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Heydorn, Kaj

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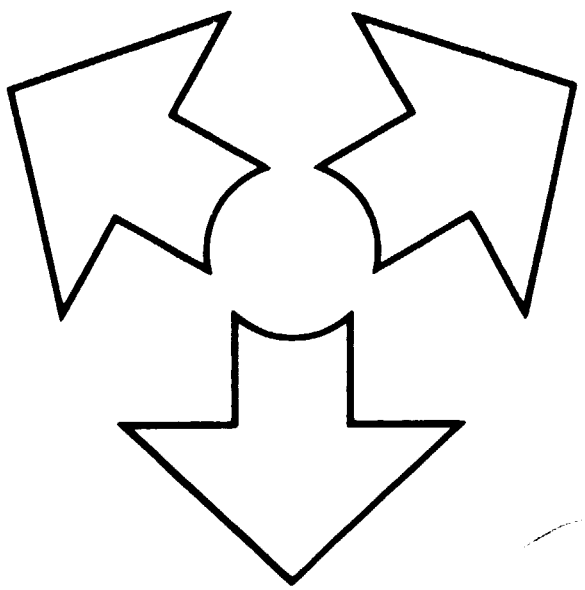
Aspects of Precision and Accuracy in Neutron Activation Analysis

Kaj Heydorn



Risø National Laboratory, DK-4000 Roskilde, Denmark
March 1980

Aspects of Precision and Accuracy in Neutron Activation Analysis

Aspects of Precision and Accuracy in Neutron Activation Analysis

Kaj Heydorn

Isotope Division
Risø National Laboratory

Roskilde, Denmark
July 1978

Denne afhandling er af Den polytekniske Læreanstalt, Danmarks tekniske Højskole, antaget til forsvar for den tekniske doktorgrad.

Lyngby, den 14. december 1979.

Peter Lawætz
Rektor

Paul Carpentier
Administrationschef

This Thesis has been accepted by the Technical University of Denmark for public defense in fulfilment of the requirements for the Degree of Doctor Technices.

Lyngby, December 14, 1979.

Peter Lawætz
President

Paul Carpentier
Secretary

Abstract

Analytical results without systematic errors and with accurately known random errors are normally distributed around their true values. Such results may be produced by means of Neutron Activation Analysis both with and without radiochemical separation.

When all sources of random variation are known a priori, their effect may be combined with the Poisson statistics characteristic of the counting process, and the standard deviation of a single analytical result may be estimated. The various steps of a complete neutron activation analytical procedure are therefore studied in detail with respect to determining their contribution to the overall variability of the final result.

Verification of the estimated standard deviation is carried out by demonstrating the absence of significant unknown random errors through analyzing, in replicate, samples covering the range of concentrations and matrices anticipated in actual use. Agreement between the estimated and the observed variability of replicate results is then tested by a simple statistic T based on the chi-square distribution. It is found that results from neutron activation analysis of biological samples can be brought into statistical control.

In routine application of methods in statistical control the same statistical test may be used for quality control when some of the actual samples are analyzed in duplicate. This Analysis of Precision serves to detect unknown or unexpected sources of variation of the analytical results, and both random and systematic errors have been discovered in practical trace element investigations in different areas of research.

Particularly, at the ultratrace level of concentration where there are few or no Standard Reference Materials for ascertaining the accuracy of results, the proposed quality control based on the Analysis of Precision combined with neutron activation analysis with radiochemical separation, with an a priori precision independent of the level of concentration, becomes a powerful tool for controlling accuracy too.

UDC 543.53 : 519.25

False facts are highly injurious to the progress of science for they often endure long; but false views if supported by some evidence, do little harm for everyone takes a salutary pleasure in proving their falseness.

Darwin

Preface

False data are probably no less common than they were 100 years ago, and methods for nullifying them are therefore still worth pursuing.

Analytical results for trace element concentrations are sometimes pejoratively referred to as random numbers [Tölg 1976]; but nothing is wrong with random numbers when they belong to a normal distribution with an accurately known standard deviation. In fact, information concerning the precision and accuracy of an analytical result marks the distinction between qualitative and quantitative analysis. However, this information is not always available, and if available it may not be reliable.

My interest in this subject was initiated while working in 1965-66 with Professor Vincent P. Guinn at General Atomic in San Diego, California, where I was engaged in neutron activation analysis, a field in which so many important contributions have been made by this laboratory.

After my return to Denmark I was fortunate enough to be able to continue work in activation analysis for medical research under the sponsorship of Professor Mogens Faber. With the support of Dr. C. F. Jacobsen, a small group was later established as part of the Risø Radioisotope Laboratory; recently, co-operation with several other scientific disciplines has been sponsored by the National Research Councils.

The present work is therefore largely based on results previously published as parts of other projects. However, a considerable number of additional analytical investigations are presented here, and the emphasis and treatment of the data differ in many respects, particularly from that of the earlier publications.

Reference is made to the most frequently quoted publications by Roman numerals [I - XI], but these papers do not in themselves form part of the dissertation.

The scientific literature is taken into account up to and including 1977, but no attempt has been made to make a complete bibliography of neutron activation analysis. Only papers with special relevance to the subject under discussion are included in the references, in particular representative or important papers on precision and accuracy that are often difficult to locate in a computer search.

In the preparation of the text the guidelines given by Vanderborght [Unisist 1976] have been combined with the formal instructions for a Risø Report [Steenbuch 1974]. The type-written text was processed by the Univac 1110 computer.

K. Heydorn
July 15, 1978

The printed version is identical to the original type-written version, except for purely editorial changes, including up-dating of literature references and the correction of misprints.

K. Heydorn
March 15, 1980

INIS-descriptors:
Accuracy
Errors
Neutron Activation Analysis
Quality Assurance

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Contents

Conversion of more conventional units to unfamiliar SI units

Concentration			
Flux of neutrons	10^{11} n/(cm^2 s)	=	1.660 nmol/(m^2 s)
Activity	1 mCi/ml	=	37 TBq/m^3
Mass	1 ng/ml	=	1 mg/m^3
Specific fraction			
Quantity	1 ppm	=	1 mg/kg
Activity	1 mCi/µg	=	37 PBq/kg
Dose	100 rad	=	1 Gy = 1 J/kg

1. Introduction

The two earliest references in a bibliographical survey of neutron activation analysis relate to works on the Action of Neutrons on the Rare Earth Elements by Georg de Hevesy and Hilde Levi when these authors were at the Institute for Theoretical Physics in Copenhagen. Instrumental neutron activation analysis (INAA) was here used for the determination of Dy in a sample of Y_2O_3 prepared by Auer von Welsbach [Hevesy 1936].

Four decades of neutron activation analysis

Not many publications in this field appeared during the *first decade* after the work of Hevesy and Levi, but the new concept and the corresponding principles of tracer methodology were combined with analytical technique, so that neutron activation analysis with radiochemical separation (RNAA), carrier addition and yield determination [Boyd 1949], could be utilized, subject to the availability of neutrons.

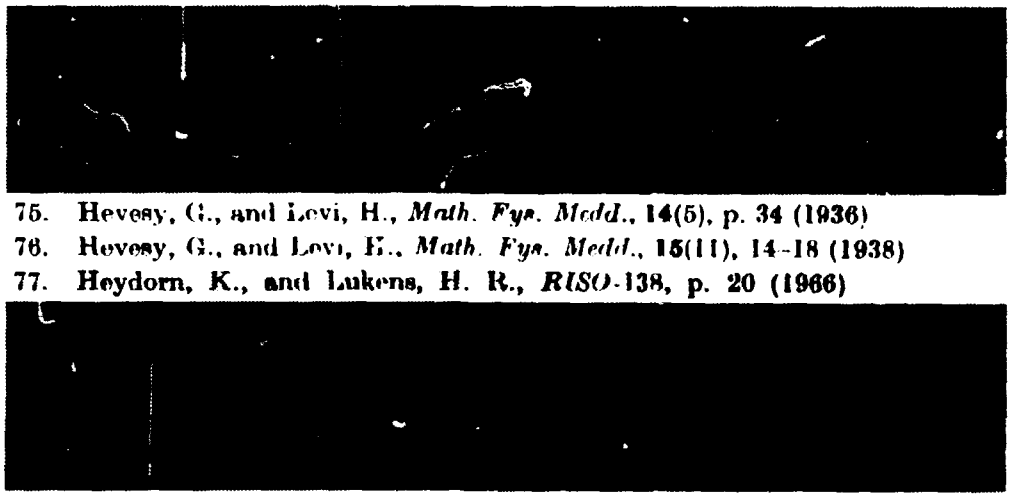
During the *next decade* access to nuclear reactors at a number of national laboratories prompted exploratory work in the trace element field, because the available neutron flux densities increased the sensitivity of the method by many orders of magnitude.

The thallium-activated sodium-iodide scintillation detector, with the Wilkinson analogue-to-digital converter [1949] for multichannel spectrometry, marked a *decade* of improved specificity and the beginning of multi-element determinations.

No other method of comparable sensitivity was available in this period, and the number of trace element determinations showed the typical exponential growth of a developing field [Braun 1977]. In the standard monograph on the subject [De Soete 1972] a bibliography lists 2716 references covering the first 3 decades, as illustrated by the triad reproduced in Fig. 1.

This was also the time of the electronic computer and of the lithium-drifted germanium detector, and both of these technical developments expanded the capability of INAA with respect to multi-element determinations, and reduced the need for radiochemical separation.

However, the most significant factor during the *last decade* of neutron activation analysis is probably the advent of alternative analytical methods with comparable sensitivity for many elements. This



75. Hevesy, G., and Levi, H., *Math. Fys. Medd.*, **14**(5), p. 34 (1936)
76. Hevesy, G., and Levi, H., *Math. Fys. Medd.*, **15**(11), 14-18 (1938)
77. Heydorn, K., and Lukens, H. R., *RISO*-138, p. 20 (1966)

Fig. 1. Excerpt of bibliography covering the first 3 decades of Neutron Activation Analysis [De Soete 1972].

has stimulated interest in the precision and accuracy of analytical results at the trace level, particularly in the field of neutron activation analysis.

Thus, the monograph on Neutron Activation Analysis [De Soete 1972] of 836 pages devotes two chapters of 122 pages to Precision and Accuracy, but the Handbuch der Spurenanalyse [Koch 1974] makes do with 17 pages out of a total of 1597 pages.

1.1. Exposition of the Subject

Strangely enough, neutron activation analysis is not usually discussed together with other analytical metods [Fahr 1976]; in the book Trace Analysis no mention is made of this technique in the comparison of 15 different analytical methods [Winefordner 1976], and the Handbuch der Spurenanalyse only describes the principles of activation analysis in general terms.

It may therefore be appropriate to recall some of the *fundamental characteristics* of neutron activation analysis (NAA) which make it differ from other analytical methods.

The high **sensitivity** for a large number of elements that can be achieved by means of moderate neutron flux densities, classifies NAA as a method for trace analysis - although the determination of major elements [V] is by no means excluded. For trace analysis, the most important quality is the absence of **blank values** from reagents added after the end of irradiation, but also the possibility of determining the **chemical yield** of the radiochemical separation by the addition of carrier is an important asset.

Results are independent of the **chemical form** of an element, but the use of comparator standards automatically assumes an identical isotopic composition of sample element and comparator, which is usually, but not always, true. In some cases abnormal **isotopic composition** is found and has been determined [III] in radionuclide preparations.

It is the purpose of the present work to show that neutron activation analysis has the additional advantage that the precision of a single analytical result can be estimated accurately; it is also intended to demonstrate the usefulness of this feature in analytical quality control at trace levels of concentration.

The reported *experimental work* is concentrated on the application of neutron activation analysis to the determination of trace elements in biological materials. Chemical separation is required either before or after irradiation, but it has been limited to the extent needed for satisfactory instrumental determination of the element sought.

Additional work was carried out on some environmental materials without chemical separation, but only for single elements.

Selection of elements

We have made no attempts to carry out real multi-element analysis of biological or other samples, but simultaneous determination of As, Se, Mn, and Cu in one sample is performed on a routine basis. These elements were chosen to represent not only a wide range of analytical problems, but also a spectrum of biological functions in man, who is the main subject of our studies.

Nuclear characteristics for these 4 elements are given in Table 1 together with data for elements with short-lived indicators, which do not lend themselves readily to radiochemical separation but which we determined instrumentally in inorganic samples.

The choice of elements having indicators with relatively short half-lives generally reduces the risk of cross-contaminating the samples and prevents the building up of an unacceptable level of contamination in the laboratory. In our particular laboratory where Curie quantities of a number of radioisotopes are handled for production purposes, such contamination risks are very serious for radionuclides with half-lives exceeding a few days.

At the same time the very low levels of some trace elements in biological tissue, particularly in human blood, can only be determined for elements with satisfactory sensitivity. However, the sensitivity for an element in NAA depends on so many factors that a single evaluation may become meaningless. Differences in neutron flux density, counting geometry, etc., may be eliminated by calculation of the relative sensitivity, and in Table 1 sodium is chosen as a reference because it is

Table 1

Nuclear characteristics of elements determined in this work

Target material			Product of irradiation			Sensitivity relative to Na		
Element	Isotope	Abundance	Isotope	Half-life	γ-energy	Meinke (1)	Yule (2)	Heydorn (3)
As	^{75}As	100 %	^{76}As	26.3 h	559 keV	1.5	1.9	0.3
Cu	^{63}Cu	69.09	^{64}Cu	12.7 h	511 keV	0.6	2.9	0.1
Mn	^{55}Mn	100 %	^{56}Mn	2.58 h	847 keV	25	80	5.0
Se	^{80}Se	49.82	^{81m}Se	57.3 min	103 keV	0.4	0.1	-
I	^{127}I	100 %	^{128}I	25.0 min	443 keV	5.6	35	-
V	^{51}V	99.76	^{52}V	3.76 min	1434 keV	2	7.6	1.5
Se	^{76}Se	9.02	^{77m}Se	17.5 s	162 keV	-	0.2	0.1
Li	^{7}Li	92.58	^{8}Li	0.84 s	β only	0.5	-	-
Na	^{23}Na	100 %	^{24}Na	15.0 h	1369 keV	1.00	1.00	1.00

Irradiation time (1) 10 hours
(2) 1 hour
(3) 1 half-life

Type of detector (1) Geiger-Müller counter
(2) Scintillation detector
(3) Ionization chamber

the dominating activity in neutron-irradiated tissue for several days after the irradiation.

It is seen that more or less arbitrary choices of irradiation strategy and detection method strongly affect the actual figures quoted for relative sensitivity. Meinke [1959] relies on the counting of β-particles immediately after a relatively long irradiation period, a situation characteristic of complete radiochemical separation and the goal of highest possible sensitivity. Yule [1965] uses a 1 hour irradiation followed by measurement of γ-rays with a NaI(Tl) scintillation detector, closely corresponding to the approach used in the present investigation of biological materials. Heydorn [1972] irradiates for one half-life and measures total ionization by hard γ-rays in an ionization chamber, which is a method only useful for the determination of major elements, such as the recovery of added carriers in a radiochemical separation.

The qualitative agreement is, however, reasonable enough to divide the elements into groups:

I	Mn,V	high sensitivity
II	As, Cu	medium sensitivity
III	Se	low sensitivity

while Li and I strongly depend upon the type of detector.

For the first group, only modest chemical separation is needed, and both Mn and V may be determined instrumentally in some samples. The second group requires an actual radiochemical separation, but the radiochemical purity of the separated sample need not be very high. Selenium requires an almost radiochemically pure sample for counting, whereas Li and I may be determined instrumentally in some samples using the right detectors.

The greatest problems in chemical separation are expected for Se and V because of their short half-lives, and this has also been found in actual practice.

Vanadium. Little is known about the possible role of vanadium in human or animal metabolism and about its distribution in the human body. Vanadium counteracts the stimulation of cholesterol synthesis induced by manganese; essentiality was demonstrated by Schwarz in 1971.

Vanadium is found as a porphyrin complex in many crude oils [Milner 1952] and enters the atmosphere from oil-fired power stations; it is associated with the smallest particles of the aerosol (≤ 0.5 μm), which can be deposited in the lungs on inhalation. The toxicity of vanadium is low.

Both tissue samples and air filter samples were included in the present study, and the most important interfering activity was ^{28}Al, which decays with a 2.3 min half-life. Separation of V from Al was carried out either by extraction with 8-hydroxyquinoline in chloroform in

the presence of aluminon [I], or with 8-hydroxyquinaldine in chloroform [Damsgaard et al. 1972].

With a detection limit of 7 ng, the method is barely sensitive enough for the determination of V in tissue samples of 1 g or less, but no other suitable methods seem to be available.

Manganese is an essential element, and a metallo-enzyme, pyruvate carboxylase, is known to contain 4 atoms of Mn per mole of protein. Manganese is not excreted through the kidneys, and its metabolism is therefore unaffected by uraemia; deficiency has been observed in man in a unique case of serendipity [Doisy 1973].

Manganese is found in plant materials, and tea leaves in particular contain high concentrations, up to 0.1 %; devoted tea drinkers may thus ingest a major fraction of a normal total body content of manganese each day. Fortunately, Mn is one of the least toxic of the trace elements, and excess intake only results in elevated concentrations in the liver, but not in any ill effects.

The range of concentrations in biological material spans more than 3 orders of magnitude, being maintained in man by a very effective homeostatic control. For tissue samples, removal of interfering ^{24}Na by hydrated antimony pentoxide is sufficient for the determination of Mn, and this procedure is part of the method for the analysis of biological materials [VII] illustrated in Fig. 2.

For serum or plasma samples, however, a greatly increased sensitivity is needed, and this was brought about by the extraction of Mn into chloroform with diethylammonium diethyldithiocarbamate [IX], shown in Fig. 3 as an overlay to Fig. 2.

The absolute detection limit for Mn is lower in neutron activation analysis than in any other analytical technique discussed by Morrison [1965]. Flameless atomic absorption has an entirely adequate sensitivity, even for serum samples, but contamination problems seem to be very difficult to overcome. With 100 % isotopic abundance Mn cannot be determined by isotope dilution mass spectrometry.

Copper. The essentiality of copper has been assumed for more than a century, and to-day many different copper-containing proteins have been identified. Together with iron, Cu is present in vital enzyme systems such as cytochrome oxidase, lysyl oxidase, etc.; in normal serum about 90 % of the copper is present as ceruloplasmin with 8 atoms of Cu per mole of protein. Like manganese, Cu is not excreted through the kidneys, and concentrations in the liver vary with intake; deficiency has never been observed in man.

