

Subsequent to spiking each sample was placed in the oven and heated at just under 100 °C until the liquid spike volume had dried out. The subsequent digestion procedure duplicated the details of Subsection 2.2.3 - (c). A blank with no spike was carried through the same procedure.

The amount of HCl pipetted was,

$$4.00 \pm 0.02 \text{ ml}$$

Each sample was made up to 100.00 ± 0.04 ml in a volumetric flask.

TABLE D-13

DETERMINATION OF LEAD IN RAINBOW TROUT BASE TISSUE BY WET PRESSURE-DIGESTION/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

Sample No.	Average* absorbance	Corrected average absorbance	**Pb in 25 ml soln. (ppm)	Lead ug/25 ml	Pb in fish base (ppm)
1.	0.035 ± 0.000	0.000 ± 0.000	---	---	---
2.	0.065 ± 0.000	0.030 ± 0.000	0.0042 ± 0.0003	$0.105^{0} \pm 0.007^{5}$	0.87 ± 0.06
3.	0.074 ± 0.000	0.039 ± 0.000	0.0054 ± 0.0003	$0.135^{0} \pm 0.007^{5}$	0.84 ± 0.05
4.	0.085 ± 0.000	0.050 ± 0.000	0.0069 ± 0.0003	$0.172^{5} \pm 0.007^{5}$	0.96 ± 0.04
5.	0.099 ± 0.001	0.064 ± 0.001	0.0088 ± 0.0006	$0.22^{0} \pm 0.01^{5}$	0.97 ± 0.07
6.	0.090 ± 0.001	0.055 ± 0.001	0.0076 ± 0.0006	$0.19^{0} \pm 0.01^{5}$	0.74 ± 0.06
7.	0.098 ± 0.000	0.063 ± 0.000	0.0087 ± 0.0003	$0.217^{5} \pm 0.007^{5}$	0.71 ± 0.02
8.	0.110 ± 0.001	0.075 ± 0.001	0.0104 ± 0.0006	$0.26^{0} \pm 0.01^{5}$	0.74 ± 0.04
9.	0.131 ± 0.000	0.096 ± 0.000	0.0133 ± 0.0003	$0.332^{5} \pm 0.007^{5}$	0.72 ± 0.02
10.	0.146 ± 0.002	0.111 ± 0.002	0.0153 ± 0.0009	$0.38^{2} \pm 0.02^{2}$	0.79 ± 0.04
11.	0.155 ± 0.000	0.120 ± 0.000	0.0166 ± 0.0003	$0.415^{0} \pm 0.007^{5}$	0.73 ± 0.01
12.	0.175 ± 0.002	0.140 ± 0.002	0.0193 ± 0.0009	$0.48^{2} \pm 0.02^{2}$	0.68 ± 0.03
13.	0.181 ± 0.003	0.146 ± 0.003	0.0202 ± 0.001^{2}	$0.50^{5} \pm 0.03^{0}$	0.68 ± 0.04
14.	0.208 ± 0.002	0.173 ± 0.002	0.0239 ± 0.0009	$0.59^{8} \pm 0.02^{2}$	0.68 ± 0.02
15.	0.226 ± 0.003	0.191 ± 0.003	0.0264 ± 0.001^{2}	0.66 ± 0.03	0.70 ± 0.03

* Average and standard deviation of five peak integration readings

** Using the linear equation from Page 294.

TABLE D-14

SAMPLE WEIGHTS OF BASE FISH TISSUE FOR WET PRESSURE-DIGESTION SPIKING TECHNIQUE

No.	Series A	Series B	Series C
1	1.0000 ± 0.0002	1.0010 ± 0.0002	1.0000 ± 0.0002
2	0.9998 ± 0.0002	0.9989 ± 0.0002	1.0001 ± 0.0002
3	1.0020 ± 0.0002	1.0000 ± 0.0002	0.9995 ± 0.0002
4	1.0002 ± 0.0002	1.0040 ± 0.0002	1.0000 ± 0.0002
5	1.0000 ± 0.0002	0.9998 ± 0.0002	1.0017 ± 0.0002

No.	Series D	Series E	Series F
1	0.9981 ± 0.0002	0.9975 ± 0.0002	1.0000 ± 0.0002
2	1.0002 ± 0.0002	1.0011 ± 0.0002	1.0006 ± 0.0002
3	1.0000 ± 0.0002	1.0020 ± 0.0002	1.0010 ± 0.0002
4	0.9990 ± 0.0002	0.9999 ± 0.0002	1.0000 ± 0.0002
5	1.0010 ± 0.0002	1.0000 ± 0.0002	1.0000 ± 0.0002

Testing of working standards by graphite furnace atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Lead (ppm)	Average absorbance*	Corr. ave. abs.
0.0000	0.005 ± 0.000	0.000 ± 0.000
0.0050 ± 0.0001	0.043 ± 0.001	0.038 ± 0.001
0.0075 ± 0.0001	0.066 ± 0.001	0.061 ± 0.001
0.0100 ± 0.0001	0.090 ± 0.003	0.085 ± 0.003
0.0150 ± 0.0004	0.127 ± 0.004	0.122 ± 0.004
0.0200 ± 0.0004	0.167 ± 0.002	0.162 ± 0.002
0.0300 ± 0.0005	0.238 ± 0.002	0.233 ± 0.002

* Average and standard deviation of five peak integration readings.

Linear regression analysis

0.0000 - 0.0300 ppm Pb

r = 0.9991
b = 0.002
m = 7.8286

Sensitivity (1% absorption) = 0.0044/7.8286 = 0.0006 ppm Pb

Reverse calculations for lead

Actual lead (ppm)	Calculated lead (ppm)	Absolute devn.
0.0000	-0.0003	0.0003
0.0050 ± 0.0001	0.0046	0.0004
0.0075 ± 0.0001	0.0075	0.0000
0.0100 ± 0.0001	0.0106	0.0006
0.0150 ± 0.0004	0.0153	0.0003
0.0200 ± 0.0004	0.0204	0.0004
0.0300 ± 0.0005	0.0295	0.0005
	Average absolute deviation	0.0003^5

Values of lead (ppm) from the above equation subject to ± 0.0003^5 ppm.

Results of lead determination of spiked wet pressure-digested fish base tissue

The absorbance results of the blank and fish base samples spiked with lead (ug) are tabulated in Table D-15.

The calculated lead recoveries for Series B to F are tabulated in Table D-16.

The value for the 100 ml each Series A samples yield the following results:-

Series and No.	*Pb in 100 ml soln. (ppm)	Pb (ug/g) in fish base
A-1	0.0078 ± 0.0007	0.78 ± 0.07
2	$0.008^{7} \pm 0.001^{0}$	$0.8^{7} \pm 0.1^{0}$
3	0.0075 ± 0.0007	0.75 ± 0.07
4	0.0088 ± 0.0007	0.88 ± 0.07
5	$0.008^{4} \pm 0.001^{0}$	$0.8^{4} \pm 0.1^{0}$

* Using the linear equation from Page 298.

These again represent fish base values and, combined with the 14 values obtained under Appendix D-4 (2) Testing of Wet Pressure-Digested Rainbow Trout Base Tissue, provide a total of 19 values. These results yield a final value for the lead content of dried rainbow trout unexposed to experimentally high contents of lead in the water and/or food of:-

$0.78^{6} \pm 0.09^{2}$ ppm or $0.78^{6} \pm 0.09^{2}$ ug Pb/g dried rainbow trout

An overall recovery percent was obtained for lead spikes on rainbow trout dried base of up to 2.5 ug/g of tissue of:-

$93.^{2} \pm 5.^{6}$ %

TABLE D-15

WET PRESSURE-DIGESTION/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

RESULTS OF LEAD DETERMINATION
ON SPIKED FISH BASE SAMPLES

Series and No.	Average* absorbance	Corrected average absorbance
blank	0.015 ± 0.000	0.000 ± 0.000
A-1	0.078 ± 0.001	0.063 ± 0.001
2	0.085 ± 0.002	0.070 ± 0.002
3	0.076 ± 0.001	0.061 ± 0.001
4	0.086 ± 0.001	0.071 ± 0.001
5	0.083 ± 0.002	0.068 ± 0.002
B-1	0.117 ± 0.002	0.102 ± 0.002
2	0.120 ± 0.001	0.105 ± 0.001
3	0.118 ± 0.001	0.103 ± 0.001
4	0.115 ± 0.002	0.100 ± 0.002
5	0.118 ± 0.002	0.103 ± 0.002
C-1	0.148 ± 0.000	0.133 ± 0.000
2	0.145 ± 0.001	0.130 ± 0.001
3	0.142 ± 0.003	0.137 ± 0.003
4	0.155 ± 0.001	0.140 ± 0.001
5	0.150 ± 0.001	0.135 ± 0.001
D-1	0.181 ± 0.001	0.166 ± 0.001
2	0.182 ± 0.003	0.167 ± 0.003
3	0.184 ± 0.002	0.169 ± 0.002
4	0.189 ± 0.003	0.174 ± 0.003
5	0.189 ± 0.003	0.174 ± 0.003
E-1	0.218 ± 0.004	0.203 ± 0.004
2	0.228 ± 0.002	0.213 ± 0.002
3	0.213 ± 0.003	0.198 ± 0.003
4	0.231 ± 0.003	0.216 ± 0.003
5	0.231 ± 0.003	0.216 ± 0.003
F-1	0.249 ± 0.002	0.234 ± 0.002
2	0.268 ± 0.003	0.253 ± 0.003
3	0.250 ± 0.002	0.235 ± 0.002
4	0.253 ± 0.003	0.238 ± 0.003
5	0.275 ± 0.004	0.260 ± 0.004

* Average and standard deviation of five peak integration readings.