Concentrations of Cu in serum are normally very stable, and deviations only occur under abnormal, clinical conditions. High serum copper levels are observed during pregnancy, but also after the intake of birth control pills. Low levels of Cu in serum may be connected with an

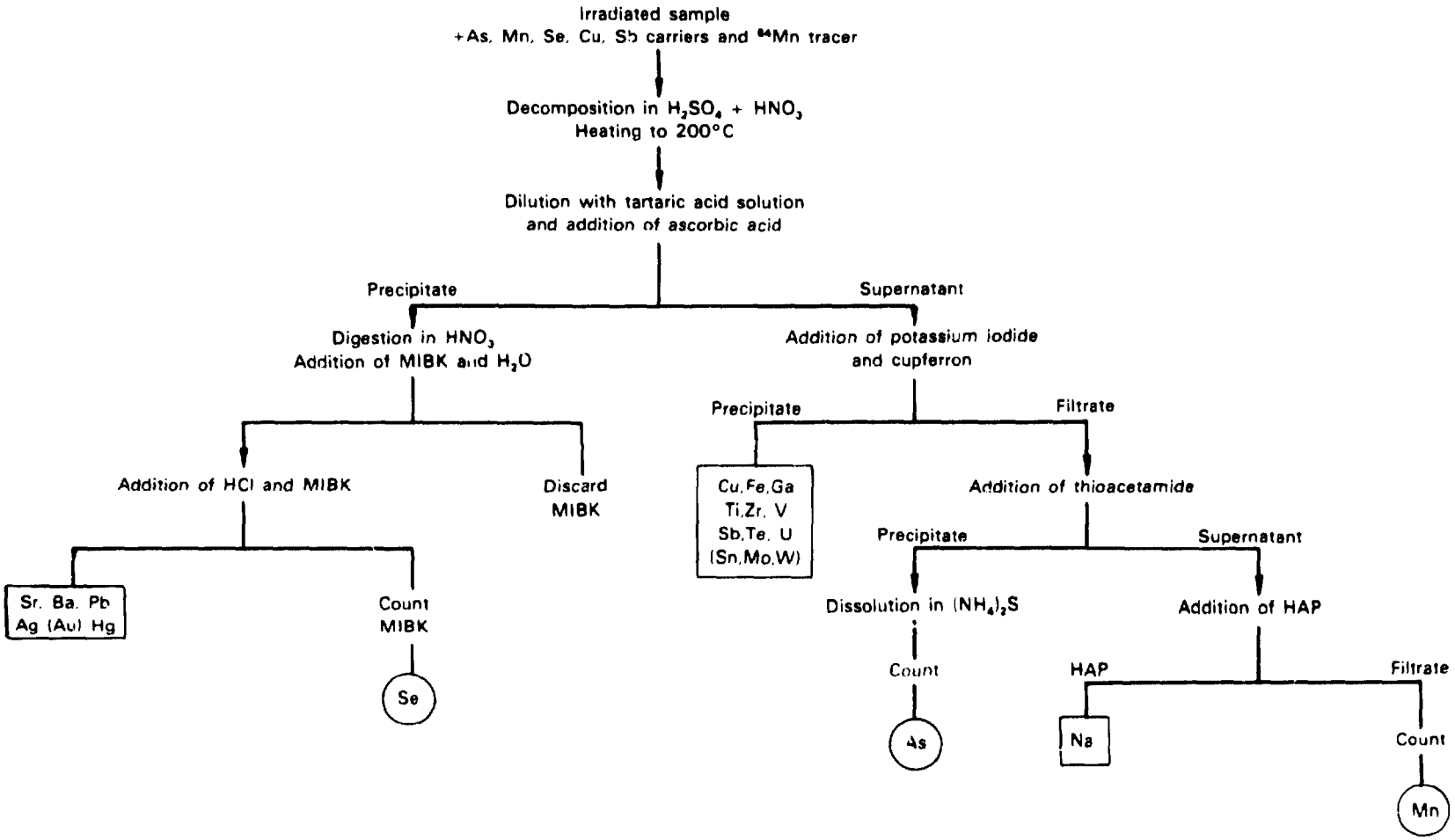


Fig. 2. Radiochemical separation scheme for arsenic, manganese, and selenium [VII].

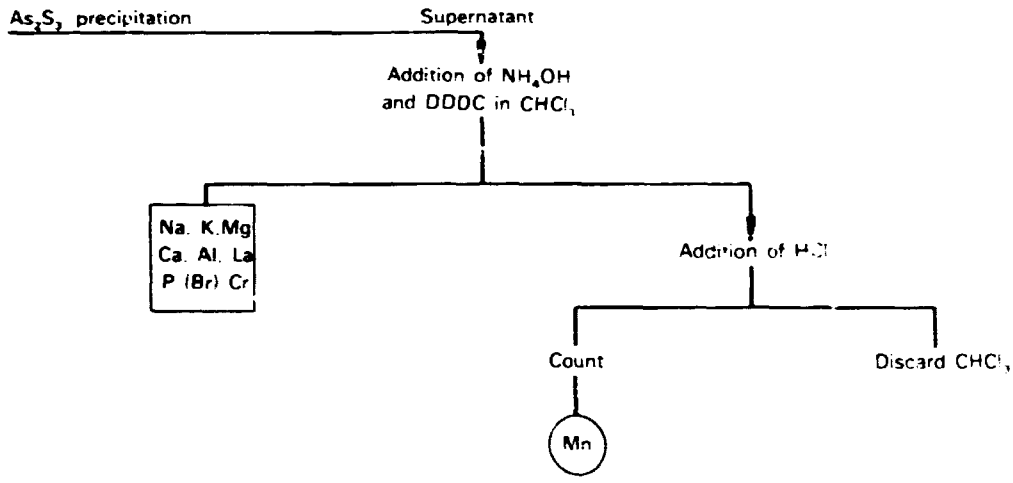


Fig. 3. Radiochemical separation of manganese [IX].

inborne defect in metabolism, such as Wilson's disease with a hereditary deficiency of ceruloplasmin, or Menkes' disease, which is currently being studied in many countries.

The determination of Cu by counting of the 511 keV annihilation peak is subject to interference from all radionuclides with high-energy γ-rays (>1.02 MeV), which may give rise to electron-positron pair formation. The worst offender is ^{24}Na, but also other elements must be considered, and several steps are therefore needed to ascertain the radiochemical purity of the sample for counting of ^{64}Cu. In Fig. 2 copper is separated from other elements as cuprous iodide in the precipitate resulting from the addition of potassium iodide and cupferron. Additional separation is carried out as shown in Fig. 4 by dissolution in NH_4OH and precipitation as the sulphide [Heydorn et al. 1976].

At the levels of Cu encountered in biological material, atomic absorption is a possible alternative to NAA. Simultaneous determination of other trace elements, as well as the absence of blank errors, made NAA slightly more favourable in our investigation of the distribution of Cu in the organs of normal and Menkes' foetuses.

Arsenic has been known as a toxic element for more than a thousand years, and its acute toxicity exceeds that of all the so-called heavy elements. Arsenic does not accumulate in the body, but is excreted partly via the kidneys or to the hair, skin or nails in the form of tricysteinyl arsine. Arsenic is generally assumed to be non-essential.

Chronic arsenic intake has been associated with many different forms of cancer, as well as with an endemic disease called Blackfoot disease. Arsenic affects the metabolism of selenium compounds, and high tissue concentrations of arsenic have been found in uraemic patients.

Table 2

Biological characteristics of trace elements selected

Element	Essentiality reported	Identified biological entity	Organ with highest concentration	Body content mg	Associated metabolic disorders
Cu	1928	Lysyloxidase	Eye	72	Menkes' Syndrome Wilson's Disease
Mn	1931	Pyruvate carboxylase	Liver	~ 12	Neonatal Ataxia
Se	1957	Glutathione peroxidase	Kidney	> 13	White Muscle Disease
V	1971	Inhibitor of cholesterol formation	Lung	unknown	Cardiovascular Disease
As	-	Tricysteinyl arsine	Hair	variable	Blackfoot Disease Cancer

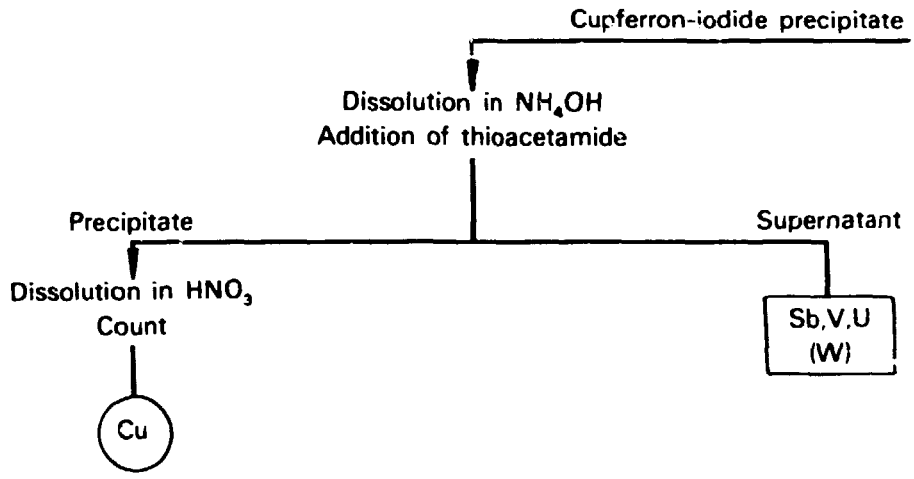


Fig. 4. Radiochemical separation of copper [Heydorn et al. 1976].

Radiochemical separation of As is carried out after scavenging with cupferron by precipitation with thioacetamide, as shown in Fig. 2. The main interfering elements are Sb and Br, but the presence of much higher concentrations of Cu in biological material must be taken into account. Methods based on distillation or solvent extraction may not always give satisfactory separation from Br.

Average concentrations of As in tissue are at the ultratrace level, less than 10 µg/kg, where the credibility of non-activation methods is very poor. Both flameless atomic absorption and X-ray fluorescense have sensitivities at the ng level, but contamination problems as well as the possibility of incomplete recovery reduce confidence in the results reported. With the 100 % isotopic abundance of ^{75}As, isotope dilution mass spectrometry is no alternative.

Selenium is accumulated from the soil in woody aster (Astragalus) and a few other seleniferous plants up to a dry-weight concentration of 1 %, and the toxicity of these plants to grazing animals was noted already by Marco Polo. It was not until 1957 that Klaus Schwarz and his co-workers proved that Se is an essential element, and deficiency symptoms in domestic animals were identified.

Selenium is the most toxic essential element with a chronic toxic level of 5 mg/kg; simultaneous ingestion of As in drinking water at 5 mg/l, however, completely prevents selenosis.

Glutathione peroxidase was identified as a seleno-enzym in 1973, but both the action of Se in normal metabolism and its interaction with other elements at toxic levels need further study. Neither chronic toxicity nor deficiency has been unequivocally identified in man. One metabolic product, dimethylselenide, is eliminated through the lungs, the major fraction of dietary Se is excreted through the kidneys.

Irradiation of selenium with thermal neutrons gives rise to 5 different radionuclides with half-lives ranging from 17.5 seconds to 120 days. The need for radiochemical separation and the desire to avoid long-lived activities led to our choice of ^{81m}Se as indicator. Previous attempts to use ^{81m}Se for biological materials [Strain 1969] were not successful, but the separation method presented in Fig. 2 proved satisfactory: Se is precipitated as the element, dissolved in HNO_3 and extracted into methyl isobutyl ketone after the addition of HCl.

Tissue concentrations of Se, as well as serum values, are usually about 0.1 mg/kg or higher and do not reach the ultratrace level. The highest sensitivities are therefore not needed, and methods based on fluorometry are quite acceptable; blank values have, however, been reported to be somewhat variable [Schroeder 1970].

Although both *iodine* and *lithium* have considerable biological interest, their inclusion in the present study is strictly based on analytical considerations.

In the first case, the problem was to determine the ratio between radioactive ^{125}I and table ^{127}I in reagents for protein iodination without regard to chemical state. Both nuclides could be determined by INAA after decay of the ^{126}I impurity; other conceivable methods were found to suffer from possible systematic errors connected with the unknown chemical state of the iodine.

The second problem was to determine element ratios for the series of alkaline elements in samples of geochemical interest. Here, the elements Na, K, Rb, and Cs can usually be readily determined by INAA, but the lightest element Li requires dissolution of the rock material with subsequent chemical separation. The number of Li results available even on a global scale has been very modest.

We found that Li could in fact also be determined by INAA when a Čerenkov counter was used in connection with a fast transfer system from irradiation to counting positions.

Outline of work

In the present work all these investigations which have been previously published in some form or other - will be reviewed with special reference to the precision and accuracy of the results. Altogether 11 papers that deal with particularly important aspects of precision and accuracy have been selected for more detailed treatment. They are presented first in the list of references, and Roman numbers I - XI are used for text reference.

Experimental results already published are only repeated here when necessary for a fuller understanding. Much additional, previously unpublished work is included in the present work, and this is described

in sufficient detail for future reference. The author realizes that this may give the presentation a certain unevenness, and therefore a relatively stringent *disposition* has been imposed on the subject in order to retain clarity.

Such clarity, however, is only possible when precise terms are used in the description of these matters. The terminology in this field is neither complete nor sufficiently accurate, and the last part of the present chapter is therefore devoted to a specification of the exact meaning of a number of terms needed in the subsequent chapters.

The main body of the present work begins in chapter 2 with an attempt to *classify* the various systematic and random errors affecting the results of neutron activation analysis. Chapter 3 describes how the precision of an analytical method is established and kept in statistical *control*. In chapter 4 methods to *verify* the accuracy of analytical results are discussed, and methods for the detection of unexpected systematic errors are presented.

> The principal THESIS to be defended is that it is possible to deduce the uncertainty of a single analytical result from first principles.
> The ANTITHESIS states that experience with actual analytical results seems to indicate that there are many exceptions to the preceding postulate.
> The SYNTHESIS concludes that at least in Neutron Activation Analysis it is possible to maintain the THESIS by taking into consideration all recognized sources of uncertainty, random as well as systematic.

Far from merely being an academic exercise, this principle is shown to form the basis for quality control with real samples, and in chapter 5 it is used to test the *reliability* of actual results at the ultratrace level.

1.2. Definition of Terms

A set of measurements whose reliability is unknown is worthless; worse, it may be dangerous [Eisenhart 1952]. The way in which to express this reliability in the quantitative manner needed for a critical evaluation of reported results, and without the ambiguity in the interpretation so often encountered, is discussed in recommendations from the National Bureau of Standards [Eisenhart 1968], and from the National Physical Laboratory [Campion 1973].

Although both sets of recommendations are intended primarily for application in the experimental physical sciences, the fundamental concepts are certainly also applicable to analytical chemistry.

The ultimate purpose of analytical chemistry, however, is to make meaningful analytical measurements on real, practical samples [Meinke 1973]; unlike physical measurements, analytical measurements often cannot be repeated exactly, and in practice a large number of replicate measurements is not possible. Information on the reliability of an analytical result must therefore be based on *a priori* knowledge.

The value of an analytical result is strongly dependent on its precision, while its validity is determined by its accuracy. A knowledge of both is required before reliability can be judged.

The relative importance of precision and accuracy depends upon the specific purpose of an investigation. From a fundamental, scientific point of view, no effort should be spared to eliminate all systematic errors, because only then can the analytical results be compared with and supplement the existing knowledge in general. The weight of the contribution to the field is determined by the total number and the overall precision of individual results.

The absolute weight, ω, of an analytical result has therefore been proposed [VIII] as a quantitative measure of its precision,

$$\omega = \frac{1}{\sigma^2} \tag{1}$$

where σ is the standard deviation of the individual result.

This has the advantage of matching the qualitative and quantitative interpretation of the term "high precision". In addition, the quantity ω is additive, so that the precision of the best mean, μ, of n independent results, becomes

$$\omega_\mu = \sum_{i}^{n} \omega_i \tag{2}$$

and the average precision of these results is

$$\bar{\omega} = \frac{\sum_{i}^{n} \omega_i}{n} \tag{3}$$

Precision and accuracy

Neither the present author's definition nor any other quantitative definition of *precision* has won general acceptance, and in order to reduce ambiguity the term precision will be considered to mean a quality rather than a quantity. Thus, precision may be excellent, good, improved, mediocre, inferior, poor, lousy - but never high or low, increased or reduced.

Under all circumstances, precision is meaningless without very detailed specification in each case, and a general definition is therefore not absolutely necessary. In this work the term is used exclusively in connection with *random* errors of analytical results.

Random errors are normally distributed with a mean value of 0 and characterized by a *standard deviation* σ used as a quantitative expression of precision - or imprecision [Eisenhart 1968].

Results are presented as $y \pm \hat{\sigma}$, where $\hat{\sigma}$ is the best - *a priori* estimate of the standard deviation of the distribution of which y is a member. The ratio $\hat{\sigma}/y$, sometimes multiplied by 100 and expressed as a percentage, is called the *relative standard deviation.*

A number n of independent, replicate measurements is called a *set,* and the reproducibility within a set represents the experimentally determined precision. The calculated - *a posteriori* - standard deviation s has n-1 degrees of freedom, and the variance ratio

$$(n-1)\frac{s^2}{\sigma^2} \quad \text{follows a} \quad \chi^2 \quad \text{distribution}$$

when the method is in *statistical control.*

When this is the case, eqs. (1)-(3) may be used to calculate σ_m, the *standard error of the mean* (SEM)

$$\sigma_m = \frac{\sigma}{\sqrt{n}} \tag{4}$$

as well as the *average standard deviation* $\bar{\sigma}$

$$\bar{\sigma}^2 = \frac{1}{\bar{\omega}} \tag{5}$$

In the final presentation of results there is no need to distinguish between the expressions

$$\bar{y} \pm \sigma_m \quad \text{and} \quad y \pm \hat{\sigma}$$

which have exactly the same meaning.

These definitions are consistent with the terms recommended by the editors of ANALYTICAL CHEMISTRY [1975].

Use of the word *accuracy* involves some of the same problems as connected with precision, and the relationship between the two concepts has not always been clearly defined. Here, accuracy always refers to non-random, *systematic errors,* but no quantitative definition of its magnitude is required.

Systematic errors are not reduced by increasing the number of replicate measurements, rather their effect or *bias* is measured as the signed difference between the limiting mean and the true value.

A special case is when the true value is 0; here a positive bias is usually referred to as a *blank value*.

Systematic, proportional errors, measured by the ratio of the limiting mean to the true value, are sometimes referred to as *calibration* errors.

These concepts are consistent with the terms recommended by Eisenhart [1952].

Analytical terminology

Analytical methodology deals with the principles underlying the development of analytical methods.

Analytical method is here defined as the set of written instructions completely defining the procedures to be adopted by the analyst in order to obtain the required analytical result [Wilson 1970].

Analytical procedures are carried out by proper application of suitable analytical techniques.

The *determinand* is the chemical species to be determined [Wilson 1965].

The *analytical result* is the value obtained for the quantity of the determinand by the corresponding analytical method [Wilson 1970].

The reliability of analytical results depends on the quality of the analytical method, which is described by its performance characteristics [Wilson 1970].

The *performance characteristics* of an analytical method used under a given set of experimental conditions are a set of quantitative and experimentally determined values for parameters of fundamental importance in assessing the suitability of the method for any given purpose.

Such performance characteristics include information on the useful *range* of the method, the effect of *interfering substances*, and the *selectivity* of the metod.

Other performance characteristics include a *calibration function* [Wilson 1974] and its first derivative, the *sensitivity* of the method [Kaiser 1972].

The *precision* of an analytical method is defined [VIII] as a set of quantitative instructions for the estimation of the standard deviation of a single analytical result.

The preceding definitions are consistent with the work of A.L. Wilson [1970 - 1974].

The *analytical sample* represents the composition of the material to be analyzed not only with respect to

	Major elements	>0.01 %
but also to	*Trace elements*	<0.01 %
and	*Ultratrace elements*	<0.01 mg/kg.

The process of *sampling* determines the validity of the results, and together with the possible lack of homogeneity of the sample material it contributes to their *variability*.

The *Analysis of Precision* [VIII] separates this contribution from known sources of variation pertaining to the analytical method.

Attempts to judge the *reliability* of analytical methods have been made from time to time [Eckschlager 1974] by combining random and systematic errors into a *total error*. Such a combination is deprecated by Campion [1973], and a much better approach is to eliminate or reduce systematic uncertainties by randomization.

In neutron activation analysis, both instrumental *(INAA)* and radiochemical *(RNAA)*, the reliability is limited by the *uncertainty* of the identity of the *specific activities* of the *indicator* isotope in the sample and the *comparator standard*.

By randomization this effect may be included in the *a priori* precision of the analytical method, and therefore it does not invalidate the conclusions based on the Analysis of Precision.

Symbols and units

In accordance with Regulation No. 320 of June 21, 1977, issued by the Danish Ministry of Commerce,[1] SI units are used throughout the text. The conversion factors of some of the conventional units to unfamiliar SI equivalents are given just after the list of contents. Traditional units are included only when they are essential for proper understanding.

Serious efforts have been made to use symbols consistent with international recommendations [Lowe 1975] and to avoid using the same symbol for different concepts; in some cases, however, the introduction of unfamiliar symbols to eliminate duplication was found to increase rather than reduce confusion. Such symbols were left unchanged, but are defined every time they appear in the text.

A number of the symbols and variables used throughout the text are explained in a list given in the Appendix.

1. Council Directive 76/770/EEC

2. Factors Affecting Precision and Accuracy

Generally speaking, Neutron Activation Analysis (NAA) is less sensitive to a number of errors than most other analytical methods, a fact that has probably led to neglect of some factors that are specific to NAA. Furthermore, it must be realised that a majority of the trivial errors affecting the precision and accuracy of classical, analytical results also have some influence on NAA.

On the one hand, it may be assumed that *all* factors affect precision and accuracy, although to varying degrees. On the other hand, many scientists have considered neutron activation analysis to be a kind of panacea that automatically assures precision and accuracy, regardless of the sloppiness with which the method is applied.

The truth is probably closer to the first assumption than to the second, although in neutron activation analysis the effect of some crucial factors may indeed be reduced to insignificance by careful planning. However, at the same time other factors previously disregarded, may assume prime importance.

Types of error

Several types of error may be identified and will be described separately, because different methods are needed to bring them under control.

The precision and accuracy of an analytical result, produced by applying a particular analytical method to an actual sample, represent the combined effect of imperfections in the analytical procedure, and the influence of conditions not specified in the analytical method. The two contributions may be referred to as inherent errors and circumstantial errors.

Inherent errors are estimated from the performance characteristics of the analytical method, which serve to ascertain the applicability of the *method* to the solution of a specific, analytical problem. Performance characteristics are intrinsic properties of the analytical method and do not depend on external conditions.

Circumstantial errors include all other sources of error and are required to evaluate the precision and accuracy of *results*. They must be determined separately for each set of circumstances under which actual samples are analyzed.

Typical errors in this category are connected with the quality and stability of electronic and other equipment needed to carry out the analysis. Such equipment may be replaced or exchanged without modifying the analytical method, and its influence on the reliability of the results is therefore circumstantial.

Specifically, in thermal neutron activation analysis, thermal neutrons are inherent, whereas fast neutrons are circumstantial. Interference from other elements activated by thermal neutrons must therefore be included in the performance characteristics of the method. The influence of interfering nuclear reactions caused by fast neutrons on the accuracy of the results, however, must be determined separately for each irradiation facility.

In general, the evaluation of circumstantial errors is postponed to the discussion of the precision and accuracy of the actual results presented in the subsequent two chapters.

Inherent errors

Inherent errors may give rise to random as well as non-random variation in analytical results, both will be discussed in the present chapter.

It is impossible, and fortunately not necessary, to study all conceivable *random errors*. Only those giving a significant contribution to the *a priori* precision of results have to be investigated.

If a significant random error has been neglected, the Analysis of Precision will detect the resulting lack of statistical control.

Systematic errors, however, or biases may not be detected by the Analysis of Precision, and therefore all possible errors of this type must be investigated very carefully by whatever appropriate means.

Once identified, the best way to deal with such errors is to randomize their effect on the results. In this way changes in their effect will be detected by the Analysis of Precision, and erroneous corrections for the effect are avoided.

By proper experimental design, most systematic errors can be randomized and thereby brought under statistical control.

Systematic errors affect all samples of a certain type, and a correction is therefore in principle possible; gross errors or mistakes affect only individual samples and must be treated differently. No correction is possible, and efforts have to be made to eliminate their occurrence.

Organization

Although straightforward in principle, NAA is made up of a number of separate *subsets*, each important for the precision and accuracy of the result, but to some extent beyond the control of the analyst.

Independent means of verifying the correct procedure are therefore of particular importance.

A typical example is the common lack of control of the orientation of samples during irradiation, such as will be discussed later.

In fact, each of the processes discussed here often involves different members of staff, not all of whom are supervised by the analyst. Precise distinction between the following eight sections is not possible in all cases, but, taken together, they include all factors of importance for estimating the *a priori* precision for an analytical method in general and for NAA in particular.

Methodology

The headings under which these factors are described are chosen so that the processes are logically simple to separate; in fact they may actually be carried out at different times, in different places, and by different persons.

For biological and environmental samples, the process of *sampling* is always separated from the analysis, and its importance has not always been realized. *Conditioning* is usually carried out in the laboratory, whereas *irradiation* is often carried out at a reactor site, which may be far from the laboratory.

Separation is again carried out in the laboratory, and the same is usually true for the *measurement* of the separated samples; however, very often different staff are responsible for the two operations. *Calculation* of the results may often be carried out by an outside computer installation by means of programs developed by other institutions.

Choice of comparator and *yield correction* are also discussed in separate sections, because both are key factors in the overall combination of both random and systematic errors in the final results.

Each section starts with some general considerations, listing the anticipated, inherent errors together with a discussion of their effect, based on Risø's own experimental work, as well as on literature studies.

The selection of conditions for the present study from these general, as well as from practical considerations, is described, and the resulting contribution to the *a priori* precision of the analytical method is estimated. An evaluation of possible improvements is made in order to identify the limiting precision and accuracy of the individual processes.

2.1. Sampling

The technique of sampling is not traditionally included in analytical methodology, although Thiers [1957] realised that:

Unless the complete history of a sample is known with certainty, the analyst should not spend his time analyzing it.

No great problems were originally thought to be involved in sampling, and even at the National Bureau of Standards [Meinke 1967] trace characterization was carried out with little attention to sampling problems. More recently [Anders 1977], an entire plenary discussion was devoted to sampling problems at the International Conference on Modern Trends in Activation Analysis held in Munich in 1976.

The problems are strongly dependent upon the type of material to be sampled, but for biological and environmental samples, with which we are mainly concerned, the complete history of a sample must include a detailed description of the sampling procedure followed.

This description should be available in advance because the process of sampling is a crucial factor in the final interpretation of the analytical results. A posteriori descriptions are usually inaccurate and always incomplete because what proved to be important observations were not made, and the necessary precautions were only taken casually - or perhaps entirely neglected.

No sampling method is universal, and the design of a specific sampling procedure requires the consideration of a number of points that affect the validity, the stability, and the quality of the sample, in order to ascertain the adequacy of the sample for the particular investigation.

Inappropriate sampling is probably responsible for more erroneous data than any other single factor in trace element neutron activation analysis of biological materials.

Quality

The quality of a sample refers to the degree to which it retains its original properties after removal from its original environment.

Not all properties can be retained simultaneously, and the quality of a sample is therefore closely linked to the particular investigations for which it is required. One important example is blood samples, which can only be prevented from clotting by the addition of an anticoagulant, and in many cases heparine is added in advance to the sampling tubes. Such heparinized blood is well suited for a great number of clinical investigations, but completely useless for the determination of trace elements [Bethard 1964].

Other anticoagulants may be used for selected trace elements, and 0.5 ml of 3.8 % sodium citrate solution added to about 9 ml of blood was used for the samples from Taiwan described in [IV]. Different preparations of sodium citrate solution used in this project were analyzed for arsenic and, with one remarkable exception, they were low enough to permit the determination of arsenic in blood plasma from patients living in the area of endemic Blackfoot disease with acceptable precision and accuracy, when results were duly corrected for citrate blank values.

For trace element analysis, samples of serum have a higher quality than plasma samples, because no anticoagulant is added, and in all subsequent investigations we have only worked with serum samples.

The concentrations of many trace elements in human serum are at the ultratrace level, less than 10 ng/ml [X] or 10 mg/m^3, even for ubiquitous elements such as Mn and As. Contamination is therefore by far the greatest problem in serum sampling, and in spite of very considerable efforts we have been unable to reduce the problem to insignificance in all cases.

Contamination is a surface effect and is therefore reduced by taking larger samples with improved surface-to-volume ratio, or by removing the surface layer of the sample after irradiation. A combination of these techniques was used for the sampling of redistilled water in [IX] for the determination of Mn, and for Cu by Damsgaard and Heydorn [1976]. For biological material the method is impractical, because sufficiently large samples are seldom available.

Redistilled water with very low trace element concentration may be used as a simulated serum sample and taken through all steps of the sampling procedure with a view to identifying possible sources of contamination. This was done in [IX] in order to evaluate contamination with Mn from equipment used for sampling human serum. Results are shown in Table 3 and illustrate the importance not only of the materials, but also of the conditions for cleaning.

It was found that contamination with Mn could be reduced to insignificance with proper precautions, but it could not be completely eliminated. Satisfactory quality of the sample was also ascertained with respect to As, but it is highly unlikely that materials can be found from which to take serum samples of satisfactory quality with respect to the determination of a large number of trace elements in the same sample.

In other biological materials, such as human tissue, trace element concentrations are usually higher than in blood, but the amount of sample available may be several orders of magnitude smaller. Autopsy samples of about 1 g are usually obtainable, but biopsy samples rarely exceed 0.01 g; control of contamination is therefore required also for these materials.

Table 3

Manganese contamination in simulated serum samples [IX]

Centrifuge tubes Material	Cleaning	Manganese contamination (1) µg/m³	(2)
Pyrex	Hospital	54.9	26.9
Pyrex	Risø	28.9	7.6
Polypropylene	Risø	45.2	13.9
Lusteroid	Risø	43.9	8.7

(1) Polyethylene ampoule cleaned at Risø and stored for 2 months at the hospital.

(2) Polyethylene ampoule cleaned at Risø the day before sample preparation.

Maletskos [1971] reported contamination with Mn from a stainless steel scalpel, which was then replaced by a knife made of boron nitride. In our studies of As, Mn and Se in human tissue [Larsen et al. 1972], we used disposable kitchen-ware polystyrene knives instead of BN. Each of the tools used in the sampling procedure was placed in a centrifuge tube with 5 ml redistilled water at 95°C for 30 minutes, and the extract was subjected to analysis for arsenic, selenium, and manganese. The results are given in Table 4 together with a blank value for the centrifuge tube and redistilled water alone. No significant contamination with As or Se was observed, and a possible contribution of about 0.5 ng of manganese was below the detection limit for all samples analyzed [VII]. It should be noted that contamination problems are not overcome by using values from simulated serum samples as blank corrections. Redistilled water is not identical to blood, and it does not even simulate other biological materials; hence, it is not contaminated to the same extent as the actual sample. In addition, contamination often exhibits considerable sample-to-sample variability, so that a constant blank correction is useless.

Only when a simulated sample confirms the absence of significant contamination is the quality of an actual sample satisfactory.

Quantity

In contrast to several other contemporary methods, NAA is capable of analyzing relatively large samples, which facilitates the analysis of micro-inhomogeneous material. The choice of sample size may therefore be determined by the intended use of the analytical results.

Table 4

Extracts from tools used for sampling [Larsen et al. 1972]

Sampling equipment	Quantity of element ng	
	As	Mn
Knife	1.5 ± 0.2	0.80 ± 0.07
Tweezers	1.7 ± 0.2	1.05 ± 0.04
Beaker	2.2 ± 0.2	0.86 ± 0.04
Blank	1.8 ± 0,2	0.53 ± 0.02

Homogenization, which may lead to the loss of volatile elements and the addition of impurities, can be avoided by choosing a larger sample for determination of *average* concentrations. Investigations of the *distribution* of an element within a body, on the other hand, require suitably small samples.

Availability may restrict the quantity analyzed in the case of biopsy material, but here the pooling of several samples may be used to arrive at an average determination, as described in Section 5.1.

Sensitivity is not usually the limiting factor for sample size in NAA, but the risk of contamination of small samples is probably the most crucial factor for ubiquitous elements such as As, Cu, etc.

In the present investigations we tried to avoid samples of less than 10 mg, and we never found it necessary to accept samples larger than 10 g. The actual quantity of sample should therefore not give rise to significant uncertainty, and it is not taken into account in the a priori precision.

Validity

The validity of a sample refers to its potential ability to confirm or disprove the expectation value for the result of the investigation for which it is intended.

A sample of human kidney may thus be of very high quality in the sense described above, but at the same time its validity may be poor for elements such as Cd [Johansen 1973], which exhibits considerable variation between Cortex and Medulla.

A related problem is the separation of serum or plasma from red cells in blood samples, where contamination of one by the other reduces the validity of the samples greatly for Fe and other constituents concentrated in one phase.

Homogeneity is seen to be an important asset in the choice of organs for the investigation of trace element levels in the body, and a liver biopsy sample is therefore expected to have far greater general validity than a kidney biopsy.

This was confirmed in co-operation with M. Simesen [1970] of the Royal Veterinary and Agricultural University for Co and Cu in bovine liver biopsies, for which the ratio of the two elements was found to vary insignificantly with the depth of sampling.

Similar observations were made by Schicha [1970] for Fe, Se, Zn and Co in human liver, using autopsy samples dried for 24 hours at 120°C; trace element ratios varied little, but actual concentrations showed moderate, but significant variation from sample to sample.

Additional investigations for Cu were made by Damsgaard and Heydorn [1976] in bovine heart with 15 samples, for which results are presented in Table 5 in µg Cu per gram wet weight.

With an average precision of 3.5 %, no sample-to-sample variation was detected, so that the validity of a single sample of heart tissue is excellent for Cu and by implication for other essential trace elements as well. This assertion is limited to trace element concentrations referring to wet - or fresh - tissue.

No generally accepted technique for drying biological material is found in the literature, and the validity of dried samples is therefore reduced because of difficulties in assigning expectation values. For example, it is impossible to decide whether the results of Schicha [1970] owe their variability to differences in the drying procedure, to the moisture content of the dried sample at the time of weighing, or to real heterogeneity of the liver tissues investigated.

Sampling procedures must be very specific in this respect, so that the exact condition of the sample at the time of weighing can be reproduced by other investigators, alternatively measurement of the dry-to-wet weight ratio may be explicitly stated.

Our own biological samples are always weighed fresh directly into the half-dram polyvials used as irradiation containers, and results are always reported on the wet weight basis. Dry-to-wet weight ratios are not determined, but wherever possible several elements are determined in one sample [VII] so that trace element ratios may be used to check whether an observed increase in a trace element is a real shift in the trace element balance, or is caused by tissue dehydration.

The validity of samples of blood or other biological fluids assumed to be - at least macroscopically - homogeneous is strongly dependent on the degree of control exerted over other factors of potential influence. The most important of these factors is food intake, which was found to affect the level of arsenic in serum; but several other factors are known to influence serum constituents [Winkel 1975].

Table 5

Test for homogeneity of bovine heart [Damsgaard and Heydorn 1976]

Results for Cu in mg/kg wet tissue		
3.96 ± 0.14	4.11 ± 0.16	4.09 ± 0.15
4.38 ± 0.16	4.37 ± 0.16	4.17 ± 0.15
3.95 ± 0.15	4.12 ± 0.15	3.83 ± 0.14
4.18 ± 0.15	4.09 ± 0.15	4.14 ± 0.15
4.29 ± 0.16	4.19 ± 0.15	4.08 ± 0.15
Test of precision T = 13.75 for 14 d.f.		
Cumulative probability $P(\chi^2 \geq T) \sim 0.47$		

For obvious reasons, the samples from Taiwan could not be controlled in these respects, and separate investigations of other sources of variation in arsenic levels were carried out under precisely known conditions in 1973 and 1975.

Stability

Without stability even the best samples may give rise to misleading results, because neither biological samples nor environmental samples can usually be analysed immediately after collection.

The transformation of one compund into another does not affect the elemental composition of the sample, unless the compound is actually lost from the sample by adsorption or volatilization. Such losses are very difficult to control, because they depend on the actual chemical form of the elements in the sample.

The effect of adsorption may be studied by preparation of the same sample, using containers of different materials. This was done in [IX] with redistilled water as a simulated serum sample, and results for manganese in Table 3 indicate no difference between the lowest values for pyrex and Lusteroid centrifuge tubes. Analysis of the same samples for arsenic showed the same agreement between results, in spite of the very different adsorption charcteristics of the two container materials.

Insignificant adsorption in both cases seems to be the most reasonable explanation, and separation of serum from cells by centrifugation of whole blood in a pyrex tube is thus unlikely to involve detectable losses of As or Mn. After weighing into the irradiation container, biological samples are immediately frozen, and no adsorption is envisaged during storage at -20°C until the sample is analyzed.

Both selenium and arsenic form volatile compounds, which can be partially or completely lost during sampling [Diplock 1973] or during storage [Woolson 1975].

Selenium has been found in the organism in the form of dimethylselenide, which is excreted by respiration. Its presence in blood and other biological material should therefore be taken into account [Parizek 1973], and the recovery of dimethylselenide added to human serum was therefore investigated in [IX].

Instrumental neutron activation analysis of Se was carried out with ^{77m}Se as indicator at the time of sampling and, at intervals, up to 80 days storage at -20°C. Although the sample remained frozen even during analysis, results plotted in Fig. 5 clearly reveal considerable losses by diffusion through the container walls. Storage at low temperature in a closed polyethylene container is consequently no guarantee against loss of volatile constituents.