TABLE D-16

WET PRESSURE-DIGESTION/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

RECOVERY OF LEAD IN SPIKED FISH BASE SAMPLES

Series and No.	*Pb in 100 ml soln. (ppm)	Total Pb (ug/100 ml)	**Pb (ug/100 ml) corr. fish base	Actual Pb spike (ug)	Recovery of Pb (%)
B-1	$0.012^{8} \pm 0.001^{0}$	$1.2^{8} \pm 0.1^{0}$	$0.4^{9} \pm 0.1^{0}$	0.50 ± 0.02	98 ± 20
2	0.0132 ± 0.0007	1.32 ± 0.07	$0.53^{5} \pm 0.09^{2}$	0.50 ± 0.02	107 ± 18
3	0.0129 ± 0.0007	1.29 ± 0.07	$0.50^{4} \pm 0.09^{2}$	0.50 ± 0.02	101 ± 18
4	$0.012^{5} \pm 0.001^{0}$	$1.2^{5} \pm 0.1^{0}$	$0.4^{6} \pm 0.1^{0}$	0.50 ± 0.02	92 ± 20
5	$0.012^{9} \pm 0.001^{0}$	$1.2^{9} \pm 0.1^{0}$	$0.5^{0} \pm 0.1^{0}$	0.50 ± 0.02	100 ± 20
C-1	$0.0167^{3} \pm 0.0003^{5}$	$1.67^{3} \pm 0.03^{5}$	$0.88^{7} \pm 0.09^{2}$	1.00 ± 0.02	88.7 ± 9.2
2	0.0164 ± 0.0007	1.64 ± 0.07	$0.85^{4} \pm 0.09^{2}$	1.00 ± 0.02	85.4 ± 9.2
3	$0.017^{2} \pm 0.001^{4}$	$1.7^{2} \pm 0.1^{4}$	$0.9^{3} \pm 0.1^{4}$	1.00 ± 0.02	93 ± 14
4	0.0176 ± 0.0007	1.76 ± 0.07	$0.97^{4} \pm 0.09^{2}$	1.00 ± 0.02	97.4 ± 9.2
5	0.0170 ± 0.0007	1.70 ± 0.07	$0.91^{3} \pm 0.09^{2}$	1.00 ± 0.02	91.3 ± 9.2
D-1	0.0209 ± 0.0007	2.09 ± 0.07	$1.30^{5} \pm 0.09^{2}$	1.50 ± 0.03	87.0 ± 6.1
2	$0.021^{1} \pm 0.001^{4}$	$2.1^{1} \pm 0.1^{4}$	$1.3^{2} \pm 0.1^{4}$	1.50 ± 0.03	88.0 ± 9.3
3	$0.021^{3} \pm 0.001^{0}$	$2.1^{3} \pm 0.1^{0}$	$1.3^{4} \pm 0.1^{0}$	1.50 ± 0.03	89.3 ± 6.6
4	$0.022^{0} \pm 0.001^{4}$	$2.2^{0} \pm 0.1^{4}$	$1.4^{1} \pm 0.1^{4}$	1.50 ± 0.03	94.0 ± 9.3
5	$0.022^{0} \pm 0.001^{4}$	$2.2^{0} \pm 0.1^{4}$	$1.4^{1} \pm 0.1^{4}$	1.50 ± 0.03	94.0 ± 9.3
E-1	$0.025^{7} \pm 0.001^{8}$	$2.5^{7} \pm 0.1^{8}$	$1.7^{9} \pm 0.1^{8}$	2.00 ± 0.03	89.5 ± 9.0
2	$0.027^{0} \pm 0.001^{0}$	$2.7^{0} \pm 0.1^{0}$	$1.9^{1} \pm 0.1^{0}$	2.00 ± 0.03	95.5 ± 5.0
3	$0.025^{0} \pm 0.001^{4}$	$2.5^{0} \pm 0.1^{4}$	$1.7^{1} \pm 0.1^{4}$	2.00 ± 0.03	85.5 ± 7.0
4	$0.027^{3} \pm 0.001^{4}$	$2.7^{3} \pm 0.1^{4}$	$1.9^{4} \pm 0.1^{4}$	2.00 ± 0.03	97.0 ± 7.0
5	$0.027^{3} \pm 0.001^{4}$	$2.7^{3} \pm 0.1^{4}$	$1.9^{4} \pm 0.1^{4}$	2.00 ± 0.03	97.0 ± 7.0
F-1	$0.029^{6} \pm 0.001^{0}$	$2.9^{6} \pm 0.1^{0}$	$2.1^{7} \pm 0.1^{0}$	2.50 ± 0.04	86.8 ± 4.0
2	$0.032^{1} \pm 0.001^{4}$	$3.2^{1} \pm 0.1^{4}$	$2.4^{2} \pm 0.1^{4}$	2.50 ± 0.04	96.8 ± 5.6
3	$0.029^{8} \pm 0.001^{0}$	$2.9^{8} \pm 0.1^{0}$	$2.1^{9} \pm 0.1^{0}$	2.50 ± 0.04	87.6 ± 4.0
4	$0.030^{1} \pm 0.001^{4}$	$3.0^{1} \pm 0.1^{4}$	$2.2^{2} \pm 0.1^{4}$	2.50 ± 0.04	88.8 ± 5.6
5	$0.033^{0} \pm 0.001^{8}$	$3.3^{0} \pm 0.1^{8}$	$2.5^{1} \pm 0.1^{8}$	2.50 ± 0.04	100.4 ± 7.2

* Using the linear equation from Page 298.

** Based on the higher of the two uncertainties:- fish base or total lead in 100 ml

If the average for each of the Series B to F is taken with respect to the "Total Pb (ug/100 ml)" column data (Table D-16) we have:-

Series	B	$1.28^{6} \pm 0.02^{5}$
	C	$1.69^{9} \pm 0.04^{6}$
	D	$2.14^{6} \pm 0.05^{1}$
	E	$2.65^{0} \pm 0.10$
	F	3.09 ± 0.15

Linear regression analysis yields:-

$r = 0.9995$
$b = 0.806$
$m = 0.9118$

Thus the zero intercept on the ordinate axis yields a value of 0.806 ug Pb/100 ml of solution or for the sample weights approximating 1.00 g of dried fish base, 0.806 ug/g dried fish base. Carrying out reverse calculations indicates an uncertainty for this value of 0.01^{8} ug, so that we have, from the method of additions, a calculated value for dried rainbow trout base content of lead of $0.80^{6} \pm 0.01^{8}$ ug/g fish base. This compares, within the respective tolerance limits, with the value of $0.78^{6} \pm 0.09^{2}$ ug Pb/g dried fish base reported on the previous page.

Using the value of $0.80^{6} \pm 0.01^{8}$ ug Pb/g dried fish base as the correction value to the "Total Pb (ug/100 ml)" data column yields an average recovery percent for spikes up to 2.5 ug on 1 g dried fish base of $91.^{7} \pm 5.^{2}$. This also agrees, within the respective tolerance limits, with the value of $93.^{2} \pm 5.^{6}$ percent reported on the previous page.

By including the average result of Series A with the average for each of the Series B to F taken with respect to the "Total Pb (ug/100 ml)" data column in a graphical determination of lead in dried rainbow trout by the method of additions, the plot on Figure D-4 was obtained. The lead content in dried rainbow trout base tissue can be extrapolated to the abscissa to 0.82 ± 0.01 ug Pb/g fish base. The value ± 0.01 represents the reading uncertainty on the graph. This extrapolated lead concentration compares, within the respective tolerance limits, with the value of $0.78^{6} \pm 0.09^{2}$ ug Pb/g dried fish base reported on the previous page.

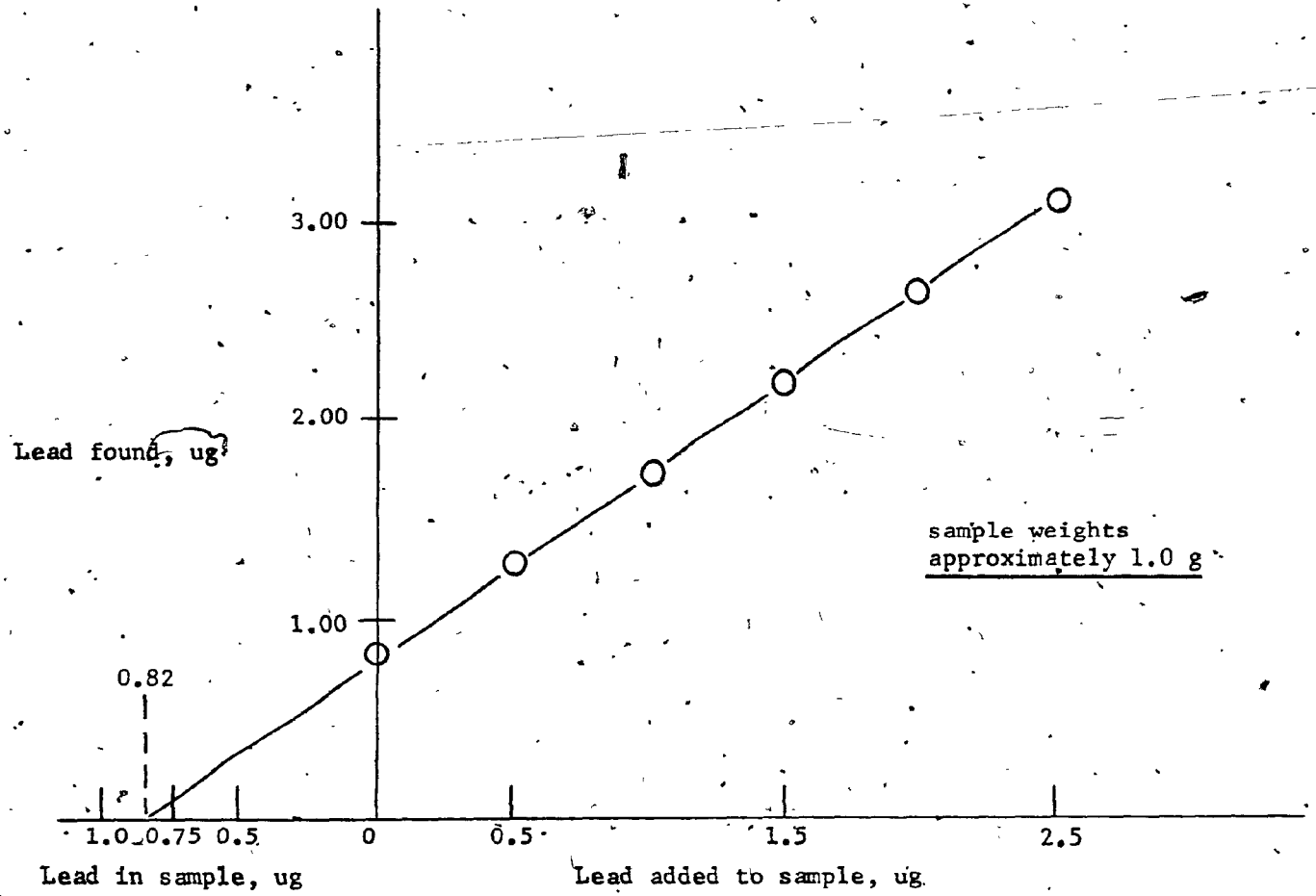


FIGURE D-4 GRAPHICAL METHOD OF ADDITIONS - LEAD DETERMINATION IN RAINBOW TROUT - WET PRESSURE-DIGESTION

4.1 Recovery of Lead from Aliquoted Spiked Fish Base Samples

Two sets of macerated and dried rainbow trout tissue were weighed out in duplicate. Each sample consisted of 1.0000 ± 0.0002 g and was placed in a Kimax screwcap 12-cm test tube.

One set (A), was spiked with 2.00 ± 0.03 ug of Pb each, while the second set (B) was spiked with 2.50 ± 0.03 ug of Pb each.

All samples were dried, digested and extracted according to the details of Sub-section 2.2.3 - (c). A blank was carried through simultaneously. The amount of HCl pipetted was 4.00 ± 0.01 ml and each sample was made up to 100.00 ± 0.04 ml in a volumetric flask.

For each set, 10 ml of the final solution was tested as-is and 50 ml diluted with 50 ml of 2.5 percent HCl and then tested. The following results were obtained:-

Set	Ave. absorbance corr. for blank		Total Pb* (ug/100 ml)		Pb corr. for** base fish (ug/g)		Actual spike (ug)	Recovery (%)	
	as-is	aliq.	as-is	aliqx2	as-is	aliqx2		as-is	aliq.
A	0.210	0.101	2.66	2.53	1.87	1.74	2.00	94	87
B	0.250	0.120	3.17	3.01	2.38	2.22	2.50	95	89

* Using the linear equation from Page 298.

** Using the correction factor of 0.78^6 uf Pb/g dried trout base

The recoveries above agree within the expected tolerance limits for lead recovery on spiked samples, so that aliquoting of tissue sample solutions, suspected to contain lead beyond the linear range maximum, should present no difficulty.

APPENDIX D-5

Determination of Cadmium in Rainbow Trout Tissue by the Wet Pressure-Digestion/Graphite Furnace Atomic Absorption Technique

1. General

The following sections outline the experimental approach applied to the testing, recovery from spiking and recovery from aliquoting on cadmium in dried rainbow trout base tissue.

The graphite furnace method was applied because of the relatively low cadmium content found for rainbow trout unexposed to experimentally high cadmium levels in the water and/or food.

General operating conditions

Certain general conditions were maintained throughout the analytical procedure. These are outlined in Appendix B-4.

Preparation of aqueous-base standard solutions

Appendix B-4 outlines the preparation of the aqueous-base standard solutions.

Preparation of fish tissue

The preparation of rainbow trout base tissue is described in Sub-section 2.2.3 - (a).

2. Testing of Wet Pressure-Digested Rainbow Trout Base Tissue

Wet ashing (pressure/digestion) of fish tissue

The general procedure is outlined in Sub-section 2.2.3 - (c). The specific details for the determination of cadmium are given below:-

The sample weights were as follows:-

Sample No.	Fish weight (g)
1.	0.0000
2.	0.0908 ± 0.0002
3.	0.0960 ± 0.0002
4.	0.1115 ± 0.0002
5.	0.1518 ± 0.0002
6.	0.1695 ± 0.0002
7.	0.4906 ± 0.0002
8.	0.7258 ± 0.0002
9.	0.7387 ± 0.0002
10.	0.7419 ± 0.0002
11.	0.7633 ± 0.0002
12.	0.9146 ± 0.0002
13.	0.9943 ± 0.0002
14.	1.0071 ± 0.0002
15.	1.0405 ± 0.0002
16.	1.4869 ± 0.0002
17.	1.6768 ± 0.0002

The amount of HCl pipetted was,

$$1.00 \pm 0.01 \text{ ml}$$

The amount of glass-distilled water pipetted was,

$$24.00 \pm 0.06 \text{ ml}$$

Testing of working standards by graphite furnace atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Cadmium (ppm)	Average absorbance*	Corr. ave. abs.
0.00000	0.009 ± 0.000	0.000 ± 0.000
0.00010 ± 0.00001	0.019 ± 0.000	0.010 ± 0.000
0.00050 ± 0.00001	0.056 ± 0.001	0.047 ± 0.001
0.00100 ± 0.00001	0.117 ± 0.002	0.108 ± 0.002
0.00150 ± 0.00004	0.178 ± 0.001	0.169 ± 0.001
0.00200 ± 0.00004	0.215 ± 0.004	0.206 ± 0.004
0.00300 ± 0.00005	0.319 ± 0.005	0.310 ± 0.005

* Average and standard deviation of three peak integration readings.

Linear regression analysis

0.00000 - 0.00300 ppm Cd

r = 0.9986[5]
b = 0.000[7]
m = 104.30

Sensitivity (1% absorption) = 0.0044/104.30 = 0.00004 ppm Cd

Reverse calculations for cadmium

Actual cadmium (ppm)	Calculated cadmium (ppm)	Absolute devn.
0.00000	0.00000	0.00000
0.00010 ± 0.00001	0.00009	0.00001
0.00050 ± 0.00001	0.00044	0.00006
0.00100 ± 0.00001	0.00103	0.00003
0.00150 ± 0.00004	0.00161	0.00011
0.00200 ± 0.00004	0.00197	0.00003
0.00300 ± 0.00005	0.00296	0.00004
	Average absolute deviation	0.00004

Values of cadmium (ppm) from the above linear equation subject to ± 0.00004 ppm.

Results of cadmium determination in wet pressure-digested fish base tissue

The results of the blank and fish base samples are tabulated in Table D-17.

The 16 values recorded in the last column yield an average of,

$$0.027^4 \pm 0.004^0 \text{ ppm}$$

or,

$$0.027^4 \pm 0.004^0 \text{ ug Cd/g dried rainbow trout base}$$

3. Recovery of Cadmium from Spiked Fish Base Samples

Wet ashing (pressure/digestion) of fish tissue

The weights of macerated and dried fish tissue weighed into 12-cm test tubes are listed in Table D-18.

These fish tissue samples were spiked with 0.100 ± 0.001 ppm Cd solution as follows:-

Series	Spike	ug cadmium added
A	none	0.000
B	0.50 ± 0.02 ml of 0.100 ppm Cd	0.050 ± 0.002
C	1.00 ± 0.02 ml " " " "	0.100 ± 0.003
D	1.50 ± 0.02 ml " " " "	0.150 ± 0.004
E	2.00 ± 0.02 ml " " " "	0.200 ± 0.004
F	2.50 ± 0.02 ml " " " "	0.250 ± 0.004

Subsequent to spiking each sample was placed in the oven and heated at just under 100 °C until the liquid spike had dried out. The subsequent digestion procedure duplicated the details of Sub-section 2.2.3 - (c). A blank with no spike was carried through the same procedure.

The amount of HCl pipetted was,

$$4.00 \pm 0.02 \text{ ml}$$

Each sample was made up to 100.00 ± 0.04 ml in a volumetric flask.

TABLE D-17

DETERMINATION OF CADMIUM IN RAINBOW TROUT BASE TISSUE BY WET PRESSURE-DIGESTION/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

Sample No.	Average* absorbance	Corrected average absorbance	**Cd in 25 ml soln. (ppm)	Cadmium ug/25 ml	Cadmium in fish base (ppm)
1.	0.020 ± 0.000	0.000 ± 0.000	---	---	---
2.	0.032 ± 0.000	0.012 ± 0.000	0.00011 ± 0.00004	$0.002^{8} \pm 0.001^{0}$	$0.03^{1} \pm 0.01^{1}$
3.	0.031 ± 0.000	0.011 ± 0.000	0.00010 ± 0.00004	$0.002^{5} \pm 0.001^{0}$	$0.02^{6} \pm 0.01^{0}$
4.	0.036 ± 0.000	0.016 ± 0.000	0.00015 ± 0.00004	$0.003^{8} \pm 0.001^{0}$	$0.03^{4} \pm 0.009$
5.	0.042 ± 0.001	0.022 ± 0.001	0.00020 ± 0.00008	0.005 ± 0.002^{1}	$0.03^{3} \pm 0.01^{3}$
6.	0.045 ± 0.000	0.025 ± 0.000	0.00023 ± 0.00004	$0.005^{8} \pm 0.001^{0}$	$0.034^{2} \pm 0.005^{9}$
7.	0.071 ± 0.002	0.051 ± 0.002	$0.0004^{8} \pm 0.0001^{2}$	0.12 ± 0.003	$0.024^{4} \pm 0.006^{1}$
8.	0.102 ± 0.002	0.082 ± 0.002	$0.0007^{8} \pm 0.0001^{2}$	$0.019^{5} \pm 0.003^{0}$	$0.026^{9} \pm 0.004^{1}$
9.	0.100 ± 0.000	0.080 ± 0.000	0.00076 ± 0.00004	0.019 ± 0.001	$0.025^{7} \pm 0.001^{3}$
10.	0.094 ± 0.001	0.074 ± 0.001	0.00070 ± 0.00008	$0.017^{5} \pm 0.002^{0}$	$0.023^{6} \pm 0.002^{7}$
11.	0.114 ± 0.003	0.094 ± 0.003	$0.0008^{9} \pm 0.0001^{6}$	$0.022^{2} \pm 0.004^{0}$	$0.029^{1} \pm 0.005^{2}$
12.	0.110 ± 0.001	0.090 ± 0.001	0.00086 ± 0.00008	$0.021^{5} \pm 0.002^{0}$	$0.023^{5} \pm 0.002^{2}$
13.	0.115 ± 0.001	0.095 ± 0.001	0.00090 ± 0.00008	$0.022^{5} \pm 0.002^{0}$	$0.022^{6} \pm 0.002^{0}$
14.	0.139 ± 0.003	0.119 ± 0.003	$0.0011^{3} \pm 0.0001^{6}$	$0.028^{2} \pm 0.004^{0}$	0.028 ± 0.004
15.	0.117 ± 0.002	0.097 ± 0.002	$0.0009^{2} \pm 0.0001^{2}$	0.023 ± 0.003	$0.022^{1} \pm 0.002^{9}$
16.	0.173 ± 0.003	0.153 ± 0.003	$0.0014^{6} \pm 0.0001^{6}$	$0.036^{5} \pm 0.004^{0}$	$0.024^{5} \pm 0.002^{7}$
17.	0.226 ± 0.005	0.206 ± 0.005	$0.0019^{7} \pm 0.0002^{4}$	$0.049^{2} \pm 0.006^{0}$	$0.029^{3} \pm 0.003^{6}$

* Average and standard deviation of five peak integration readings.