Actual samples have been checked for such possible losses in two different ways. Samples of serum stored for between 1 and 4 weeks before analysis showed no correlation between the length of the storage period and the observed concentrations of As and Se, which indicates that no significant losses were involved.

Remaining duplicates of nerve tissue samples orginally analyzed in 1970 were analyzed for As, Se and Mn after 5 years of storage at -20°C. Ratios of selenium to manganese are presented in Table 6 which shows that they have remained in statistical control over the entire period. Arsenic showed greater variation, perhaps because the original data were not corrected for Br interference; of the 6 duplicates, 3 results were highest in 1970, 3 in 1975. Thus there was no indication of any loss of the 3 elements during storage.

Sampling procedure

A satisfactory sampling procedure is the result of detailed considerations of the factors discussed here in the light of practical experience, combined with experimental efforts to verify actual performance. An exact description of the procedure is an essential condition for producing meaningful results with any analytical method, and published accounts of analytical investigations should include records of unfamiliar sampling techniques [Anders 1977].

As an example, the procedure we followed for taking samples of human serum is included as Fig. 6; without being perfect, it has proved capable of yielding samples without significant [VIII] adulteration, at least as regards As, Se, and Mn.

Our usual procedure for taking autopsy samples is given by Larsen et al. [1972], and with slight modifications by Heydorn et al. [1975].

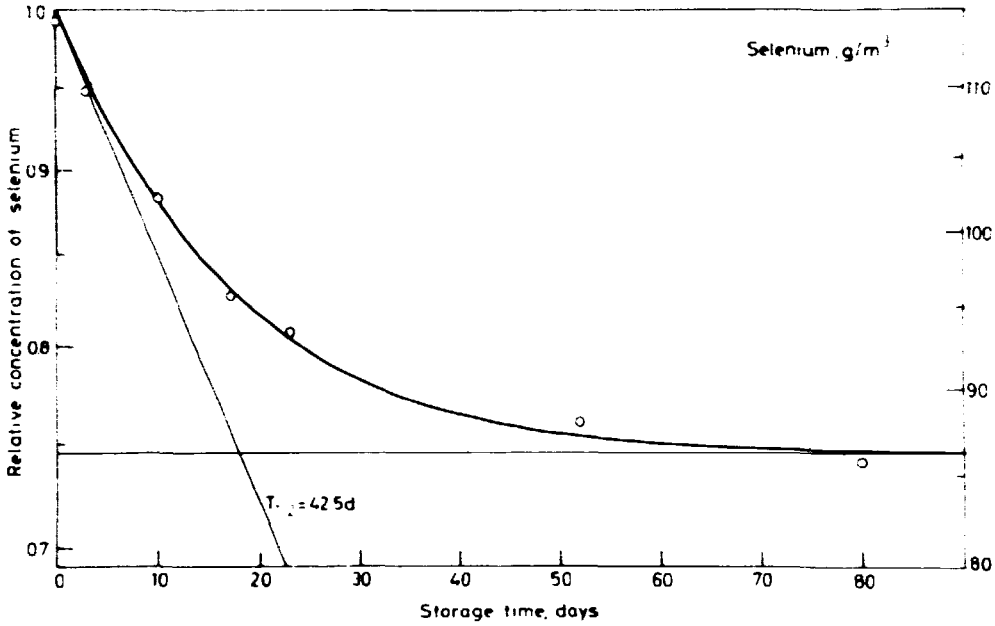


Fig. 5. Loss of dimethylselenide from serum during storage at -20°C [IX].

Samples should always - where possible - be taken in duplicate so that an Analysis of Precision can be performed to the extent needed.

Sampling errors are usually not random but systematic; what is more, they are not easily randomized. Such errors are therefore not included in the a priori precision of the analytical method - but they may be detected by the Analysis of Precision in some cases.

2.2. Choice of Comparator

The purpose of a comparator standard is to provide a conversion factor from counts to micrograms under conditions identical to those under which the sample was counted.

The *quality* of the comparator is determined by the accuracy with which the quantity of determinand is known at the time of counting.

Lack of control in this matter is surprisingly often the real cause of discrepancies between the analytical results of different laboratories.

The *validity* of a comparator is determined by the exactitude with which the irradiation and counting conditions of the sample are duplicated.

Complete identicality in both time and space is obviously not possible, and a choice between practical alternatives has to be made.

Table 6

Stability of Se to Mn ratios in samples of human tissue before and after storage for 5 years at -20°C

Nerve tissue	Ratios observed Se/Mn 1970	Se/Mn 1975
Cortex cerebri	0.51 ± 0.03	0.47 ± 0.03
Plexus lumbosacralis	1.38 ± 0.14	1.51 ± 0.13
Plexus lumbosacralis	1.85 ± 0.18	2.44 ± 0.24
Nervus tibialis	1.57 ± 0.13	1.68 ± 0.35
Plexus lumbosacralis	2.89 ± 0.25	2.66 ± 0.21
Nervus tibialis	1.58 ± 0.14	1.69 ± 0.15
Test of precision	T = 6.10 for 6 d.f.	
Cumulative probability	$P(\chi^2 \geq T) \sim 0.41$	

Principles

Counting conditions must be stable in time, so that successive countings of sample and comparator are fully compatible when duly corrected for decay.

Irradiation conditions, however, normally change with time outside of the control of the analyst and cannot be accurately reproduced afterwards, except under very favourable conditions.

A comparator standard can be dispensed with under such conditions, and *absolute activation analysis*, based on the detailed mapping of irradiation facilities with respect to neutron flux density and flux spectrum in combination with tabulated data, can be carried out with calibrated counting equipment.

Until quite recently [Ricci 1975], this method was only used for exploratory work, because the required data were not of satisfactory quality. Still, the accuracy of analytical results is difficult to ascertain, because there is no positive control of individual irradiations.

Such individual control may be introduced by adding a suitable *flux monitor* to each irradiation, usually with a relatively long-lived indicator isotope. In this way the integrated neutron fluence is determined for each irradiation, and corrections can be made for sample-to-sample differences with respect to long-lived indicators. Changes in the ratio of

Equipment

Platinum cannulae with gold-plated brass heads, Simonsen & Weel

After cleaning, repeatedly flushed with redistilled water, and placed in similarly treated glass tubes, stoppered with hydrophobic cotton; sterilized in a steam autoclave.

Pyrex centrifuge tubes, round-bottomed, 15 ml

After cleaning, immersed in a 3% hydrogenperoxyde solution for at least 3 hours, rinsed with redistilled water, and dried upside-down on tissue-paper at 100°C; stored individually in sealed polyethylene bags.

Polyethylene ampoules, 5 ml, Atomic Industrial Co.

Rinsed with redistilled water, and dried upside-down on a tissue-paper at room temperature in a dust-free place; stored individually in heat-sealed polyethylene bags.

Pasteur pipettes, Harshaw, disposable

Stored in original box until use.

Parafilm M covers, 3M

Cut, when required.

Serum Sampling

Venipuncture is made with a sterile platinum cannula through normal, clean skin, no disinfectants being applied. The first milliliter of blood is discarded, and about 10 ml is collected in a centrifuge tube, cut out from its protective polyethylene bag.

The centrifuge tube is immediately covered with Parafilm, and the blood is allowed to clot. After clotting, serum is separated by centrifugation at 800 g (2000 r/min) for 10 minutes.

In a dust-free room a Pasteur pipette is flushed repeatedly with redistilled water and used to transfer the serum to a polyethylene ampoule, cut out from its protective bag. The Parafilm cover is only removed from the centrifuge tube to allow introduction of the pipette, and the polyethylene ampoule is closed with Parafilm, as soon as it has been filled with about 4.5 ml serum.

Storage

As soon as possible after completion of the transfer operation the sample is frozen, and the polyethylene ampoule is placed in a larger polyethylene receptacle.

Storage takes place in a freezer at -20°C until the time of analysis.

Fig. 6. Sampling procedure for human serum [IX].

epithermal to thermal neutrons require additional monitors for correction, and DeCorte [1969] used a triple comparator based on Co, Au, and In in order to cover a wide range of resonance energies. More recently, the use of Ru as a multi-isotopic comparator was proposed by Van der Linden [1974] to serve the same purpose, but the presence of Ir as an impurity in Ru compounds prevents accurate measurements at low epithermal fluxes.

Changes in reactor power level during irradiation, and in particular the intermitte' operation of many research reactors including the DR 2 reactor at Risø, affects indicator isotopes with different half-lives differently. The only way to correct for such differences is to use a *comparator standard* containing the element to be determined.

For very short-lived indicator isotopes, the comparator cannot be irradiated at the same time as the sample, but it can be irradiated in exactly the same position. Here the activation and decay during the passage to and from the irradiation position can only be faithfully approximated by a comparator of the element to be determined.

Finally, the presence of the determinand in the comparator allows much greater freedom in the choice of counting conditions without loss of accuracy, both as regards counting equipment and decay time.

The validity of a comparator containing the element to be determined is greater than other types of comparators [Robertson 1974].

Composition

In *single element* determinations the composition of the comparator is chosen so that no other significant activity is formed during irradiation; in addition to the pure element, oxides, hydroxides, carbonates, nitrates or other compounds with H, C, N and O may be used, either as such or in aqueous solution. Simultaneous determination of several elements in the same sample requires several comparators, but only a limited number can be irradiated reasonably close to the sample, and their validity is therefore impaired. Our usual irradiation containers at Risø can accommodate up to a maximum of 6 comparators together with a sample [Heydorn et al. 1976] in roughly the same relative positions.

When more than 5 elements are determined in the same sample, some of them are generally determined in the same counting, and therefore it is convenient to use the same comparator for them. A *multi-element* comparator standard containing these elements in carefully chosen ratios reduces the number of individual comparators without jeopardizing the validity of the comparison [Otoshi 1976].

Instrumental neutron activation analysis with high resolution Ge(Li) detectors sometimes permits determination of a large number of elements, and special comparators with suitable concentrations of 21 elements were prepared by Mosulishvili [1975]. Batch to batch variation limited the accuracy to about 10 %, and precision was limited by a heterogeneity of 4-6 %.

A comparator of similar or better quality may be prepared by using Kale [Bowen 1965] or Standard Reference Materials from the National Bureau of Standards. However, elemental ratios are less favourable, and correct certification is not available for all elements of interest.

Generally speaking, the quality of multi-element comparators is lower than that of comparators for individual elements, and the former have not been included in our work.

In cases where elements of secondary interest may be determined in the same countings as the primary determinands, we prefer to use the *single comparator method* [Girardi 1965]. Experimentally determined calibration factors were used in [XI] for Al, Cl, and Mn relative to the single vanadium comparator. Constant irradiation conditions and rigidly standardised counting procedures eliminate most of the uncertainties associated with this method.

Form

Both the chemical and the physical form of the comparator should be roughly similar to that of the sample during irradiation, so that the same specific activity of determinand results in both.

For instrumental neutron activation analysis, the same similarity is required during counting, and in some cases this will only be possible by the addition of comparator to an actual sample [I]. For geological and other abundant materials, this addition is no problem, and thus Li was added to geochemical standards [Heydorn et al. 1977] in order to reproduce the counting geometry faithfully. For archaeological, as well as some biological materials, substitute sample materials must be used; in environmental samples it is usually possible to make the addition to a blank [XI].

In neutron activation analysis with radiochemical separation, the comparator should also be similar to the separated sample during counting. This may be achieved by subjecting the irradiated comparator to the same radiochemical separation as the samples, which is always a necessity for multi-element comparators such as Kale or other reference materials.

However, if the separated sample is similar to the original sample, the comparator standard will be similar to both, and no radiochemical processing of the comparator is required. For an original sample of 1 g biological material and a separated sample of 1 ml aqueous solution, a comparator standard of 1 ml aqueous solution in the same half-dram polyvial as the others was considered a good solution.

Furthermore, when re-irradiation yield determination is used, the form of the original and of the separated samples is no longer important; as long as the comparator standard and the yield comparator have identical forms, e.g. as the same volume of aqueous solution in a polyvial.

This last situation seems to be the most satisfactory comparator method and has been used for As, Se [VII], and Cu [Heydorn et al.

1976]. No processing of comparators is required to ensure validity, and thus the quality of the comparators at the counting stage is unimpaired.

Use of a quality control system based on comparators, as described in [X!], thereby becomes a permanent part of the analytical procedure.

Quality

The accuracy with which the determinand is known at the time of counting depends on a number of factors, starting with the isotopic composition of the starting material.

In most cases, of course, it can safely be assumed that analytical grade reagents have normal isotopic compositions, but there are a few notable exceptions, including Pb and Li. For the elements with which we are mostly concerned As, Se, Mn, Cu and V, no abnormal isotopic composition has been considered. On the other hand, the ^{125}I comparator standard used in [II] represents an extreme case, where the stable isotope ^{127}I is almost absent.

In all other cases standard chemical reagents were used for the preparation of comparator standards; the assay should be high and the stoichiometric composition precise, but the presence of traces of other elements is unimportant - with the exception of some rare earth elements - so that conventional *analytical grade* is more than sufficient.

Wherever possible, aqueous solutions are preferred to solids, both to reduce problems of homogeneity and to facilitate dilution. In this way accurately prepared stock solutions may be used as comparator basis for extended periods of time. At a mass concentration of 5 - 10 kg of element per m^3, the solution is directly applicable to carrier addition and yield comparator, and for the comparator standard it may be diluted as needed.

Stock solutions are stable systems when stored in rubber-stoppered glass bottles, and their concentration can easily be verified periodically by conventional chemical analysis, as was done for As in [Damsgaard and Heydorn 1973]. The accuracy of *stock comparator* solutions is maintained to within ± 1 % or better, and this is a level commensurate with the standard solutions commercially available from the major suppliers of analytical reagents.

Although the composition and volume of the comparator should be similar to those of the sample, the concentration of determinand may be much higher, and this has significant advantages for the quality of the comparator.

For the determination of As in gasoline, a comparator was prepared at the approximate level anticipated by dissolution of $AsCl_3$ in n-heptane and dilution to 20 and 100 mg of As per m^3. Irradiation and radiochemical separation produced the yield-corrected, relative specific

activities shown in Table 7. A very satisfactory agreement between the two comparators lent support to their accuracy, but after 2 months the relative specific activity produced in the strongest comparator had apparently dropped to 7.5 kcounts/mg As. With the hope of improving the stability, another comparator was prepared in ethanol which resulted in the relative specific activity shown in Table 7.

These results show that more than 95 % of the added As was lost from heptane, presumably by adsorption, even before the first analyses were made.

Losses by adsorption to container walls are significant sources of error in most dilute aqueous solutions, and the concentration of reference solutions from which comparator standards are taken should not be lower than absolutely necessary. This also reduces the risk of accidental contamination from impure diluents - which in a particular case was responsible for a 20 % increase of the nominal concentration of Cl in a dilute comparator standard.

When stored in screw-cap polyethylene bottles most stock comparator solutions are stable at 10 000 times *dilution* with redistilled water, which means that they change significantly less than 1 % per month [VII]. For Cu, acidification to 100 mol/m^3 with HNO_3 proved necessary [Heydorn et al. 1976] to keep the change below 1 % per month even at 5 g/m^3, and for Hg and Au storage in polyethylene bottles is not possible.

The upper level of concentration is determined by the quantity of indicator that can be counted in the same counting position as the sample at a sensible dead-time, together with the minimum quantity of comparator standard that can be dispensed with satisfactory precision. The use of prolonged decay to match comparator and sample leads to inaccuracies, because the half-life of short-lived indicators is not known exactly.

In practice we found 10 mm^3 to be suitable for the addition of comparator to air filter samples [XI], whereas aqueous comparators were usually 100 - 1000 mm^3 in volume. The lowest concentration of comparator in regular use was Mn at 0.2 g/m^3, which was replaced by freshly diluted solution every month in order to keep the accuracy to better than 1 %.

Most comparator standards are transferred to an unirradiated counting vial after irradiation in order to eliminate unwanted activity from the irradiation container or, particularly for the relatively short-lived indicators, from ^{41}Ar produced by irradiating atmospheric air. The comparator standard must therefore be stable under reactor *irradiation* conditions. This is no matter of course, as shown by Lukens [1966] in an investigation of halogen comparators. All 4 halogens showed significant losses of activity when transferred, in particular I with a

Table 7

Apparent specific activity of As from different comparators [Dalager 1969]

Medium	Nominal concentration	Relative specific activity
Heptane	20 mg/m^3	106 kcounts/mg
Heptane	100 mg/m^3	115 kcounts/mg
Ethanol	22 mg/m^3	2770 kcounts/mg

recovery of 50 % or less from an aqueous solution. In the determination of the specific activity of ^{125}I preparations [III], iodide comparators were therefore not transferred but purged with CO_2 to reduce the contribution from Ar to the activity before irradiation.

Similar results were found with Hg comparators irradiated in aqueous solution in polyethylene containers; in this case a transfer is usually unnecessary, because ^{41}Ar and other radionuclides from the irradiation container may be left to decay before the comparator is counted.

As the stability of Hg reference solutions is not satisfactory, it is tempting to dispense a large number of comparator standards into half-dram polyvials immediately after dilution of the stock comparator, and then to use the sealed containers over a period of time as they are.

However, considerable losses of Hg were found to take place from the sealed containers [Heydorn 1975] even before irradiation began, as shown in Fig. 7 for 4 different concentrations of HNO_3. In addition, irradiation was found to cause further losses, as shown in Fig. 8, without the polyvials even being opened. Similar problems were encountered in the preparation of Hg comparator standards for air-filter samples by the deposition of a small drop of reference solution on a blank filter. Considerable losses of Hg as the nitrate were found during irradiation, but the conversion of nitrate to sulphide by exposure to H_2S gas may have all but eliminated this problem.

In cases where no stable chemical system can be found for the irradiation of a comparator standard, the use may be warranted of a sealed quartz container for irradiation. This is actually required in Denmark in the officially recognized method for the determination of Hg by neutron activation analysis.[1)]

1. Statens tekniske Prøvenævn, 1975.

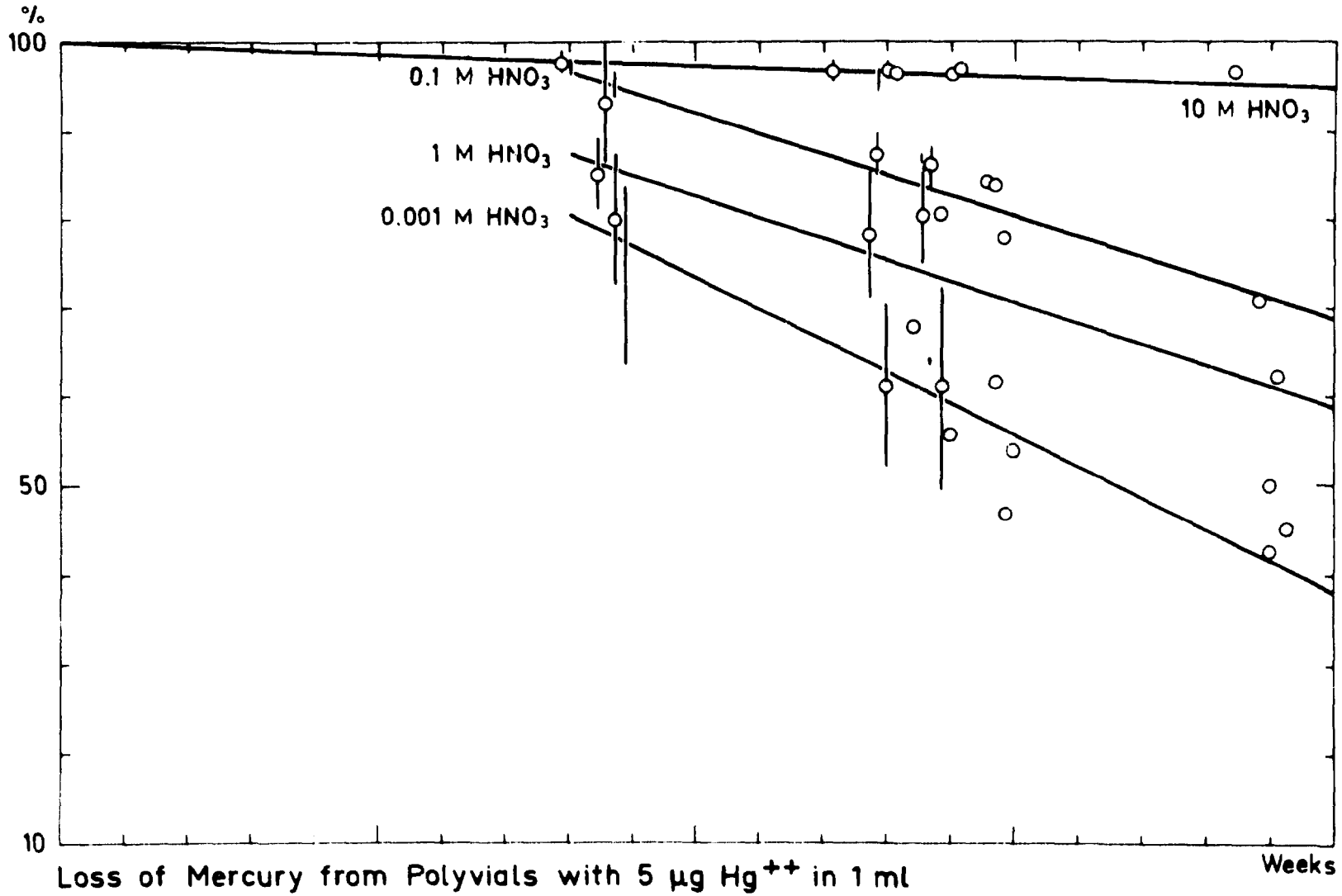


Fig. 7. Loss of Hg by diffusion from sealed, half-dram polyethylene containers determined by means of ^{203}Hg tracer at 4 different levels of nitric acid concentration [Heydorn 1975].

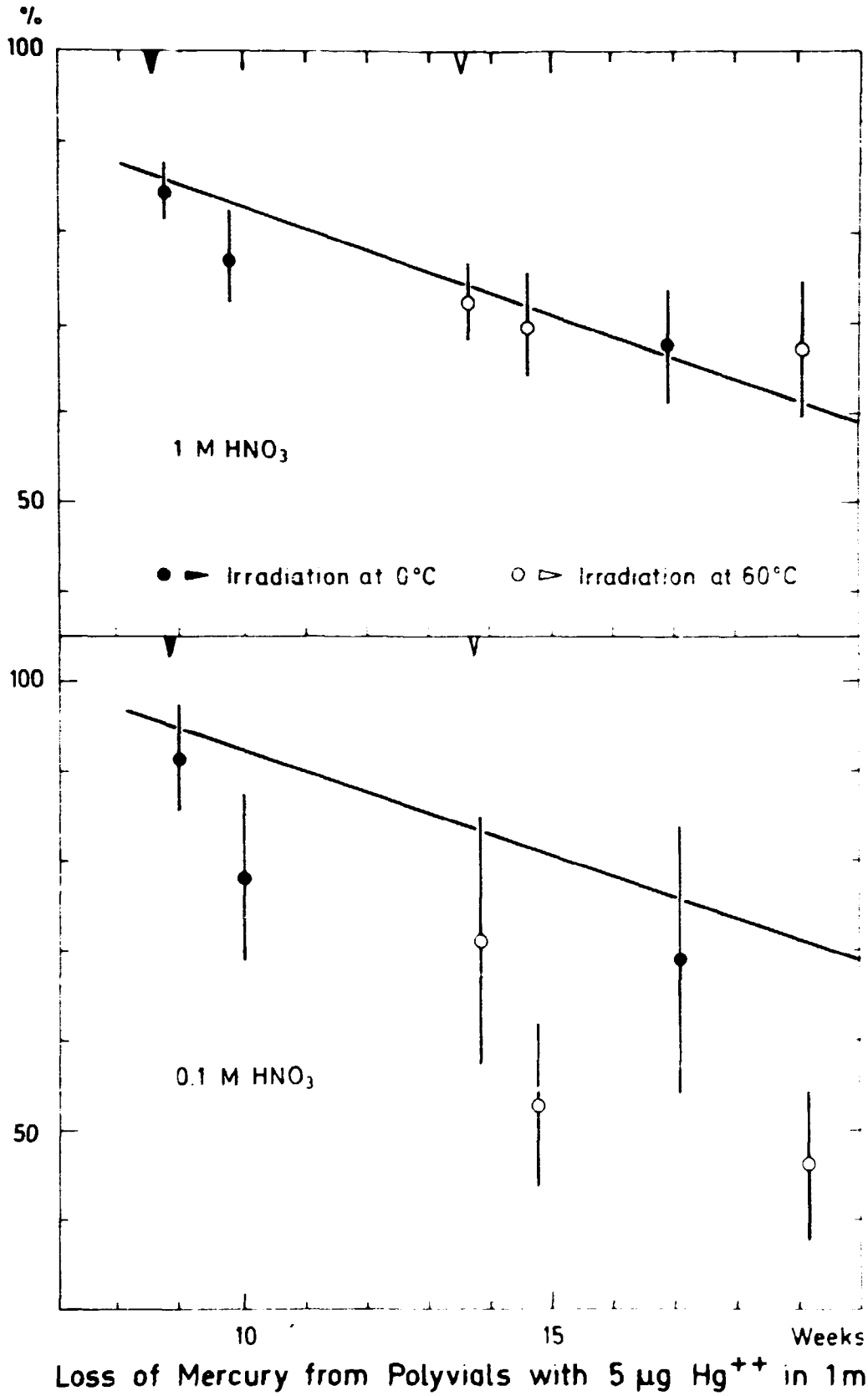


Fig. 8. Effect of irradiation on the rate of Hg loss from sealed polyethylene containers [Heydorn 1975].

The precision and accuracy of the comparator standard can be controlled to within very close limits without much effort, and the quality of the comparator should therefore always be superior to even the most well-mannered real sample, including Standard Reference Materials.

Quality control of the accurately prepared stock comparators by chemical analysis from time to time verifies the inherent accuracy of the comparator standards to required specifications.

Dilution can be carried out with almost any desired precision by suitable combinations of volumetric and gravimetric procedures, and quality control is carried out by comparing present and previous comparator standards by neutron activation analysis.

Dispensing of individual comparator standards is usually done by volume, by means of constriction pipettes, and the precision depends somewhat on the volume needed. In our routine applications the contribution of the comparator standard to the a priori precision of analytical results is from 1 to 2 %.

Further improvements in precision are quite possible by using weighing throughout, but other contributions to the precision of the analytical methods are much more difficult to reduce correspondingly, and the volumetric comparator was used in all cases.

2.3. Conditioning

The basic idea in activation analysis is to leave all treatment of the sample until after it has been irradiated. This is exactly what we do when analyzing biological material [VII] and serum [IX], where samples are taken directly to the *irradiation container*.

Here, the conditioning of the samples only consists of heat-sealing the individual polyvials and packing them together with comparators in the proper geometrical arrangement to suit the rabbit or other type of irradiation can. The polyvials are packed in a polyethylene bag, which again is heat-sealed tightly around the vials to maintain their relative position. The bag also acts as an extra containment for possible leakage from liquid samples or comparators.

Polyvials containing comparators or samples for instrumental neutron activation analysis, from which no transfer is made, must be treated with the same precautions against contamination as the samples themselves. True enough the vials may be thoroughly cleaned after irradiation, but only for contamination on the exterior surface. Heat sealing of polyvials is therefore carried out by means of radiant heat from a hot wire, which does not touch the vial and therefore avoids contamination; polyethylene foil, which serves as irradiation containers

for cellulose-acetate membrane filters [XI], is heat-sealed between teflon-lined jaws.

Low-density polyethylene is the preferred material, but where its temperature or radiation stability is insufficient, fused quartz or silica is used for irradiation. Synthetic quartz materials, like Vitreosil, Spectrosil, and Suprasil are superior to natural quartz, and after fabrication of ampoules the trace element content may be further reduced by etching for 5 min with 40 % HF. After cleaning and transfer of the sample, the quartz ampoule is sealed with a hydrogen-oxygen burner and supported by quartz wool to reduce the risk of breakage during automatic transport in the pneumatic tube system.

Treatment

In some cases, however, more extensive treatment is required to bring the sample into a condition suitable for irradiation. Usually, but not always, the purpose is to *remove material* from the sample in order to

(a) reduce the volume because of space limitations in the irradiation facility,
(b) avoid irradiation of materials with insufficient radiation stability,
(c) eliminate nuclear interference from fast neutron reactions,
(d) lower the limit of detection in activation spectrometry.

Removal of material always entails the risk of losing some of the determinand, which can only be checked by the addition of a tracer, such as was done in [Damsgaard et al. 1972] for the determination of V in biological samples. Here the 3.8 minute half-life of the ^{52}V indicator made pre-irradiation separation of vanadium an attractive possibility to avoid the difficulties of rapid radiochemical separations.

Vanadium in biological material is a particularly interesting analytical problem, because a chemical separation is always required, but it can be carried out partly, as well as entirely, before as well as after irradiation. Indicators with a half-life longer than about 10 minutes, including for example ^{60m}Co, lose so little during a radiochemical separation that pre-irradiation separation is seldom considered. On the other hand, losses from the decay of indicators with half-lives of less than a minute, such as ^{167m}Er [Graber et al. 1966] and ^{77m}Se [Dickson 1967], etc., barely permit transfer of the activated sample before activation spectrometry, and chemical separation must be included completely in the processing of the sample before irradiation.

Processing may be only the dissolution of samples of cellulosenitrate in pyridine [XI] to avoid spontaneous irradiation decomposition [Ricci 1975], or purging the sample with CO_2 to reduce interference from Ar in the determination of I by activation spectrometry [III].

Biological samples are often dried to make the sample more acceptable to the reactor safety committee, but this process is not very well defined [Becker 1970], and where possible *lyophilization* is preferable [Speecke 1976]. Both processes could give rise to losses of elements such as As, Se, and Hg, and it is difficult to prove that such losses are negligible [Fourie 1977].

Dialysis may be used for removing elements such as Na, Cl, etc., from samples, but most trace elements including V require a real chemical separation from other activable elements, which must be preceded by complete mineralization of the sample.

Dry ashing at high temperature is always associated with the risk of losing a significant fraction of a number of elements, whereas low temperature ashing with electronically excited oxygen has been found to give complete recovery of many elements. Walsh [1976], however, reports a considerable matrix effect for As with occasionally substantial losses even at low excitation power levels. Only *wet ashing* offers reasonable assurance against losses of trace elements, but decomposition mixtures are entirely unsuitable for irradiation and must be subjected to an appropriate chemical separation. This was done in [I] by extraction of VO_2^+ from aqueous solution at pH=4.75 into chloroform with 8-hydroxy-quinoline or quinaldine [Damsgaard et al. 1972]. Chloroform is, however, also unsuitable as solvent for samples to be irradiated, and it must therefore be entirely removed from the vanadium complex. A final dissolution in pyridine was found to give a satisfactory solution to be irradiated for instrumental determination of vanadium.

Blank problems

Conditioning of a sample usually involves the introduction of blanks.

Addition of reagents is the most obvious source of blank value, but even when no reagents are added, blank values may originate from the possible addition of tracer for determination of recovery [Damsgaard et al. 1972], or simply from containers [Zief 1972], the laboratory atmosphere [Scott 1972], or the analysts themselves [Zief 1976].

As pointed out by Thiers [1957], dry ashing of organic samples is preferable to wet ashing in this respect because no reagents are needed usually. Both wet and dry methods were, however, used in our determination of V in human serum [I] as well as in SRM 1571 Orchard Leaves. In this way it was found that the use of porcelain crucibles for dry ashing resulted in a significant contamination of serum samples with V that was not found in the redistilled water blank sample.

This could be attributed to a slight reaction between the alkaline ash of the serum samples and the porcelain glaze, which was subsequently found to contain about 2 μg vanadium per cm^2 surface.

A similar reaction also takes place in quartz crucibles [Wood 1949], but the absence of V in the surface of Vitreosil crucibles eliminates the blank problem for this element, but probably not for Cr, Co, etc. [Maziere 1977].

Wet ashing with nitric-sulphuric acid gives another type of blank problem, because the fuming nitric acid used was found to contain significant concentrations of V. Distillation was sufficient for purification, but some degradation takes place simultaneously, and the decomposition of serum or other biological samples takes place much more slowly.

The absolute magnitude of a blank as such is not important for the precision of results, but the variability of blanks enters directly into the absolute part of the *a priori precision*, thereby determining the limit of detection for the analytical method.

This appears clearly from [XI], where membrane filters dissolved in pyridine had a higher detection limit than membrane filters in sealed polyethylene bags, although the latter had blank values more than 7 times higher.

The standard deviation of the blank value is sometimes referred to as the power of detection [Wilson 1973], and it was used in [I] to test whether the reduction in blank value achieved by transferring the irradiated sample to an unirradiated polyethylene container before counting gave a significant lowering of the detection limit.

The validity of a blank depends upon the reproducible addition of identical reagents to all samples, and the a priori precision of a sample blank is calculated from the absolute standard deviation σ_i with which the reagents (1,m) with known impurity concentration of determinand, C_i, are added to the sample during conditioning,

$$\hat{\sigma}_o^2 = \sum^{m} \sigma_i^2 \times C_i^2 \qquad (6)$$

In practice, a particular reagent often proves to be the critical one, and it must therefore be added with greater precision than required for others for which an upper impurity level is now sufficient information [I]. If this is not sufficient, purification must be carried out, such as was done for fuming nitric acid in [I] and for 8-hydroxy-quinaldine in subsequent studies.

In equation (6) must be included not only the addition of reagents, etc., but also inherent blank contributions from the actual, necessary sample constituents, such as the cellulose filter in samples of air particulates or the citrate present in plasma samples. The variability of citrate concentration was estimated to be about 20 %, and in some cases of As-determination [IV] this factor was the main constituent of the a priori precision.

Experience with the use of polyethylene for sample containment shows that blank variability may be reduced to insignificance by careful selection of origin and batch; conditions will not be satisfactory for all trace elements simultaneously, and the problem should not be overlooked [Piotrowicz 1975].

As a final note of caution it must be realised that the estimated a priori precision of the blank value does not include the uncertainty from variations in the recovery of determinand from a complete separation procedure. The contribution from reagents added at different stages of the separation cannot be properly corrected by means of a yield correction factor pertaining to determinand in the sample proper.

Blank values therefore have decisive influence on the accuracy of the results, and this will be discussed further in Section 4.3.

2.4. Irradiation

The purpose of irradiation is to activate the nuclides in the sample to create the indicators required for the determination of the elements of interest.

Activation by neutrons is most efficient when using neutrons of low energy, and the most powerful source of such neutrons is the nuclear reactor, which is also the most widely used installation for neutron activation analysis.

Nuclear reactor activation

Irradiation facilities for activation analysis should permit the insertion into and extraction of samples from locations with a high flux density of slow neutrons and a minimum of undesirable factors [Becker and La Fleur 1974]. Such factors include fast neutrons, which are a principal source of nuclear interference and a typical circumstantial error.

Reactor γ-radiation is the major factor determining the dose absorbed in a sample, although in biological material or similar samples with considerable hydrogen content the slowing down of fast neutrons may give a comparable dose.

High radiation doses lead to sample decomposition, ranging from the violent disintegration of cellulose nitrate membrane filters [Ricci 1975] to the continuous build-up of internal pressure in ammonium bromide crystals [Heydorn 1963].

High dose rates lead to a rise in the temperature of the sample over the surrounding temperature, which in turn may result in the loss of some elements by volatilization or diffusion. Cooling during irradiation reduces the ambient temperature, but not the heat generated in the

sample, and improved heat transfer from sample to environment may be required to keep the temperature of the sample below the maximum permissible level.

Slow neutrons comprise both thermal and epithermal energies, and their respective contributions to the activity produced in a sample may be expressed by the cadmium ratio

$$R_{Cd} = 1 + \frac{\Phi_{th}}{\Phi_{epi}} \times \frac{\sigma_0}{I + 0.45\sigma_0} \qquad (7)$$

where Φ_{th} and Φ_{epi} are the respective neutron flux densities, σ_0 is the 2200 m/s activation cross-section and I is the resonance integral with the factor 0.45 giving the 1/v contribution to epithermal activation.

Epithermal neutrons are useful for the determination of nuclides with high values of I/σ_0 by instrumental neutron activation analysis. Irradiation in a cadmium container reduces the interference from other elements activated by thermal neutrons, and this technique was used by Steinnes [1972] for the analysis of silicate rocks. For biological materials [Brune 1976] the technique is only applicable in very few cases, such as the determination of Cd in liver biopsy samples that was recently[2] carried out in our water-cooled irradiation facility in the core of DR 3.

Epithermal neutron flux density gradients are usually greater than thermal neutron flux density gradients, and the well thermalized neutron spectrum in a graphite column is thus more suitable for irradiation of biological or environmental samples. This also eliminates problems with possible differences in the moderating power of sample and comparator with resulting differences in specific activity. Differences in self-shielding between biological samples and aqueous comparator standards of the same volume are negligible for small samples.

For the irradiation of biological materials, sample decomposition is the limiting factor, and both ambient temperature and the ratio of dose rate to flux density should be as low as practicable.