** Using the linear equation from Page 308.

TABLE D-18

SAMPLE WEIGHTS OF BASE FISH TISSUE FOR WET PRESSURE-DIGESTION SPIKING TECHNIQUE

No.	Series A	Series B	Series C
1	1.0001 ± 0.0002	1.0018 ± 0.0002	0.9979 ± 0.0002
2	0.9999 ± 0.0002	0.9965 ± 0.0002	1.0008 ± 0.0002
3	1.0026 ± 0.0002	0.9990 ± 0.0002	1.0006 ± 0.0002
4	0.9998 ± 0.0002	1.0048 ± 0.0002	0.9994 ± 0.0002
5	1.0000 ± 0.0002	1.0008 ± 0.0002	1.0051 ± 0.0002

No.	Series D	Series E	Series F
1	0.9979 ± 0.0002	0.9970 ± 0.0002	1.0007 ± 0.0002
2	1.0010 ± 0.0002	1.0060 ± 0.0002	1.0070 ± 0.0002
3	0.9997 ± 0.0002	1.0059 ± 0.0002	0.9991 ± 0.0002
4	0.9987 ± 0.0002	1.0013 ± 0.0002	1.0002 ± 0.0002
5	1.0007 ± 0.0002	0.9995 ± 0.0002	1.0000 ± 0.0002

Testing of working standards by graphite furnace atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Cadmium (ppm)	Average absorbance*	Corr. ave. abs.
0.00000	0.008 ± 0.000	0.000 ± 0.000
0.00050 ± 0.00001	0.057 ± 0.001	0.049 ± 0.001
0.00075 ± 0.00001	0.085 ± 0.001	0.077 ± 0.001
0.00100 ± 0.00001	0.112 ± 0.003	0.105 ± 0.003
0.00150 ± 0.00004	0.162 ± 0.002	0.154 ± 0.002
0.00200 ± 0.00004	0.208 ± 0.004	0.200 ± 0.004
0.00300 ± 0.00005	0.309 ± 0.004	0.301 ± 0.004

* Average and standard deviation of five peak integration readings.

Linear regression analysis

0.00000 - 0.00300 ppm Cd

r = 0.9998
b = 0.001
m = 100.20

Sensitivity (1% absorption) = 0.0044/100.20 = 0.00004 ppm Cd

Reverse calculations for cadmium

Actual cadmium (ppm)	Calculated cadmium (ppm)	Absolute devn.
0.00000	-0.00001	0.00001
0.00050 ± 0.00001	0.00048	0.00002
0.00075 ± 0.00001	0.00076	0.00001
0.00100 ± 0.00001	0.00104	0.00004
0.00150 ± 0.00004	0.00153	0.00003
0.00200 ± 0.00004	0.00198	0.00002
0.00300 ± 0.00005	0.00299	0.00001
	Average absolute deviation	0.00002

Values of cadmium (ppm) from the above linear equation subject to ± 0.00002 ppm.

Results of cadmium determination of spiked wet pressure-digested fish base tissue

The absorbance results for the blank and fish base samples spiked with cadmium (ug) are tabulated in Table D-19.

The calculated cadmium recoveries for Series B to F are tabulated in Table D-20.

The value for the 100 ml each Series A samples yield the following results:-

Series and No.	Cd in 100 ml soln. (ppm)*	Cd (ug/g) in fish base
A-1	0.00031 ± 0.00004	0.031 ± 0.004
2	0.00030 ± 0.00004	0.030 ± 0.004
3	0.00029 ± 0.00006	0.029 ± 0.006
4	0.00024 ± 0.00004	0.024 ± 0.004
5	0.00028 ± 0.00004	0.028 ± 0.004

* Using the linear equation from Page 312.

These again represent fish base values and combined with the 16 results obtained under Appendix D-5 (2) Testing of Wet Pressure-Digested Rainbow Trout Base Tissue, provide a total of 21 values. These yield a final value for the cadmium content of dried rainbow trout unexposed to experimentally high cadmium contents in the water and/or food of:-

$0.027^{6} \pm 0.003^{7}$ ppm or $0.027^{6} \pm 0.003^{7}$ ug Cd/g dried rainbow trout base

An overall recovery percent for cadmium spikes on rainbow trout dried base of up to 0.25 ug/g dried fish base resulted in:-

$$92.^{4} \pm 8.^{2}\ \%$$

TABLE D-19

WET PRESSURE-DIGESTION/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

RESULTS OF CADMIUM DETERMINATION
ON SPIKED FISH BASE SAMPLES

Series and No.	Average* absorbance	Corrected average absorbance
blank	0.030 ± 0.000	0.000 ± 0.000
A-1	0.062 ± 0.001	0.032 ± 0.001
2	0.061 ± 0.001	0.031 ± 0.001
3	0.060 ± 0.002	0.030 ± 0.002
4	0.055 ± 0.001	0.025 ± 0.001
5	0.059 ± 0.001	0.029 ± 0.001
B-1	0.112 ± 0.000	0.082 ± 0.000
2	0.114 ± 0.001	0.084 ± 0.001
3	0.110 ± 0.001	0.080 ± 0.001
4	0.110 ± 0.002	0.080 ± 0.002
5	0.100 ± 0.002	0.070 ± 0.002
C-1	0.163 ± 0.003	0.133 ± 0.003
2	0.163 ± 0.001	0.133 ± 0.001
3	0.141 ± 0.002	0.111 ± 0.002
4	0.146 ± 0.002	0.116 ± 0.002
5	0.150 ± 0.003	0.120 ± 0.003
D-1	0.191 ± 0.001	0.161 ± 0.001
2	0.187 ± 0.003	0.157 ± 0.003
3	0.180 ± 0.001	0.150 ± 0.001
4	0.191 ± 0.004	0.161 ± 0.004
5	0.195 ± 0.003	0.165 ± 0.003
E-1	0.240 ± 0.004	0.210 ± 0.004
2	0.240 ± 0.001	0.210 ± 0.001
3	0.237 ± 0.002	0.207 ± 0.002
4	0.259 ± 0.003	0.229 ± 0.003
5	0.240 ± 0.001	0.210 ± 0.001
F-1	0.296 ± 0.004	0.266 ± 0.004
2	0.265 ± 0.003	0.235 ± 0.003
3	0.289 ± 0.003	0.259 ± 0.003
4	0.289 ± 0.004	0.259 ± 0.004
5	0.279 ± 0.003	0.249 ± 0.003

* Average and standard deviation of five peak integration readings.

TABLE D-20

WET PRESSURE-DIGESTION/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

RECOVERY OF CADMIUM IN SPIKED FISH BASE SAMPLES

Series and No.	*Cd in 100 ml soln. (ppm)	Total Cd (ug/100 ml)	**Cd (ug/100 ml) corr. fish base	Actual Cd spike (ug)	Recovery of Cd (%)
B-1	0.00081 ± 0.00002	0.081 ± 0.002	0.053 ± 0.004	0.050 ± 0.002	106.0 ± 8.0
2	0.00083 ± 0.00004	0.083 ± 0.004	0.055 ± 0.004	0.050 ± 0.002	110.0 ± 8.0
3	0.00079 ± 0.00004	0.079 ± 0.004	0.051 ± 0.004	0.050 ± 0.002	102.0 ± 8.0
4	0.00079 ± 0.00006	0.079 ± 0.006	0.051 ± 0.006	0.050 ± 0.002	102 ± 12
5	0.00069 ± 0.00006	0.069 ± 0.006	0.041 ± 0.006	0.050 ± 0.002	82 ± 12
C-1	0.00132 ± 0.00008	0.132 ± 0.008	0.104 ± 0.008	0.100 ± 0.003	104.0 ± 8.0
2	0.00132 ± 0.00004	0.132 ± 0.004	0.104 ± 0.004	0.100 ± 0.003	104.0 ± 4.0
3	0.00110 ± 0.00006	0.110 ± 0.006	0.082 ± 0.006	0.100 ± 0.003	82.0 ± 6.0
4	0.00115 ± 0.00006	0.115 ± 0.006	0.087 ± 0.006	0.100 ± 0.003	87.0 ± 6.0
5	0.00119 ± 0.00008	0.119 ± 0.008	0.091 ± 0.008	0.100 ± 0.003	91.0 ± 8.0
D-1	0.00160 ± 0.00004	0.160 ± 0.004	0.132 ± 0.004	0.150 ± 0.004	88.0 ± 2.7
2	0.00156 ± 0.00008	0.156 ± 0.008	0.128 ± 0.008	0.150 ± 0.004	85.3 ± 5.3
3	0.00149 ± 0.00004	0.149 ± 0.004	0.121 ± 0.004	0.150 ± 0.004	80.7 ± 2.7
4	$0.0016^{0} \pm 0.0001^{0}$	$0.16^{0} \pm 0.01^{0}$	$0.13^{2} \pm 0.01^{0}$	0.150 ± 0.004	88.0 ± 6.7
5	0.00164 ± 0.00008	0.164 ± 0.008	0.136 ± 0.008	0.150 ± 0.004	90.7 ± 5.3
E-1	$0.0020^{8} \pm 0.0001^{0}$	$0.20^{8} \pm 0.01^{0}$	$0.18^{0} \pm 0.01^{0}$	0.200 ± 0.004	90.0 ± 5.0
2	0.00208 ± 0.00004	0.208 ± 0.004	0.180 ± 0.004	0.200 ± 0.004	90.0 ± 2.0
3	0.00206 ± 0.00006	0.206 ± 0.006	0.178 ± 0.006	0.200 ± 0.004	89.0 ± 3.0
4	0.00228 ± 0.00008	0.228 ± 0.008	0.200 ± 0.008	0.200 ± 0.004	100.0 ± 4.0
5	0.00208 ± 0.00004	0.208 ± 0.004	0.180 ± 0.004	0.200 ± 0.004	90.0 ± 2.0
F-1	$0.0026^{4} \pm 0.0001^{0}$	$0.26^{4} \pm 0.01^{0}$	$0.23^{6} \pm 0.01^{0}$	0.250 ± 0.004	94.4 ± 4.0
2	0.00234 ± 0.00008	0.234 ± 0.008	0.206 ± 0.008	0.250 ± 0.004	82.4 ± 3.2
3	0.00257 ± 0.00008	0.257 ± 0.008	0.229 ± 0.008	0.250 ± 0.004	91.6 ± 3.2
4	$0.0025^{7} \pm 0.0001^{0}$	$0.25^{7} \pm 0.01^{0}$	$0.22^{9} \pm 0.01^{0}$	0.250 ± 0.004	91.6 ± 4.0
5	0.00248 ± 0.00008	0.248 ± 0.008	0.220 ± 0.008	0.250 ± 0.004	88.0 ± 3.2

* Using the linear equation from Page 312.

** Based on the higher of the two uncertainties:- fish base or total cadmium in 100 ml.

If the average for each of the Series B to F is taken with respect to the "Total Cd (ug/100 ml)" column data (Table D-20) we have:-

Series	B	0.078 ± 0.005
	C	$0.12^{2} \pm 0.01^{0}$
	D	0.158 ± 0.006
	E	0.212 ± 0.009
	F	$0.25^{2} \pm 0.01^{2}$

Linear regression analysis yields:- $r = 0.9985$, $b = 0.033$, $m = 0.876$

Thus the zero intercept on the ordinate axis yields a value of 0.033 ug Cd/100 ml of solution or, for the sample weights approximating 1.00 g of dried fish base, 0.033 ug Cd/g dried fish base. Carrying out reverse calculations indicates an uncertainty for this value of $\pm$ 0.002 ug, so that we have, from the method of additions, a calculated value for dried rainbow trout content of cadmium of 0.033 ± 0.002 ug/g fish base. This falls, within the respective tolerance limits, well in line with the value of $0.027^{6} \pm 0.003^{7}$ ug Cd/g dried fish base reported on the previous page.

Using the value of 0.033 ± 0.002 ug Cd/g dried fish base as the correction value to the "Total Cd (ug/100 ml)" data column yields an average recovery percent for spikes up to 0.25 ug on 1 g dried fish base of $87.^{9} \pm 7.^{3}$. Inspite of being relatively low, this value of recovery also agrees, within the respective tolerance limits, with the value of $92.^{4} \pm 8.^{2}$ percent reported on the previous page.

By including the average result of Series A with the average for each of the Series B to F taken with respect to the "Total Cd (ug/100 ml)" data column in a graphical determination of cadmium in dried rainbow trout by the method of additions, the plot on Figure D-5 was obtained. The cadmium content in dried fish base tissue can be extrapolated to the abscissa to $0.032^{5} \pm 0.002^{5}$ ug Cd/g dried fish base. The value of $\pm$ 0.002^{5} represents the reading uncertainty on the graph. This extrapolated cadmium concentration compares, within the respective tolerance limits, with the value of $0.027^{6} \pm 0.003^{7}$ ug Cd/g dried fish base reported on the previous page.

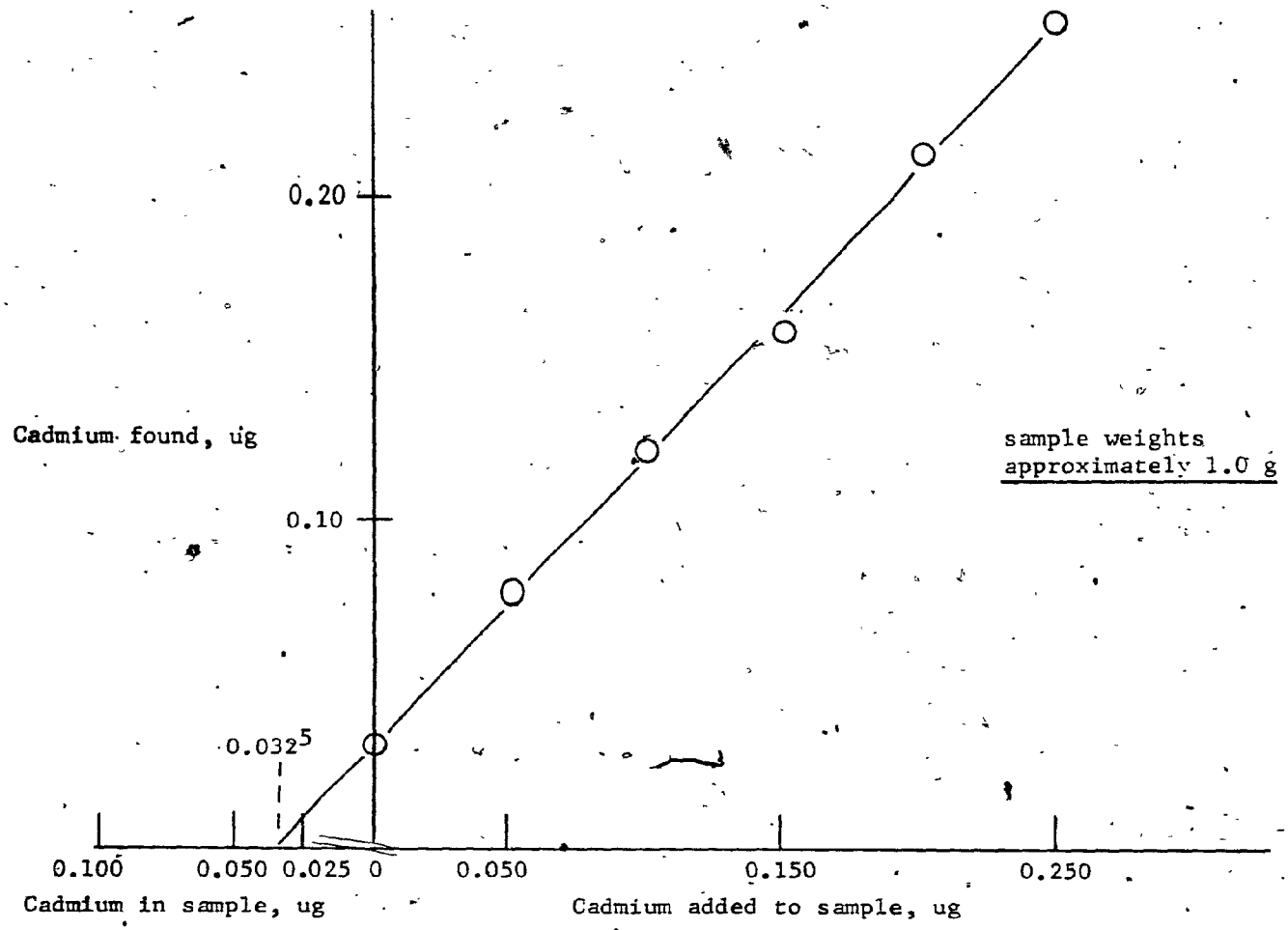


FIGURE D-5 GRAPHICAL METHOD OF ADDITIONS - CADMIUM DETERMINATION IN RAINBOW TROUT - WET PRESSURE-DIGESTION

4. Recovery of Cadmium from Aliquoted Spiked Fish Base Samples

Two sets of macerated and dried rainbow trout tissue were weighed out in duplicate. Each sample consisted of 1.0000 ± 0.0002 g and was placed in a Kimax 12-cm test tube.

One set (A) was spiked with 0.200 ± 0.004 ug of cadmium each, while the second set (B) was spiked with 0.250 ± 0.004 ug of cadmium each.

All samples were dried, digested and extracted according to the details of Sub-section 2.2.3 - (c). A blank was carried through simultaneously. The amount of HCl pipetted was 4.00 ± 0.02 ml and each sample was made up to 100.00 ± 0.04 ml in a volumetric flask.

For each set, 50 ml of the final solution was tested as-is and 50 ml was diluted with 50 ml of 2.5 percent HCl and then tested. The following results were obtained:-

Set	Ave. absorbance corr. for blank		Total Cd* (ug/100 ml)		Cd corr. for** base fish (ug/g)		Actual spike (ug)	Recovery (%)	
	as-is	aliq.	as-is	aliqx2	as-is	aliqx2		as-is	aliq.
A	0.215	0.110	0.214	0.218	0.186	0.190	0.200	93	95
B	0.250	0.135	0.248	0.267	0.220	0.239	0.250	88	96

* Using the linear equation from Page 312.

** Using the correction value of $0.027^6 \pm 0.003^7$ ug Cd/g dried rainbow trout base

The recovery percent values agree within the expected tolerance limits for cadmium recovery on spiked samples, so that aliquoting of tissue sample solutions, suspected to contain cadmium in ppm beyond the linear range maximum, should present no difficulty.

APPENDIX D-6

Determination of Vanadium in Rainbow Trout Tissue by the Wet Pressure-Digestion/Graphite Furnace Atomic Absorption.

1. General

The following sections outline the experimental approach applied to the testing, recovery from spiking and recovery from aliquoting on vanadium in dried rainbow trout base tissue.

The graphite furnace method was applied because of the relatively low vanadium content found for rainbow trout unexposed to experimentally high vanadium levels in the water and/or food.

General operating conditions

Certain general operating conditions were maintained throughout the analytical procedures. These are outlined in Appendix B-5.

Preparation of aqueous-base standard solutions

Appendix B-5 outlines the preparation of the aqueous-base standard solutions.

Preparation of fish tissue

The preparation of rainbow trout base tissue is described in Sub-section 2.2.3 - (a).