Inherent errors of irradiation therefore pertain to thermal neutrons only, and flux density gradients are the most significant offenders. Spatial gradients are eliminated by irradiating sample and comparator successively in exactly the same position, such as was done in the determination of V [XI] and Li [Heydorn et al. 1977], but precise timing is essential for short-lived indicators.

Variations with time are inconsequential where sample and comparator are irradiated simultaneously, which is usually the case, but flux density gradients must be known or otherwise compensated for.

Activation by slow neutrons is accompanied by the emission of capture γ-radiation and the corresponding nuclear recoil; this easily

breaks chemical bonds, and the recoiling nuclide ("hot atom") may end up in a different chemical state than originally present. This Szilard-Chalmers effect may lead to preferential loss of activated nuclides, for example by adsorption on the container walls or by volatilization after the container is opened. Other chemical effects of radiation include redox processes in aqueous media, such as the dissolution of Cu or Mn from the surface of the irradiation container, or the precipitation of elementary Hg from nitrate solutions.

Even mild irradiation conditions prevent the subsequent determination of the original chemical forms of the activated nuclide, and there is no reason not to choose the highest possible thermal neutron flux density for activation analysis.

Irradiation and decay times

The half-life of a radionuclide determines its rate of formation during activation as well as its disappearance after irradiation. Proper choice of irradiation and decay times therefore favours a nuclide with a particular half-life and thus helps to discriminate against interfering radionuclides with shorter or longer half-lives. Irradiation time, decay times, and to some extent also counting times, strongly influence the precision as well as the accuracy of the analytical results.

For one particular indicator isotope these parameters may be chosen so that a specific performance characteristic is maximized or minimized relative to other isotopes produced in a given sample. No single combination of times represents a true optimum, and different approaches to enhancing the precision or accuracy of single element determinations have been reported.

In the general case only the properties of the element to be determined can be taken into account in the selection of proper conditions.

Improvement of *accuracy* under given irradiation conditions is achieved by choosing a decay time at which the indicator isotope is closer to saturation than any other isotope [Okada 1961]. Minimum interference from other isotopes is found when the irradiation time is small compared with the half-life of the indicator isotope, [V] and Vozzhenikov [1973], and the decay time is one mean life. This is in agreement with calculations made by Hansen [1964] and by Pollack [1965] from rather different points of view.

With thermal neutron irradiation of a sample for a time t_i, the activity of a radioisotope at the time t_c after the end of irradiation becomes

$$A = a(1 - e^{-\lambda t_i})e^{-\lambda t_c} \qquad (8)$$

where a is the saturation activity and λ the decay constant.

For fixed t_i and t_c the activity varies with half-life so that

$$\frac{\delta A}{\delta \lambda} = a e^{-\lambda t_c} \left[(t_i + t_c) e^{-\lambda t_i} - t_c \right] \tag{9}$$

and the activity of an isotope with

$$\lambda t_i = \ln \left(1 + \frac{t_i}{t_c}\right) \tag{10}$$

is maximized relative to isotopes with shorter or longer half-lives. In the special case of $t_i = t_c = T_{1/2}$, the isotope with half-life $T_{1/2}$ is maximized.

The influence of half-life on the activity can be enhanced by maximizing $\delta A/\delta\lambda$ with respect to t_i

$$\frac{\delta^2 A}{\delta \lambda \delta t_i} = a e^{-\lambda (t_i + t_c)} \left[1 - \lambda (t_i + t_c) \right] = 0 \tag{11}$$

for

$$\lambda t_i = 1 - \lambda t_c \tag{12}$$

By elimination of t_c from Eqs. (10) and (12) we find

$$-\lambda t_i = \ln (1 - \lambda t_i) \tag{13}$$

which shows that t_i must be small in comparison with the half-life of the isotope to be determined, while $t_i + t_c$ equals λ^{-1}. Under these conditions Eq. (8) is reduced to

$$A = a \frac{\lambda t_i}{e} \tag{14}$$

If t_i is a small fraction f of the half-life $T_{1/2}$, Eq. (14) becomes

$$A = fa \frac{\lambda T_{1/2}}{e} = fa \frac{\ln 2}{e} \simeq f \frac{a}{4} \tag{15}$$

In the special case of $t_i = t_c = T_{1/2}$, Eq. (8) becomes

$$A^* = \frac{a}{4} \tag{16}$$

which shows that for

$$A \simeq f A^* \qquad\qquad t_i = f T_{1/2} \tag{17}$$

The graph in Fig. 9 presents $A/A^{\star}$ as a function of $f = t_i/T_{1/2}$ for $\delta A/\delta\lambda = 0$ and illustrates that the approximate Eq. (17) holds within 2 % up to $f = 1$, i.e. $t_i = T_{1/2}$. The validity of Eq. (12), however, is restricted to small values of f.

In specific cases of instrumental analysis, the selection of optimum conditions for the determination of a particular element depends on a priori information on the composition of the sample. When such information is available, deviations from the expected concentration of a particular element can be determined with optimum precision, Isenhour [1964] and Fedoroff [1971].

When the composition of the sample is known, irradiation and decay times may be chosen to give maximum selectivity [Isenhour 1964, Abdullaev 1970], which means that the indicator isotope activity relative to all other activities has its highest value. The effect of counting time was first taken into account by Ivanov [1971 and 1973], and Zikowsky [1974] went on to include cases of double counting with a waiting time in between. With so many parameters to choose, calculations become very tedious, and have only been demonstrated for the case of 3 elements, cf. Fig. 12.

With known sample composition, such as in cases with radiochemical separation (RNAA), *precision* seems a more appropriate target for improvement than accuracy, and various factors contributing to the estimated standard deviation of a single determination have been brought forward. Tsirlin [1966] took a constant background into account, while Tomov [1966] treated the case of one interfering isotope; Quittner [1967] combined the two and also accounted for the effect of counting time on the choice of irradiation and decay time. The general case was described by Tyurnin [1971], but the minimization of the standard deviation has not produced any generally useful system for choosing irradiation and decay times.

The special case of one interfering isotope was studied by Fedoroff [1971] to achieve the lowest possible value for the detection limit, and useful results are presented in graphical form. Similarly, conditions for maximum relative precision for counting with a constant background are given by this author in a later article [Fedoroff 1973].

Recently, Watterson [1975] reintroduced the general case with the important modification that counting geometry is chosen to yield the highest acceptable count-rate, or minimum source-detector distance, while counting time is kept constant. These conditions have a profound influence on the selected irradiation and decay times. Gordadze [1975] introduced a number of supplementary sources of variation and arrived at a generalized criterion for choosing appropriate times; alternative approaches are included as special cases, but no practical examples are presented.

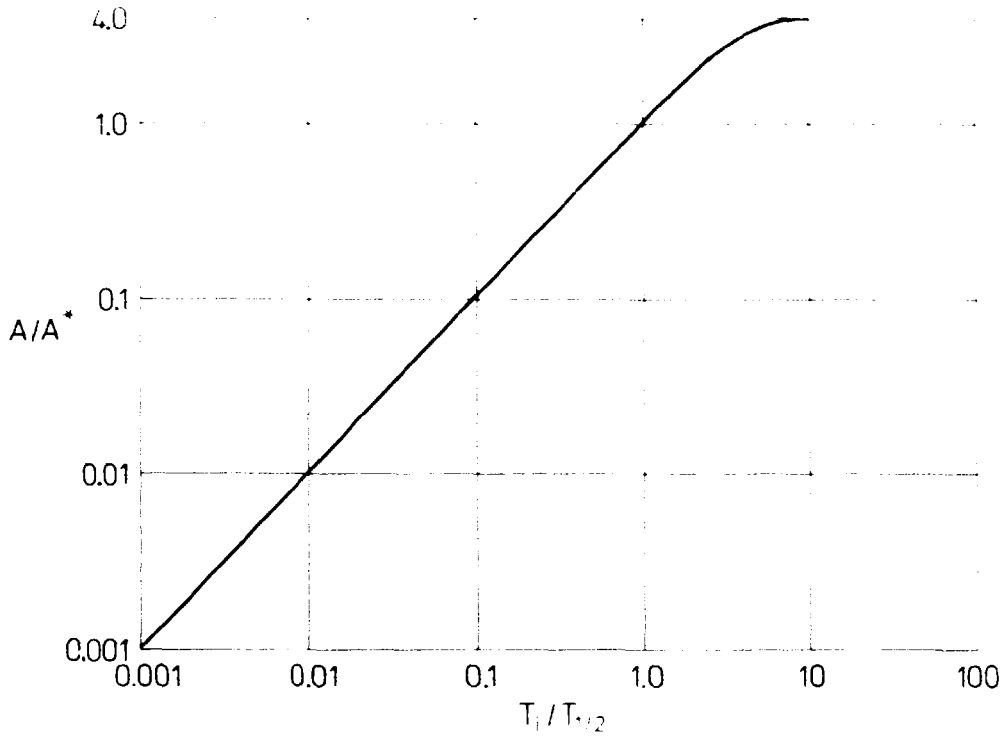


Fig. 9. Relative activity at the selected time of measurement as a function of fractional half-life irradiation [V].

Petrenko [1973] *chose to minimize a combination of random counting* statistics and systematic errors, similar to the *total error* endorsed by Eckschlager [1972]. Like most other approaches of this type, the optimum irradiation, decay, and counting times calculated are of little general interest, strongly dependent as they are on specific sample composition. Kishi [1976] and Davydov [1976] introduced the additional *parameter of cost* for the allocation of irradiation and counting times in multi-element analysis; but the necessarily arbitrary choice of pricing schedules prevents any generally valid conclusions.

In the general case of unknown composition such as is normal in instrumental neutron activation analysis (INAA), where accuracy as the minimum systematic error originating from interference is the prime consideration, the choice of irradiation and decay times according to [V] seems preferable to the other methods reported, because

(a) the application is simple, yet it may be used for successive measurements of all indicator isotopes in the same sample when half-lives exceed ~100 times the irradiation time,

(b) although no knowledge of sample composition is required, utilization of such information to correct for possible interference is very easy.

The half-life discrimination achieved by this method is independent of irradiation conditions and is therefore of general applicability.

A short irradiation time t_i followed by a decay time $t_c = 1/\lambda - t_i$ provides discrimination against radioisotopes with decay constants λ_j different from λ.

For
$$t_i = f T_{1/2} = f_j T_{1/2}^j \tag{18}$$

according to Eq. (17)

$$A = fA^* \quad \text{at} \quad t_c = \frac{1}{\lambda} - t_i \tag{19}$$

$$A_j = f_j A_j^* \quad \text{at} \quad t_c + \Delta t_c \tag{20}$$

At the time t_c the ratio between the activities becomes

$$\frac{A_j}{A} = \frac{A_j^*}{A^*} \times \frac{f_j}{f} \, e^{\lambda_j \Delta t_c} \tag{21}$$

For f_j also small, Eq. (12) is fulfilled, and

$$\Delta t_c = \frac{1}{\lambda_j} - \frac{1}{\lambda} = \tau_j - \tau \tag{22}$$

where
$$\tau = \frac{T_{1/2}}{\ln 2}$$

Now Eqs. (18) and (21) become

$$\frac{A_j}{A} = \frac{A_j^*}{A^*} \times \frac{\tau}{\tau_j} \, e^{1 - \tau/\tau_j} \tag{23}$$

The graph in Fig. 10 shows the discrimination factor

$$q_j = \frac{\tau}{\tau_j} \, e^{1 - \tau/\tau_j} \quad \text{as a function of} \quad \frac{\tau_j}{\tau} \tag{24}$$

For increasing f_j, Eq. (22) is not applicable, but the effect on the discrimination factor is comparatively small; for $f_j = 1$, the factor q_j should be multiplied by $4/e = 1.47$.

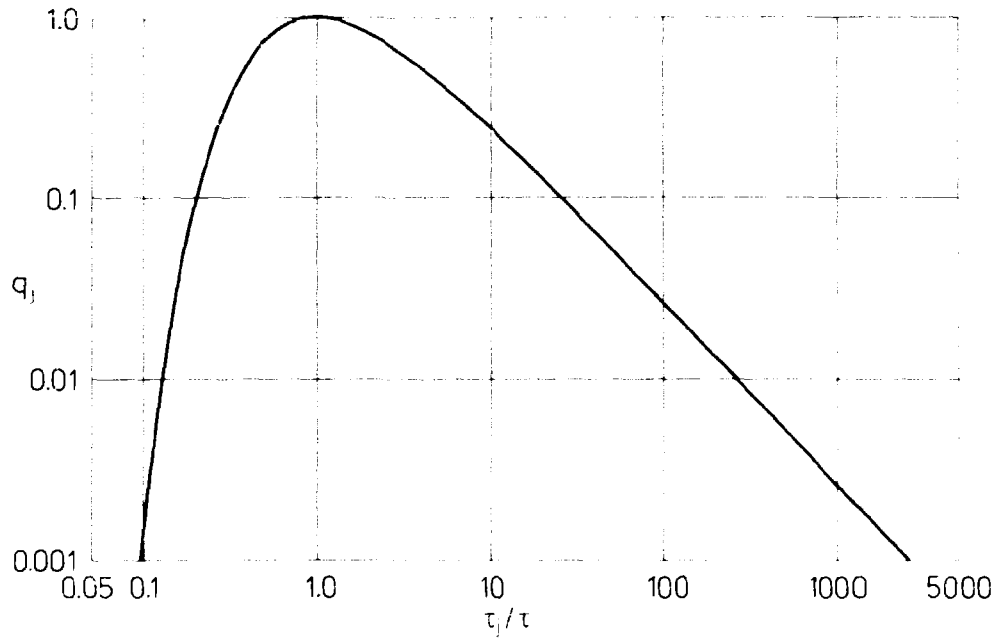


Fig. 10. Half-life discrimination at the selected time of measurement for short irradiation times [V].

For increasing τ_j, the factor q_j becomes a linear function of the half-life ratio

$$q_j \longrightarrow e\,\frac{\tau}{\tau_j}$$

For the instrumental determination of vanadium in separated samples, [I] and [Damsgaard et al. 1972], or in air filter samples [XI] by means of a 3" x 3" sodium-iodide scintillation detector, additional half-life discrimination was required to minimize the interference from longer-lived radionuclides, such as ^{41}Ar and ^{24}Na, as can be seen from Fig. 11. This was brought about by introducing a second counting period, beginning after a total decay time of $t_c + t_d$, and using the difference spectrum between the two spectra recorded with a time difference t_d for the determination of V.

Optimum values for t_i, t_c and t_d were found by trial and error to be 2, 3, and 7.5 minutes with a counting time of 1 minute. The half-life discrimination attained by using the resulting difference spectrum, relative to the spectrum in Fig. 11, is shown as the full line in Fig. 12.

A general approach to the problem of choosing the best irradiation, decay, and counting times, including *subtraction* of a second counting after some waiting time, was first presented by Zikowsky [1974]. His method is based on minimizing the relative contribution to the activity for one radionuclide with shorter half-life and one with longer half-life than the radionuclide to be determined.

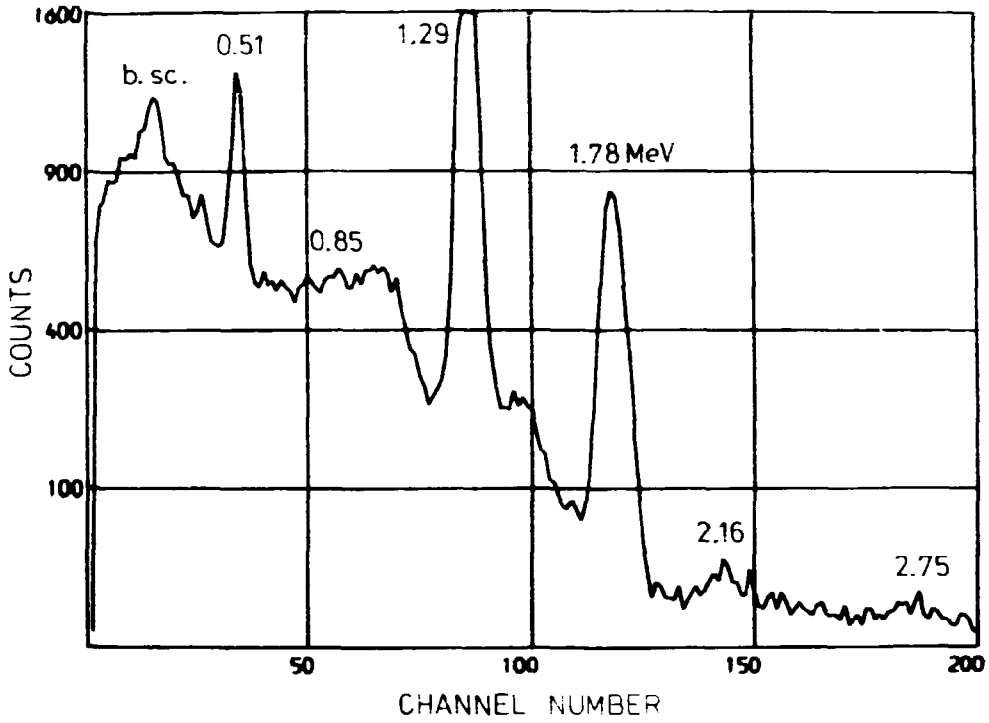


Fig. 11. γ-spectrum of a vanadium blank counted for 1 min, beginning 1 min after the end of a 1 min irradiation at a thermal neutron flux density of 4.3×10^{12} n/(cm^2 s) using a 3" × 3" NaI(Tl) scintillation detector coupled to a 200-channel analyzer set at 15 keV per channel.

For the determination of vanadium, the choice of ^{28}Al for the short-lived and ^{38}Cl for the long-lived interfering isotope, produced the recommended times for vanadium shown in Table 8.

These conditions may be compared with those chosen in the original method for instrumental determination of vanadium, presented in the first column. In the accompanying Fig. 12 are shown the half-life discriminations achieved by the two sets of conditions.

The maximum is displaced slightly towards a half-life of about 5.5 minutes, thereby improving the discrimination against ^{28}Al, but at the same time impairing the discrimination towards longer half-lives, including ^{38}Cl.

In practice, however, the important difference between the two sets of conditions is the much longer times required by the new set. While the original method permits a turn-over of 20 samples per hour of reactor operation with 1 analyzer, the new method allows less than 4 samples under the same conditions.

It should be noted that for counting times exceeding one half-life, such as those recommended by Zikowsky [1974], a correct decay time correction is no longer obtained by referring to the midpoint of the

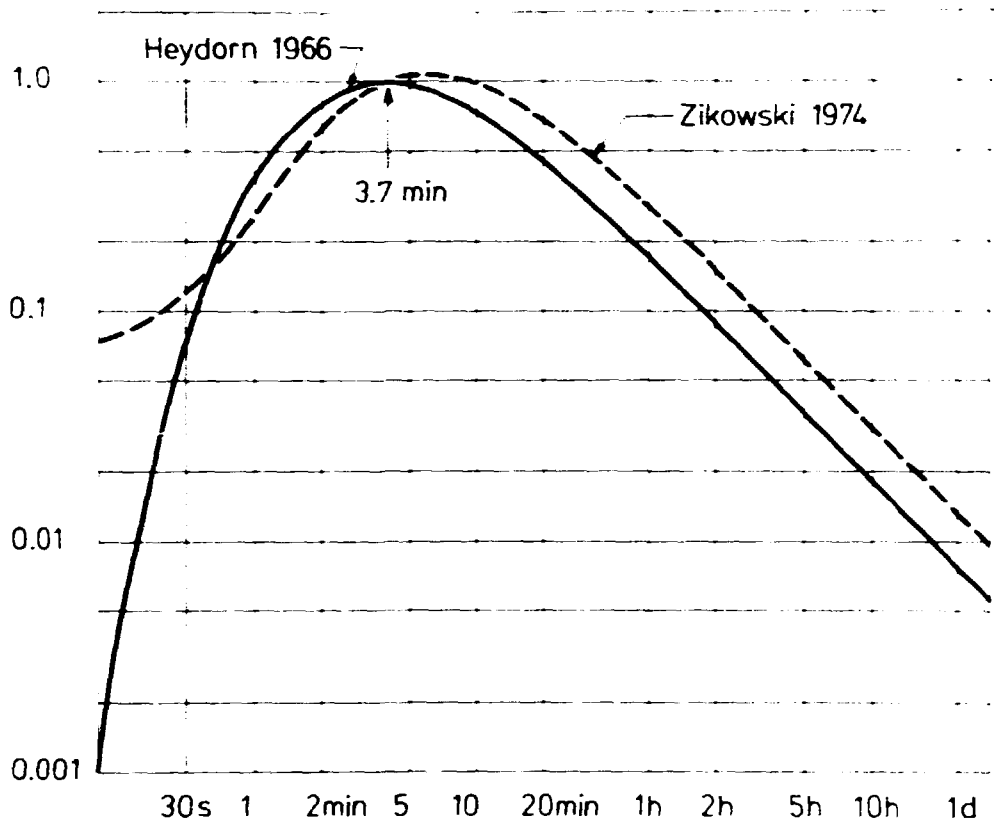


Fig. 12. Normalized reduction of contribution to a γ-spectrum as a function of half-life, achieved by using the difference between successive spectra, recorded as shown in Table 8, relative to a spectrum recorded 1 minute after the end of a 1 minute irradiation.

counting interval; this is of special importance when sample and comparator are counted for different lengths of time, such as for ^{81m}Se in [VII].

Irradiation conditions

With the exception of some positions in the thermal column of DR 2, no other irradiation facility available to the Isotope Laboratory features a lower γ-dose rate to thermal neutron flux density than the *pneumatic tube system R2*, directly accessible from laboratory room 6 in the building. The arrangement of this and other irradiation facilities in the DR 2 reactor is shown on Figs. 13 and 14 [Heydorn 1960], but with the exception of determinations of Pb and Li [Heydorn et. al. 1977] all irradiations referred to in this work are carried out in the pneumatic tube system R2.

Table 8

Recommended irradiation and counting schedules for the determination of V by INAA

Reference	Heydorn 1966 [I]	Zikowsky 1974
Irradiation time	2 min	7 min
Decay time	3 min	1 min
1st counting	1 min	7 min
Waiting time	6.5 min	1 min
2nd counting	1 min	7 min

The irradiation position of the 1.5" tube is 50 mm above the CCP (core centre plane), which means that the neutron flux decreases over the length of the transport rabbit. It has its centerline at 365 mm from the core surface or about 175 mm from the front end of the thermal column. The 15 cm lead shield between the thermal column and the core significantly reduces the primary γ-radiation relative to the neutron flux densities. The transport rabbit is shown in Fig. 15. It can take samples up to a length of 100 mm, so that very significant systematic errors from differences in activation between samples and comparator result from the existing flux gradient.

A correction for this error may of course be made, but a more satisfactory solution is to randomize this effect. This can be done, when yield correction by re-irradiation is made, by irradiating samples and yield comparator in the same geometry as the original activation. Perfect compensation for flux differences is hereby achieved under the assumption of reproducible flux distribution in the reactor.

This assumption was found to be justified by occasional experimental checks of the flux gradient, but it is also anticipated because the control rods are located in the reactor core at considerable distance from this irradiation position. The contribution to the *a priori* precision from variations in flux gradient was determined to be about 0.6 % relative standard deviation.

The movement of the regulating rods of the reactor to keep the power level constant had absolutely no effect on the flux density in the R2 facility, whereas the movement of the shim control rods did have a slight effect. A batch of samples for vanadium determination based on the same comparator standard was therefore always irradiated at constant shim rod position; the variability of the comparators over several years from changing control rod positions only corresponded, however, to a standard deviation of 2-2.5 % [XI].

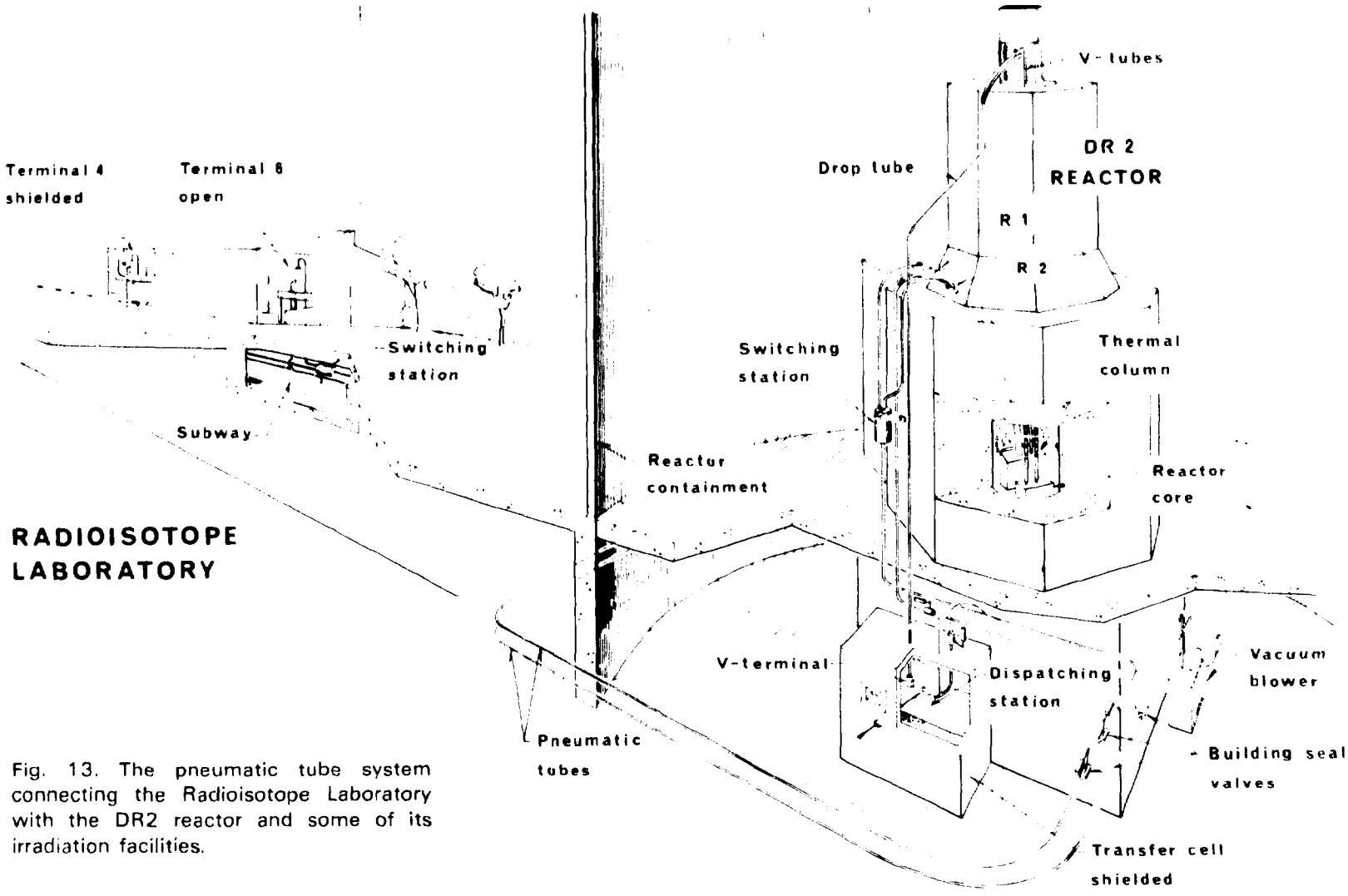


Fig. 13. The pneumatic tube system connecting the Radioisotope Laboratory with the DR2 reactor and some of its irradiation facilities.

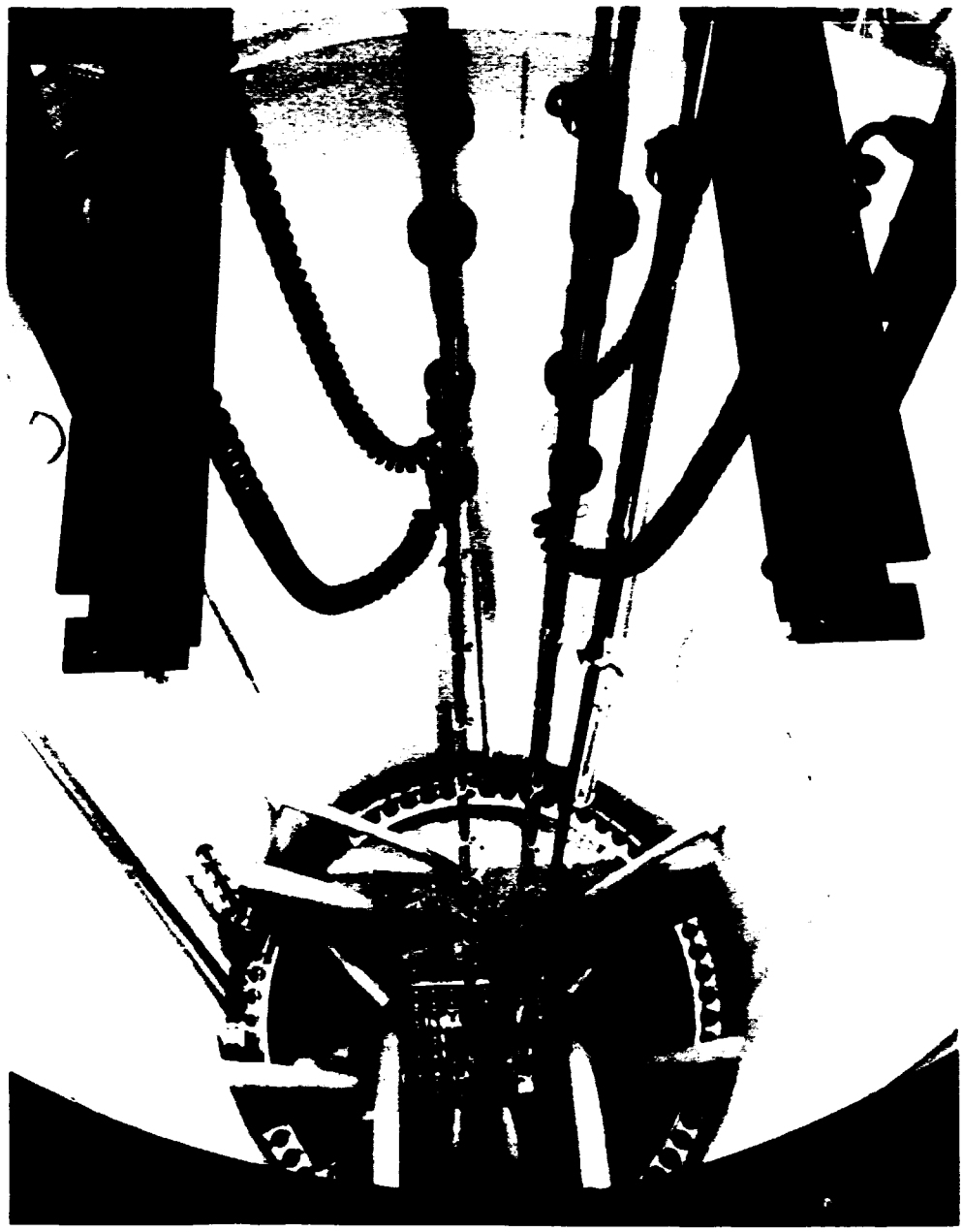


Fig. 14. View from the top of DR 2 into the tank, showing experimental tubes surrounding the reactor core.

The data in Table 9 make it possible to calculate the energy imparted to a sample of known composition and weight. For biological material with the approximate composition of Reference Man [ICRP 1975], the mass energy absorption coefficient for secondary γ-rays with an average energy of about 4 MeV is only about 2 % higher than in

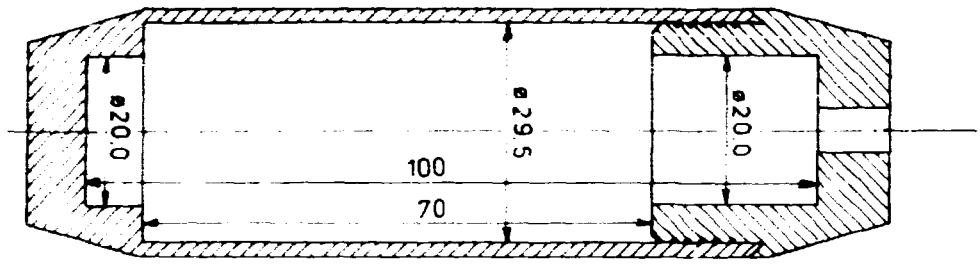


Fig. 15. Polyethylene rabbit for pneumatic tube irradiations.

aluminium, and the dose rate from γ-radiation becomes ~3.6 Mrad/h. Energy absorption from fast neutrons is practically only determined by hydrogen content, and for biological materials like soft tissue with ~10% hydrogen, the dose-rate from fast neutrons in R2 amounts to about 1.3 Mrad/h. The total dose-rate from circumstantial radiation effects is thus close to 5 Mrad/h.

The inherent dose-rate from the γ-rays emitted by the capture of slow neutrons depends on the actual size and shape of the sample; for a 1 g biological sample in R2 an approximate dose-rate of 1 Mrad/h was estimated.

The decomposition caused by this radiation intensity is well below 0.5 % per hour of irradiation. A dose of 1 Mrad is just perceptible in meat, and doses of 4-5 Mrad have been used for radiation curing of bacon in the United States. Gelation of serum and plasma occurs at 45 minutes irradiation, but no problems were encountered with irradiation times up to 30 minutes.

An energy absorption of 6 Mrad/h corresponds to a heat production in the sample of 15 cal/g·h or 17 W/kg. Adiabatic heating from the surrounding temperature of 33°C would produce a maximum temperature in an aqueous sample of 48°C after 1 hour.

The actual temperatures reached during irradiation of simulated samples of serum and tissue were 38±1°C and 42°C.

Consequently, neither the temperature nor the radiation decomposition give rise to any significant loss of determinands during irradiation in the pneumatic tube system.

Special, short irradiations were carried out at temperatures below 0°C, as referred to in Section 2.1, and by using solid carbon dioxide in the rabbit an aqueous sample could be irradiated in a frozen condition for 1 hour in R2. In this way contamination from the interior of the polyvial to the sample can be completely eliminated, as described in [IX].

In most cases irradiation time does not have to be known very accurately, although for short-lived indicators, where samples and comparators are irradiated separately, precision is important.

Table 9

Irradiation conditions in DR 2 at 5 MW

Pneumatic tube facility	CGS system	SI units
Thermal neutrons		
flux density	7×10^{12} n/(cm^2·s)	115 nmol/(m^2·s)
axial gradient	1 %/cm	1 m^{-1}
Fast neutrons		
flux density	1.6×10^{11} n/(cm^2·s)	2.6 nmol/(m^2·s)
cadmium ratio for Au	5.5	5.5
γ-heating in aluminium	10 mW/g	10 Gy/s

However, for multi-element determinations with the single comparator method described in Section 2.2, accuracy becomes crucial. Both the precision and the accuracy of the Microflex Timer used to control irradiation times were therefore studied[3] in the interval 2.5 seconds to 20 minutes.

The overall precision determined from 150 measurements is expressed by a standard deviation of 0.2 seconds, so that an a priori precision of less than 1 % requires irradiation times longer than 20 seconds.

Accuracy was within ±0.5 seconds over the whole range, and even better at times exceeding 2 minutes.

For sub-second indicator isotopes such as ^{8}Li and ^{207m}Pb, precision is of paramount importance and it was carefully checked for the fast transfer system in DR 2 [Heydorn et al. 1977]. The standard deviation of an irradiation time of 2.4 s was found to be 3 ms, and for the transfer time only 2.4 ms. This contribution to the overall a priori precision is less than 0.3 %.

The total contribution to the a priori precision from irradiation conditions can be kept at less than 1 %, and systematic errors can be compensated, randomized, or reduced to insignificance.

Attempts to *improve* irradiation *conditions* in connection with the design of new facilities in the DR 3 reactor at Risø resulted in a pneumatic tube facility with a thermal neutron flux density about 4 times as high as in R2 without an increase in fast neutron flux. This is possible because of the high moderating ratio of heavy water compared with that of ordinary water. Variations in the epithermal to thermal ratio were studied by means of a zirconium monitor [Simonits 1976] and found to be small [Damsgaard and Heydorn 1978].

No improvements could be made in the thermal neutron flux gradient or in the γ-flux to thermal flux ratio in spite of the installation of a 50 mm Bi-absorber surrounding the irradiation position, but the total absorbed dose in biological material per unit thermal neutron flux density was unchanged. Cooling with air was necessary to keep the temperature below 40°C for the irradiation of serum samples, and the best possible heat transfer is needed to keep the temperature of solid samples lower than 50°C.

For most purposes, our new facility in the DR 3 reactor is preferable to the old one in the DR 2 giving shorter irradiation times and less nuclear interference, but slightly poorer precision because of the steeper flux gradient.

2.5. Separation

All steps between the end of irradiation and the start of counting of the sample, which are taken to improve the *precision and accuracy* of the analytical results, may be characterised as a separation procedure.

Instrumental neutron activation analysis (INAA) is characterised by the complete absence of such separation, but in return it carries a blank value in addition to the inherent blank value from conditioning or sampling, namely from the irradiation container.

The separation of a sample from the irradiation container is the first step of a separation procedure; in its simplest form this is only its transfer to an unirradiated counting vial [I].

Additional efforts may be required, such as dissolution of the sample to achieve a satisfactory physical form for counting. However, even in the absence of significant interference, precision may not be satisfactory, and a real radiochemical separation may have to be carried out.