2. Testing of Wet Pressure-Digested Rainbow Trout Base Tissue

Wet ashing (pressure/digestion) of fish tissue

The general procedure is outlined in Sub-section 2.2.3 - (c). The specific details for the determination of vanadium are given below:-

The sample weights were as follows:-

Sample No.	Fish weight (g)
1.	0.0000
2.	0.4906 ± 0.0002
3.	0.5021 ± 0.0002
4.	0.7258 ± 0.0002
5.	0.7374 ± 0.0002
6.	0.7387 ± 0.0002
7.	0.9146 ± 0.0002
8.	0.9692 ± 0.0002
9.	0.9943 ± 0.0002
10.	1.1878 ± 0.0002
11.	1.3003 ± 0.0002
12.	1.4490 ± 0.0002
13.	1.4869 ± 0.0002
14.	1.5105 ± 0.0002
15.	1.6768 ± 0.0002
16.	1.8543 ± 0.0002
17.	2.0574 ± 0.0002

The amount of HCl pipetted was,

$$0.20 \pm 0.01 \text{ ml}$$

The amount of glass-distilled water pipetted was,

$$4.80 \pm 0.02 \text{ ml}$$

Testing of working standards by graphite furnace atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Vanadium (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.050 ± 0.002	0.013 ± 0.000
0.100 ± 0.002	0.022 ± 0.000
0.250 ± 0.002	0.058 ± 0.001
0.500 ± 0.003	0.118 ± 0.000
0.750 ± 0.004	0.178 ± 0.002
1.000 ± 0.004	0.236 ± 0.002

* Average and standard deviation of three peak integration readings.

Linear regression analysis

0.000 - 1.000 ppm V

$r = 0.9999$
$b = -0.000^2$
$m = 0.2366^2$

Sensitivity (1% absorption) = $0.0044/0.2366^2 = 0.01^8$ ppm V

Reverse calculations for vanadium

Actual vanadium (ppm)	Calculated vanadium (ppm)	Absolute devn.
0.000	-0.001	0.001
0.050 ± 0.002	0.056	0.006
0.100 ± 0.002	0.094	0.006
0.250 ± 0.002	0.246	0.004
0.500 ± 0.003	0.500	0.000
0.750 ± 0.004	0.754	0.004
1.000 ± 0.004	0.999	0.001
	Average absolute deviation	0.003

Values of vanadium (ppm) from the above linear equation subject to ± 0.003 ppm.

Results of vanadium determination in wet pressure-digested fish base tissue

The results for the blank and fish base samples are tabulated in Table D-21.

The 16 values recorded in the last column yield an average of,

$$0.247 \pm 0.044 \text{ ppm}$$

or,

$$0.247 \pm 0.044 \text{ ug V/g dried rainbow trout base}$$

3. Recovery of Vanadium from Spiked Fish Base Samples

Wet ashing (pressure-digestion) of fish tissue

The weights of macerated and dried fish tissue weighed into 12-cm test tubes are listed in Table D-22.

These fish tissue samples were spiked with 1.000 ± 0.004 ppm V solution as follows:-

Series	Spike	ug vanadium added
A	none	0.00
B	0.50 ± 0.02 ml of 1.000 ppm V	0.50 ± 0.02
C	1.00 ± 0.02 ml " " " "	1.00 ± 0.02
D	1.50 ± 0.02 ml " " " "	1.50 ± 0.03
E	2.00 ± 0.02 ml " " " "	2.00 ± 0.03
F	2.50 ± 0.02 ml " " " "	2.50 ± 0.03
G	4.00 ± 0.02 ml " " " "	4.00 ± 0.04

Subsequent to spiking each sample was placed in the oven and heated at just under 100 °C until the liquid spike volume had dried out. The subsequent ashing procedure duplicated the details of Sub-section 2.2.3 - (c). A blank with no spike was carried through the same procedure.

The amount of HCl pipetted was,

$$0.20 \pm 0.01 \text{ ml}$$

The amount of glass-distilled water pipetted was,

$$4.80 \pm 0.02 \text{ ml}$$

TABLE D-21

DETERMINATION OF VANADIUM IN RAINBOW TROUT BASE TISSUE BY WET PRESSURE-DIGESTION/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

Sample No.	Average* absorbance	Corrected average absorbance	**V in 5 ml soln. (ppm)	Vanadium ug/5 ml	Vanadium in fish base (ppm)
1.	0.001 ± 0.000	0.000 ± 0.000	---	---	---
2.	0.008 ± 0.000	0.007 ± 0.000	0.026 ± 0.003	$0.13^{0} \pm 0.01^{5}$	$0.26^{5} \pm 0.03^{0}$
3.	0.009 ± 0.000	0.008 ± 0.000	0.033 ± 0.003	$0.16^{5} \pm 0.01^{5}$	$0.32^{8} \pm 0.03^{0}$
4.	0.012 ± 0.001	0.011 ± 0.001	$0.045^{6} \pm 0.006^{0}$	0.23 ± 0.03	$0.31^{6} \pm 0.04^{1}$
5.	0.010 ± 0.000	0.009 ± 0.000	0.037 ± 0.003	$0.18^{5} \pm 0.01^{5}$	$0.25^{1} \pm 0.02^{0}$
6.	0.011 ± 0.000	0.010 ± 0.000	$0.041^{4} \pm 0.003^{0}$	$0.20^{7} \pm 0.01^{5}$	0.28 ± 0.02
7.	0.010 ± 0.001	0.009 ± 0.001	0.037 ± 0.006	$0.18^{5} \pm 0.03^{0}$	$0.20^{2} \pm 0.03^{2}$
8.	0.012 ± 0.001	0.011 ± 0.001	$0.045^{6} \pm 0.006^{0}$	0.23 ± 0.03	$0.23^{7} \pm 0.03^{1}$
9.	0.017 ± 0.001	0.016 ± 0.001	$0.066^{7} \pm 0.006^{0}$	0.33 ± 0.03	0.33 ± 0.03
10.	0.013 ± 0.000	0.012 ± 0.000	0.050 ± 0.003	$0.25^{0} \pm 0.01^{5}$	0.21 ± 0.01
11.	0.015 ± 0.000	0.014 ± 0.000	0.058 ± 0.003	$0.29^{0} \pm 0.01^{5}$	$0.22^{3} \pm 0.01^{1}$
12.	0.016 ± 0.001	0.015 ± 0.001	$0.062^{5} \pm 0.006^{0}$	0.31 ± 0.03	$0.21^{4} \pm 0.02^{1}$
13.	0.015 ± 0.000	0.014 ± 0.000	0.058 ± 0.003	$0.29^{0} \pm 0.01^{5}$	0.20 ± 0.01
14.	0.016 ± 0.001	0.015 ± 0.001	$0.062^{5} \pm 0.006^{0}$	0.31 ± 0.03	$0.20^{5} \pm 0.02^{0}$
15.	0.020 ± 0.002	0.019 ± 0.002	$0.079^{4} \pm 0.009^{0}$	$0.39^{7} \pm 0.04^{5}$	$0.23^{7} \pm 0.02^{6}$
16.	0.022 ± 0.000	0.021 ± 0.000	0.088 ± 0.003	$0.44^{0} \pm 0.01^{5}$	0.237 ± 0.008
17.	0.022 ± 0.002	0.021 ± 0.002	0.088 ± 0.009	$0.44^{0} \pm 0.04^{5}$	$0.21^{4} \pm 0.02^{1}$

* Average and standard deviation of three peak integration readings

** Using the linear equation from Page 322.

TABLE D-22

SAMPLE WEIGHTS OF BASE FISH TISSUE FOR WET PRESSURE-DIGESTION SPIKING TECHNIQUE

No.	Series A	Series B	Series C
1	0.9997 ± 0.0002	1.0015 ± 0.0002	0.9981 ± 0.0002
2	1.0004 ± 0.0002	0.9967 ± 0.0002	1.0009 ± 0.0002
3	1.0023 ± 0.0002	0.9988 ± 0.0002	1.0004 ± 0.0002
4	0.9998 ± 0.0002	1.0048 ± 0.0002	0.9994 ± 0.0002
5	1.0000 ± 0.0002	1.0010 ± 0.0002	1.0046 ± 0.0002

No.	Series D	Series E	Series F	Series G
1	0.9981 ± 0.0002	0.9973 ± 0.0002	1.0007 ± 0.0002	0.9990 ± 0.0002
2	1.0011 ± 0.0002	1.0064 ± 0.0002	1.0066 ± 0.0002	1.0006 ± 0.0002
3	1.0001 ± 0.0002	1.0064 ± 0.0002	0.9993 ± 0.0002	1.0000 ± 0.0002
4	0.9990 ± 0.0002	1.0011 ± 0.0002	1.0000 ± 0.0002	1.0010 ± 0.0002
5	1.0007 ± 0.0002	0.9999 ± 0.0002	1.0000 ± 0.0002	0.9999 ± 0.0002

Testing of working standards by graphite furnace atomic absorption

The set of working standards prepared for this series of tests gave the following results:-

Vanadium (ppm)	Average absorbance*
0.000	0.000 ± 0.000
0.050 ± 0.002	0.012 ± 0.001
0.100 ± 0.002	0.024 ± 0.000
0.250 ± 0.002	0.060 ± 0.003
0.500 ± 0.003	0.119 ± 0.005
0.750 ± 0.004	0.175 ± 0.004
1.000 ± 0.004	0.232 ± 0.004

* Average and standard deviation of five peak integration readings.

Linear regression analysis

0.000 - 1.000 ppm V

r = 0.9999
b = 0.001
m = 0.23214

Sensitivity (1% absorption) = 0.0044/0.23214 = 0.01^9 ppm V

Reverse calculations for vanadium

Actual vanadium (ppm)	Calculated vanadium (ppm)	Absolute devn.
0.000	-0.004	0.004
0.050 ± 0.002	0.047	0.003
0.100 ± 0.002	0.099	0.001
0.250 ± 0.002	0.254	0.004
0.500 ± 0.003	0.508	0.008
0.750 ± 0.004	0.750	0.000
1.000 ± 0.004	0.995	0.005
	Average absolute deviation	0.004

Values of vanadium (ppm) from the above linear equation subject to ± 0.004 ppm.

Results of vanadium determination of spiked wet pressure-digested fish base tissue

The absorbance results for the blank and fish base samples spiked with vanadium (ug) are tabulated in Table D-23.

The calculated vanadium recoveries for Series B to C are tabulated in Table D-24.

The values for the 5 ml each Series A yield the following results:-

Series and No.	*V in 5 ml soln. (ppm)	V (ug/g) in fish base
A-1	0.056 ± 0.004	0.28 ± 0.02
2	0.047 ± 0.004	0.24 ± 0.02
3	0.060 ± 0.008	0.30 ± 0.04
4	0.056 ± 0.004	0.28 ± 0.02
5	0.052 ± 0.008	0.26 ± 0.04

* Using the linear equation from Page 326.

These again represent fish base values and combined with the 16 values obtained under Appendix D-6 (2) Testing of Wet Pressure-Digested Rainbow Trout Base Tissue, provide a total of 21 values. These yield a final value for the vanadium content of dried rainbow trout unexposed to experimentally high vanadium contents in the water and/or food of:-

$0.25^{3} \pm 0.04^{2}$ ppm or $0.25^{3} \pm 0.04^{2}$ ug V/g dried rainbow trout base

An overall recovery percent for vanadium spikes on rainbow trout dried base of up to 4.00 ug/g dried fish base resulted in:-

$$107.^{2} \pm 4.^{8}\ \%$$

TABLE D-23

WET PRESSURE-DIGESTION/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

RESULTS OF VANADIUM DETERMINATION
ON SPIKED FISH BASE SAMPLES

Series and No.	Average* absorbance	Corrected average absorbance
blank	0.014 ± 0.000	0.000 ± 0.000
A-1	0.028 ± 0.000	0.014 ± 0.000
2	0.026 ± 0.000	0.012 ± 0.000
3	0.029 ± 0.001	0.015 ± 0.001
4	0.028 ± 0.000	0.014 ± 0.000
5	0.027 ± 0.001	0.013 ± 0.001
B-1	0.052 ± 0.001	0.038 ± 0.001
2	0.049 ± 0.000	0.035 ± 0.000
3	0.051 ± 0.000	0.037 ± 0.000
4	0.052 ± 0.000	0.038 ± 0.000
5	0.049 ± 0.001	0.035 ± 0.001
C-1	0.072 ± 0.002	0.058 ± 0.002
2	0.076 ± 0.001	0.062 ± 0.001
3	0.075 ± 0.001	0.061 ± 0.001
4	0.076 ± 0.000	0.062 ± 0.000
5	0.077 ± 0.000	0.063 ± 0.000
D-1	0.105 ± 0.003	0.091 ± 0.003
2	0.106 ± 0.000	0.092 ± 0.000
3	0.107 ± 0.001	0.093 ± 0.001
4	0.103 ± 0.001	0.089 ± 0.001
5	0.105 ± 0.002	0.091 ± 0.002
E-1	0.127 ± 0.000	0.113 ± 0.000
2	0.127 ± 0.001	0.113 ± 0.001
3	0.125 ± 0.001	0.111 ± 0.001
4	0.128 ± 0.000	0.114 ± 0.000
5	0.125 ± 0.003	0.111 ± 0.003
F-1	0.158 ± 0.002	0.144 ± 0.002
2	0.153 ± 0.002	0.139 ± 0.002
3	0.158 ± 0.002	0.144 ± 0.002
4	0.147 ± 0.002	0.133 ± 0.002
5	0.155 ± 0.002	0.141 ± 0.002
G-1	0.222 ± 0.003	0.208 ± 0.003
2	0.223 ± 0.002	0.209 ± 0.002
3	0.224 ± 0.004	0.210 ± 0.004
4	0.228 ± 0.002	0.214 ± 0.002
5	0.222 ± 0.004	0.208 ± 0.004

* Average and standard deviation of five peak integration readings.

TABLE D-24

WET PRESSURE-DIGESTION/GRAPHITE FURNACE ATOMIC ABSORPTION TECHNIQUE

RECOVERY OF VANADIUM IN SPIKED FISH BASE SAMPLES

Series and No.	V in 5 ml* soln. (ppm)	Total V (ug/5 ml)	**V (ug/5 ml) corr. fish base	Actual V spike (ug)	Recovery of V (%)
B-1	0.159 ± 0.008	0.80 ± 0.04	$0.54^{6} \pm 0.04^{2}$	0.50 ± 0.02	$109.^{2} \pm 8.^{4}$
2	0.146 ± 0.004	0.73 ± 0.02	$0.47^{8} \pm 0.04^{2}$	0.50 ± 0.02	$95.^{6} \pm 8.^{4}$
3	0.155 ± 0.004	0.78 ± 0.02	$0.52^{7} \pm 0.04^{2}$	0.50 ± 0.02	$105.^{4} \pm 8.^{4}$
4	0.159 ± 0.004	0.80 ± 0.02	$0.54^{6} \pm 0.04^{2}$	0.50 ± 0.02	$109.^{2} \pm 8.^{4}$
5	0.146 ± 0.008	0.73 ± 0.04	$0.47^{7} \pm 0.04^{2}$	0.50 ± 0.02	$95.^{4} \pm 8.^{4}$
C-1	$0.24^{6} \pm 0.01^{2}$	1.23 ± 0.06	$0.97^{8} \pm 0.06^{0}$	1.00 ± 0.02	$97.^{8} \pm 6.^{0}$
2	0.263 ± 0.008	1.32 ± 0.04	$1.06^{7} \pm 0.04^{2}$	1.00 ± 0.02	$106.^{7} \pm 4.^{2}$
3	0.258 ± 0.008	1.29 ± 0.04	$1.03^{7} \pm 0.04^{2}$	1.00 ± 0.02	$103.^{7} \pm 4.^{2}$
4	0.263 ± 0.004	1.32 ± 0.02	$1.06^{7} \pm 0.04^{2}$	1.00 ± 0.02	$106.^{7} \pm 4.^{2}$
5	0.267 ± 0.004	1.34 ± 0.02	$1.08^{6} \pm 0.04^{2}$	1.00 ± 0.02	$108.^{6} \pm 4.^{2}$
D-1	$0.38^{8} \pm 0.01^{6}$	1.94 ± 0.08	$1.68^{8} \pm 0.08^{0}$	1.50 ± 0.03	$112.^{5} \pm 5.^{3}$
2	0.392 ± 0.004	1.96 ± 0.02	$1.70^{7} \pm 0.04^{2}$	1.50 ± 0.03	$113.^{8} \pm 2.^{8}$
3	0.396 ± 0.008	1.98 ± 0.04	$1.72^{7} \pm 0.04^{2}$	1.50 ± 0.03	$115.^{1} \pm 2.^{8}$
4	0.379 ± 0.008	1.90 ± 0.04	$1.64^{7} \pm 0.04^{2}$	1.50 ± 0.03	$109.^{8} \pm 2.^{8}$
5	$0.38^{8} \pm 0.01^{2}$	1.94 ± 0.06	$1.68^{7} \pm 0.06^{0}$	1.50 ± 0.03	$112.^{5} \pm 4.^{0}$
E-1	0.482 ± 0.004	2.41 ± 0.02	$2.15^{8} \pm 0.04^{2}$	2.00 ± 0.03	$107.^{9} \pm 2.^{1}$
2	0.482 ± 0.008	2.41 ± 0.04	$2.15^{5} \pm 0.04^{2}$	2.00 ± 0.03	$107.^{8} \pm 2.^{1}$
3	0.474 ± 0.008	2.37 ± 0.04	$2.11^{6} \pm 0.04^{2}$	2.00 ± 0.03	$105.^{8} \pm 2.^{1}$
4	0.487 ± 0.004	2.44 ± 0.02	$2.18^{7} \pm 0.04^{2}$	2.00 ± 0.03	$109.^{4} \pm 2.^{1}$
5	$0.47^{4} \pm 0.01^{6}$	2.37 ± 0.08	$2.11^{7} \pm 0.08^{0}$	2.00 ± 0.03	$105.^{8} \pm 4.^{0}$
F-1	$0.61^{6} \pm 0.01^{2}$	3.08 ± 0.06	$2.82^{7} \pm 0.06^{0}$	2.50 ± 0.03	$113.^{1} \pm 2.^{4}$
2	$0.59^{4} \pm 0.01^{2}$	2.97 ± 0.06	$2.71^{5} \pm 0.06^{0}$	2.50 ± 0.03	$108.^{6} \pm 2.^{4}$
3	$0.61^{6} \pm 0.01^{2}$	3.08 ± 0.06	$2.82^{7} \pm 0.06^{0}$	2.50 ± 0.03	$113.^{1} \pm 2.^{4}$
4	$0.56^{9} \pm 0.01^{2}$	2.84 ± 0.06	$2.58^{7} \pm 0.06^{0}$	2.50 ± 0.03	$103.^{5} \pm 2.^{4}$
5	0.603 ± 0.008	3.02 ± 0.04	$2.76^{7} \pm 0.04^{2}$	2.50 ± 0.03	$110.^{7} \pm 1.^{7}$
G-1	$0.89^{2} \pm 0.01^{6}$	4.46 ± 0.08	4.21 ± 0.08	4.00 ± 0.04	$105.^{3} \pm 2.^{0}$
2	$0.89^{6} \pm 0.01^{2}$	4.48 ± 0.06	$4.22^{7} \pm 0.06^{0}$	4.00 ± 0.04	$105.^{7} \pm 1.^{5}$
3	0.90 ± 0.02	4.5 ± 0.1	$4.2^{5} \pm 0.1^{0}$	4.00 ± 0.04	$106.^{2} \pm 2.^{5}$
4	$0.91^{8} \pm 0.01^{2}$	4.59 ± 0.06	$4.33^{7} \pm 0.06^{0}$	4.00 ± 0.04	$108.^{4} \pm 1.^{5}$
5	$0.89^{2} \pm 0.02^{0}$	$4.4^{6} \pm 0.1^{0}$	4.21 ± 0.1^{0}	4.00 ± 0.04	$105.^{2} \pm 2.^{5}$

* Using the linear equation from Page 326.

** Based on the higher of the two uncertainties:- fish base or total vanadium in 5 ml.

If the average for each of the Series B to G is taken with respect to the "Total V (ug/5 ml)" column data (Table D-24) we have:-

Series	B	$0.76^{8} \pm 0.03^{6}$
	C	1.30 ± 0.04
	D	$1.94^{4} \pm 0.03^{0}$
	E	2.40 ± 0.03
	F	$2.99^{8} \pm 0.09^{9}$
	G	$4.48^{6} \pm 0.06^{5}$

Linear regression analysis yields:- $r = 0.9992$, $b = 0.276$, $m = 1.0644$

Thus the zero intercept on the ordinate axis yields a value of 0.276 ug V/5 ml of solution or, for the sample weights approximating 1.00 g of dried fish base, 0.276 ug V/g dried fish base. Carrying out reverse calculations indicates an uncertainty for this value of $\pm 0.04^{3}$ ug, so that we have, from the method of additions, a calculated value for dried rainbow trout content of vanadium of $0.27^{6} \pm 0.04^{3}$ ug/g fish base. This compares, within the respective tolerance limits, with the value of $0.25^{3} \pm 0.04^{2}$ ug V/g dried fish base reported on the previous page.

Using the value of $0.27^{6} \pm 0.04^{3}$ ug V/g dried fish base as the correction value to the "Total V (ug/5 ml)" data column yields an average recovery percent for spikes up to 4.0 ug on 1 g dried fish base of $105.^{6} \pm 5.^{6}$. This value also agrees well, within the respective tolerance limits, with the value of $107.^{2} \pm 4.^{8}$ percent reported on the previous page.

By including the average result of Series A with the average for each of the Series B to G taken with respect to the "Total V (ug/5 ml)" data column in a graphical determination of vanadium in dried rainbow trout by the method of additions, the plot on Figure D-6 was obtained. The vanadium content in dried fish base tissue can be extrapolated to the abscissa to 0.24 ± 0.02 ug V/g dried fish base. The value of ± 0.02 represents the reading uncertainty on the graph. This extrapolated vanadium concentration compares, within the respective tolerance limits, with the value of $0.25^{3} \pm 0.04^{2}$ ug V/g dried fish base reported on the previous page.

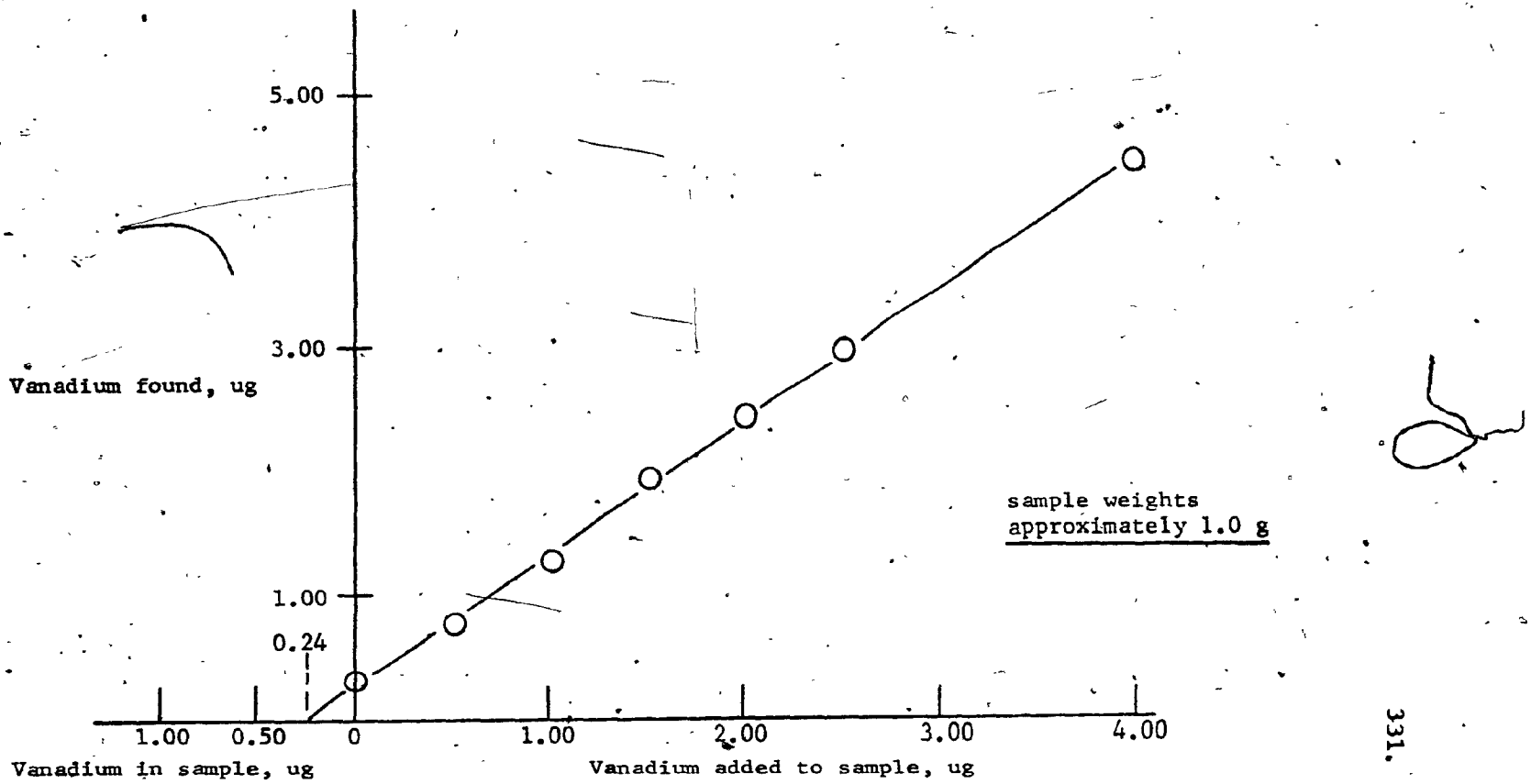


FIGURE D-6 GRAPHICAL METHOD OF ADDITIONS - VANADIUM DETERMINATION IN RAINBOW TROUT - WET PRESSURE-DIGESTION

4. Recovery of Vanadium from Aliquoted Spiked Fish Base Samples

Two sets of macerated and dried rainbow trout tissue were weighed out in duplicate. Each sample consisted of 1.0000 ± 0.0002 g and was placed in a Kimax 12-cm test tube.

One set (A) was spiked with 2.50 ± 0.03 ug of V each, while the second set (B) was spiked with 4.00 ± 0.04 ug of V each.

All samples were dried, digested and extracted according to the details of Sub-section 2.2.3 - (c). A blank was carried through simultaneously. The amount of HCl pipetted was 0.20 ± 0.01 ml while the amount of glass-distilled water pipetted was 4.80 ± 0.02 ml.

For each set, 2 ml of the final solution was tested as-is, and 3 ml was diluted with 6 ml of 2.5 percent HCl and then tested. The results were:-

Set	Ave. absorbance corr. for blank		Total V* (ug/5 ml)		V corr. for** base fish (ug/g)		Actual spike (ug)	Recovery (%)	
	as-is	aliq.	as-is	aliqx3	as-is	aliqx3		as-is	aliq.
A	0.140	0.045	2.99	2.84	2.74	2.59	2.50	110	104
B	0.206	0.071	4.42	4.52	4.17	4.27	4.00	104	107

* Using the linear equation from Page 326.

** Using the correction factor of 0.25^3 ug V/g dried rainbow trout base.

The recovery percent values agree within the expected tolerance limits for vanadium recovery on spiked samples, so that aliquoting a tissue sample solutions, suspected to contain vanadium in ppm beyond the linear range maximum, should present no difficulty.

APPENDIX E

List of Equipment and Materials Used for Experimentation

Atomic Absorption:- (Perkin-Elmer Corporation of Canada Limited)

- Atomic Absorption Spectrophotometer Model 503
- Graphite Furnace - HGA 2000
- Graphite Furnace - HGA 2100
- HGA Temperature Ramp Assembly
- Deuterium Arc Background Corrector
- Pyro-Coating Gas Control
- Automatic Gas Control Box
- Burner Heads - Flat single slot 10-cm air/acetylene burner with safety pin interlock
  - Flat single slot 5-cm nitrous oxide/acetylene burner with safety pin interlock
- Adjustable Flow Nebulizer
- Pre-mix Burner Assembly
- Hollow Cathode Lamps - copper lead
  - nickel cadmium
  - zinc vanadium
- Hollow Cathode Lamp Warm-up Assembly - capacity of up to 4 lamps
- Graphite Tubes - for HGA 2000 and pyrolizable tubes for HGA 2100
- Recorder Model 056
- Eppendorf Micropipette - capacity 20 ± 1 ul

General-use Equipment:-

- Mettler Balance - capacity 99.9999 ± 0.0002 g
- Hot Plates - Fisher Scientific Company of Canada
- Temperature Control Oven - Fisher Scientific Co. of Canada
- Furnace (max. temp. 1300 °C) from Lindberg Company Ltd.
- Standard Stock Solutions - (1000.0 ± 0.1 ppm)
  - copper
  - lead
  - nickel
  - cadmium
  - zinc
  - vanadium
- Gases - acetylene (Matheson Company of Canada)
  nitrous oxide (Matheson Company of Canada)
  Nitrogen (Linde - Union Carbide)
  P-10 (Linde - Union Carbide)

Glassware:-

Volumetric flasks - 100.00 ± 0.05 ml
250.00 ± 0.05 ml

Transfer pipettes - 5.000 ± 0.005 ml
10.00 ± 0.01 ml
20.00 ± 0.02 ml
25.00 ± 0.02 ml

Graduated pipettes - 1.000 ± 0.005 ml
2.00 ± 0.01 ml
5.00 ± 0.01 ml
10.00 ± 0.05 ml

Beakers - Pyrex 50-ml beakers

Graduated cylinders - 10 ml
50 ml
100 ml

- Kimax test tubes with polyethylene screw - cap (capacity 15 ml)
- 12-inch test tubes
- Polyethylene beakers - capacity 500 ml

Calculating Equipment:-

- Texas Instrument programmable calculators
  Models SR 56
  TI 59

Reagents:-

- Nitric, perchloric and hydrochloric acids (reagent grade) obtained from J. T. Baker Chemicals.
- Glass-distilled water obtained from Concordia University, Montreal, Quebec, Biochemistry Department.