Radiochemical separation

The proper design of a radiochemical separation procedure requires information on the sample composition, but the elemental make-ups of biological materials have so much in common that general procedures can be worked out [VII]. However, concentrations of trace elements such as Mn and As in serum are so low that additional efforts are required [IX]. Conversely, concentrations in liver are so much higher that some elements may be determined instrumentally.

Based on the elemental concentrations given by the ICRP [1975] for liver and serum, Parr [1973] calculated count-rates for most prominent

photo-peaks when 1 g samples were irradiated at a neutron flux density of 2 x 10^{13} n/(cm^2·s), and counted on a 20 cm^3 Ge(Li) detector. He showed that 8 elements could be determined by INAA in all tissues, while an additional 11 elements, including Mn and Se, could be determined in some tissues only. All other activable trace elements, including As, Cu, and V, require chemical separation in every case.

Deviations from the conditions chosen by Parr [1973] will tend to reduce the number of elements that can be determined without separation, and the degree of separation required for any one element will depend on the type of counting equipment available.

In principle, elements with reasonably long half-lives can be determined with high precision by means of a GM-counter or similar unspecific detector, provided that the radionuclidic purity of the separated sample is ascertained by a reliable separation procedure. Accuracy is controlled by verification of the half-life, but obviously only one element is determined at a time, and the procedure is very time-consuming - and now practically never used.

More satisfactory combinations of radiochemistry and counting equipment, etc., can usually be found in a modern radioanalytical laboratory. As indicated in the accompanying Table 10, the performance characteristics of the separation method may be chosen so that the effects of 3 main factors on precision and accuracy are reduced to insignificance with a minimum of analytical effort.

The matrix effect is eliminated or reduced by removing other radionuclides from the indicator, which is the principal object of the radiochemical separation. In some cases it is sufficient to remove a single interfering radionuclide, such as the removal of ^{24}Na from irradiated biological material by means of Hydrated Antimony Pentoxide [VII]. In other cases a limited number of radionuclides - a group - can be determined without mutual interference. Finally, complete separation from all other radionuclides may be necessary, for example where a pure β-emitter like ^{204}Tl is the only available indicator.

The result of a chemical separation is strongly affected by the concentration of the determinand, particularly when this concentration is very low and adsorption effects begin to assume importance. In order to reduce these effects, carriers may be added to accompany the determinand without interfering with its final determination, such as for example the use of Ca as a carrier for rare-earth elements separated by a fluoride precipitation [Graber et al. 1966].

In the case of a radiochemical separation, the inactive element of the indicator radionuclide may be added as a truly isotopic carrier. The separation procedure must be designed so that complete isotopic exchange is ensured, thus giving the possibility of individual yield determinations.

Table 10

Factors and their influence on the precision and accuracy of results using different radiochemical techniques

Composition of matrix	Transfer — Interference removal — Group separation — Complete → Degree of separation
Level of concentration	None — Non-isotopic — Isotopic carrier — Multiple → Carrier addition
Radiochemical recovery	None — Average yield — Individual yield — Re-irradiation → Yield correction
Analytical results	High — Medium — Low — None ← Influence

Simple transfer of an irradiated sample may be assumed to take place quantitatively, and when carrier is added the reproducibility of the separation procedure may justify the use of an average yield based on occasional checking with a radioactive tracer, or perhaps on the use of substoichiometric separations.

When every sample counts, or when accuracy is a sine qua non, individual yield determinations should be carried out, and the separation procedure must take into account the requirements of the analytical technique selected for this purpose.

This includes the choice of carrier concentrations and of a suitable medium for the separated sample, but also of the physical form of the counting sample, which should be similar to that of the original sample.

This applies in particular to the re-irradiation yield determination, where the preceding radiochemical separation procedure is equivalent to the sample conditioning described in Section 2.3; the composition of the sample, however, may in this case be chosen so that interference is minimized [11].

Multiple carrier addition

Activation-analysis determination of trace elements by post-irradiation radiochemical separation followed by re-irradiation yield measurement is subject to interference from:

(a) insufficient radioisotopic purity of the separated sample, which tends to produce too high analytical results by increasing the count-rate in the first counting period,
(b) insufficient chemical purity of the separated sample, which tends to produce too low analytical results by increasing the apparent chemical yield after re-irradiation.

Let a sample contain *a* micrograms of the element A to be determined, together with *b*, *c*, *d*, ...*z* micrograms of interfering elements B,C, D, ...Z. After irradiation, ā, b̄, etc., milligrams of carrier of these elements are added to the sample, and after radiochemical separation the recovery of A is R, and the recoveries of interfering elements are Q_B, Q_C, etc.

The initial activation of the sample will, under the selected counting conditions, give rise to S_A, S_B, etc., counts per microgram of A, B, etc. Reactivation will, under the same counting conditions, give rise to $f \cdot S_A$, $f \cdot S_B$, etc., counts per milligram of carrier, provided both activation periods are short compared with the half-life of the radioisotopes in question.

The number of counts recorded in the measurement of A after separation is now

$$a \cdot S_A \cdot R + \sum_{B}^{Z} x \cdot S_x \cdot Q_x \qquad (25)$$

The observed chemical yield after re-irradiation will be

$$\frac{\bar{a} \cdot fS_A \cdot R + \sum^{Z} \bar{x} \cdot fS_x \cdot Q_x}{\bar{a} \cdot fS_A} \qquad (26)$$

The result of the determination of A becomes

$$a \frac{S_A \cdot R + \sum^{Z} \frac{x}{a} S_x Q_x}{S_A \cdot R + \sum_{B} \frac{\bar{x}}{\bar{a}} S_x Q_x} = a \quad \text{for} \quad \frac{x}{a} = \frac{\bar{x}}{\bar{a}} \Bigg]_{B}^{Z} \qquad (27)$$

which means that complete elimination of interference is achieved when carriers are added to all elements in the ratio in which they occur in the sample.

If carrier is only added to the element to be determined, or if the chemical yield is measured by an interference-free chemical method, the result of the determination of A is

$$a \frac{S_A \cdot R + \sum^{Z} \frac{x}{a} S_x Q_x}{S_A \cdot R} = a \quad \text{for} \quad x = 0 \Bigg]_{B}^{Z} \qquad (28)$$

which means that interference-free determination is only possible when no interfering elements are present.

Obviously, the relative trace element composition in a particular sample would not normally be known, and the addition of carriers must be based on a probable sample composition derived from knowledge of the general composition of sample material. The result will be a statistical improvement of accuracy, depending upon the distribution of trace element ratios around the most probable values.

The relative error as based on re-irradiation yield, Form. (27), becomes

$$\frac{\sum^{Z}\left(\frac{x}{a}-\frac{\bar{x}}{\bar{a}}\right)S_x Q_x}{S_A \cdot R+\sum_{B}\frac{\bar{x}}{\bar{a}}S_x Q_x} \tag{29}$$

and the relative error as based on chemical yield, Form. (28)

$$\frac{\sum^{Z}\frac{x}{a}S_x Q_x}{S_A \cdot R} \tag{30}$$

On substitution of

$$\xi=\frac{x}{\sigma}\times\frac{S_x Q_x}{S_A \cdot R} \tag{31}$$

the relative errors, Form. (29) and (30), are expressed as

$$\frac{\sum^{Z}\xi-\sum^{Z}\bar{\xi}}{1+\sum_{B}\bar{\xi}} \quad \text{and} \quad \sum_{B}^{Z}\xi \tag{32}$$

An improvement of the relative accuracy is always obtained when

$$0 \leq \sum_{B}^{Z}\bar{\xi} \leq 2\sum_{B}^{Z}\xi \tag{33}$$

If independent standard distributions are assumed for all elements concerned, Fig. 16 represents the distribution of $\Sigma_B^Z \xi$ around its most probable value $\Sigma_B^Z \bar{\xi}$ and the unhatched area represents the area of improvement of accuracy.

This area is always greater than 50 %, but its real size depends on the standard deviation of the distribution, σ, which is limited by the relative standard deviations σ_x of the concentrations of the elements:

$$\sigma=\sum_{B}^{Z}\bar{\xi}\times\sqrt{\frac{\sum^{Z}(\sigma_x\cdot\bar{x}\cdot S_x Q_x)^2}{(\sum_{B}\bar{x}\cdot S_x Q_x)^2}+\sigma_A^2} \leq k\sqrt{2}\times\sum_{B}^{Z}\bar{\xi} \tag{34}$$

where k is the largest relative standard deviation of these elements.

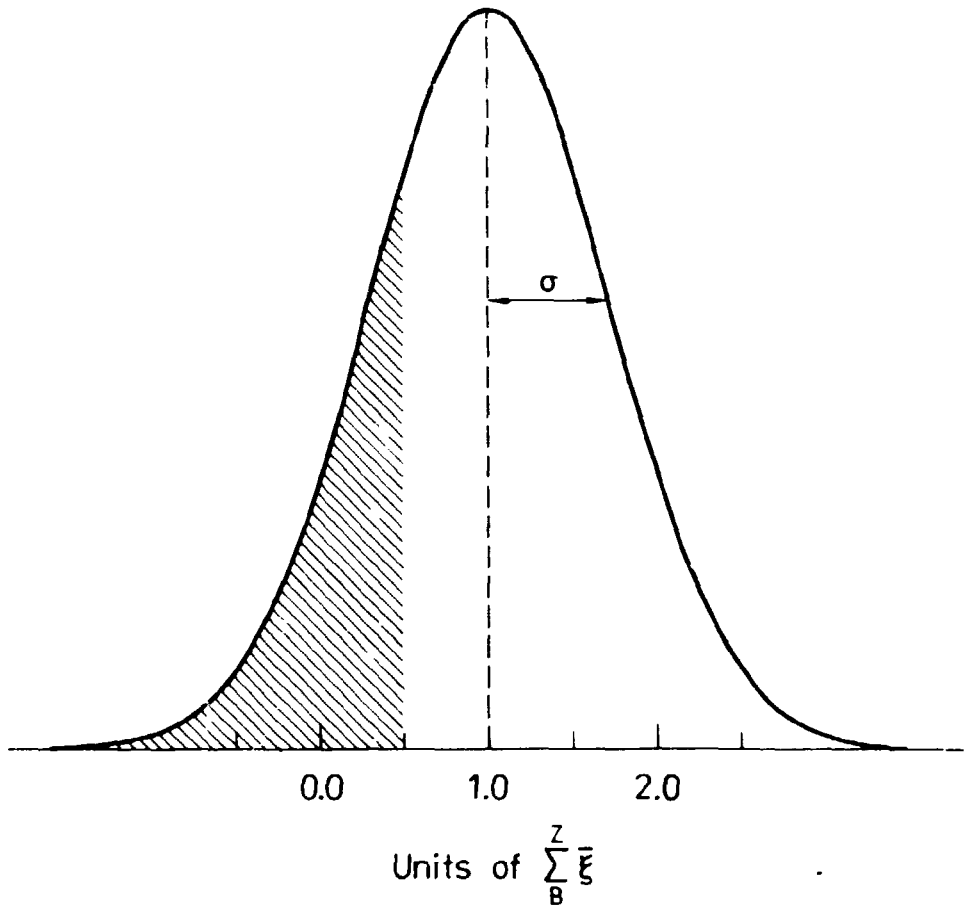


Fig. 16. Distribution of $\sum_{B}^{Z} \xi$ with no correlation of trace element concentrations [11].

Equation (33) is now fulfilled for $t \geq -\frac{1}{2k\sqrt{2}}$, and

Table 11 gives the minimum fraction of analyses with improved accuracy for various maximum relative standard deviations of element concentrations.

For logarithmic standard distributions of all elements, similar considerations lead to

$\sigma_{ln} \leq k\sqrt{2}$ *in units of the mean value,*

where k is the largest logarithmic standard deviation of the elements concerned.

Equation (33) is here fulfilled for $t \geq -\frac{\ln 2}{k\sqrt{2}}$, and

Table 11

Fraction of analyses with improved accuracy for some logarithmic and relative standard deviations of element concentrations [11]

Relative standard deviation %	Minimum improved analyses %	Logarithmic standard deviation	
		Factor	Logarithm
100	64	4	1.386
50	76	2	0.693
20	96	1.32	0.277
10	99.98	1.15	0.139

Table 11 gives the minimum fraction of analyses with improved accuracy for various logarithmic standard deviations of the element concentrations.

Performance characteristics

The design stage of a separation procedure is only completed when a comprehensive and detailed description of the procedure has been made and can be incorporated into the analytical method as a whole. Only when all processes are specified can the precision and accuracy of the method be investigated.

Investigation of the performance characteristics of the analytical method is a systematic test of its applicability to various analytical problems, of its robustness if unskilfully handled, as well as of other factors that can be described in quantitative terms, such as time requirements. The performance characteristics serve to:

(a) select the proper procedure
(b) estimate its precision
(c) prove its accuracy.

For the present investigations we have selected procedures with adequate, but not necessarily complete separation, together with the highest precision and accuracy. This means that multiple carrier addition is employed not only for determinands, but also for some interfering elements, in order to make performance characteristics, particularly interference, independent of the varying concentrations in the sample.

Perhaps the most crucical property of the radiochemical separation is the degree of *equilibration* between indicator and carrier that is

achieved; this applies particularly to biological material where losses may occur during - or before - the decomposition is complete, and where they may be quite different for different chemical entities. This possible error is not easy to disprove, but usually it is assumed that agreement between results obtained by different decomposition methods is adequate proof of complete exchange.

After complete decomposition, exchange is usually brought about by quantitative reduction or oxidation. Thus in the method described in [VII] all forms of As are oxidized to As^{V} during the nitric-sulphuric acid decomposition, and all Mn is reduced to Mn^{II} by the addition of ascorbic acid. However, Se^{IV} is partly oxidized to Se^{VI} by nitric acid, and Se^{VI} is not reduced by ascorbic acid; the accuracy of the selenium analysis therefore depends on the complete exchange between Se^{IV} and Se^{VI}.

This was checked by adding 20 kBq (0.5 μCi) of $^{75}Se^{VI}$ as selenate to an unirradiated sample, which was then processed according to the specified procedure; the separated selenium sample was counted, and the chemical yield determined by re-irradiation. The recovery of ^{75}Se and the chemical yield agreed well within counting statistics, proving that complete exchange between Se^{IV} and Se^{VI} had taken place during sample decomposition.

Additional performance characteristics of importance are *duration* of separation, sample *size*, and average *dose*.

The separation time does not affect precision or accuracy, as long as it is shorter than the half-life of the indicator to be separated, so that counting can take place at the selected decay time discussed in Section 2.4. In general, longer separation times give higher doses not because of the longer exposure times involved, but rather because separation must start at a shorter decay time after irradiation, which means a higher total activity. This again may be off-set by carrying out the first phases of the separation by means of remote handling equipment. With a maximum of about 2 mCi of total activity produced in biological samples [VII] this was deemed unnecessary; the average dose per sample is about 20 μGy for tissue or 1 ml serum.

The procedures that we adopted for this study are designed for biological samples of about one gram wet weight, with the exception of serum for which 4 cm^3 is used. No problems are anticipated with smaller samples - which were actually used in the study of arsenic in skin biopsies discussed in Section 5.1. Larger samples are limited by the size of the irradiation containers, and no significant increase above the anticipated quantities seems justifiable - far more information is obtained by replicate analysis of subsamples for the purpose of analytical quality control.

Interference

Another important performance characteristic is interference from other elements, which is expressed as the ratio between a quantity of a particular element and the corresponding error of the analytical result. Interference in activation analysis is therefore the product of a *separation factor* [Mizuike 1965] K and an effective value f of the interfering element irradiated and counted as a sample, and calculated by reference to a comparator standard of the element to be determined.

The experimental determination of a separation factor is carried out in two steps: measurement of a decontamination factor D, followed by determination of chemical yield R, where

$$D \times R = 1/K \qquad (35)$$

The decontamination factor was determined by adding 10 - 100 µCi, ~0.5-5 MBq, of a radioactive tracer of the highest possible specific activity of the interfering element to an unirradiated sample which was then processed according to the specified procedure. The ratio between the added activity and the activity of the separated sample is D.

The chemical yields of arsenic and selenium were determined by re-irradiation, while a fixed yield of 4 % was assumed for manganese.

The effective value was determined by irradiating an aqueous sample of the interfering element together with comparator standards. A known quantity *m* of the irradiated interfering element was transferred to a half-dram polyvial and diluted to 1.0 ml with water; this sample as well as a comparator standard were counted according to the specified counting procedure, and calculations were made to produce the apparent quantity of the element to be determined, $m\bigstar$. The *effective value* f is the ratio $m\bigstar/m$, and may be negative.

The error arising from the interference can now be expressed as f · K µg of determinand per µg of interfering element present, or its reciprocal D · R/f expressing the concentration of interfering element not to be exceeded to keep the error in the determination of As, Mn and Se below 1 µg/kg.

The choice of interfering elements [Wilson 1974] selected for investigation was made on the basis of expected effective values >0.01, or comparatively poor separation factors. Experimentally determined interferences for the elements arsenic, selenium and manganese from [VII] are listed in Tables 12 - 14, and additional interferences in the determination of Cu by an extension of the previous method are presented by Heydorn et al. [1976] and shown in Table 15.

The separation factor is a specific performance characteristic for the radiochemical separation procedure, whereas the effective value of an

Table 12

Experimentally determined interferences in arsenic analysis [VII]

Interfering element	Radioactive tracer	Activity MBq	Mass µg	Separation factor K	Effective value f	mg of element ≡1 µg of As
Cu	^{64}Cu	10	*	2.3×10^{-5}	0,29	150
Br	^{80m}Br	4	3	$1,1 \times 10^{-3}$	0,09	10
Sb	^{122}Sb	0.04	*	$5,3 \times 10^{-3}$	0,72	0,25
W	^{187}W	0,8	0,1	$7,2 \times 10^{-3}$	-0,29	0,5

*Irradiated carrier

Table 13

Experimentally determined interferences in manganese analysis [VII]

Interfering element	Radioactive tracer	Activity MBq	Mass µg	Separation factor K	Effective value f	mg of element ≡1 µg of Mn
Na	^{24}Na	*		$1,0 \times 10^{-2}$	$1,6 \times 10^{-4}$	600
K	^{42}K	*		1	$0,2 \times 10^{-5}$	500
Ga	^{72}Ga	8	7	$5,8 \times 10^{-3}$	$5,8 \times 10^{-4}$	300
Br	^{80m}Br	4	3	$1,2 \times 10^{-1}$	$2,0 \times 10^{-3}$	4

*Irradiated sample

Table 14

Experimentally determined interferences in selenium analysis [VII]

Interfering element	Radioactive tracer	Activity MBq	Mass µg	Separation factor K	Effective value f	mg of element ≙1 µg of Se
Cl	^{38}Cl	*		2.0×10^{-5}	-0.01	5000
Br	^{80m}Br	4	3	5.6×10^{-4}	-0.01	>40
Mo	^{99}Mo	2	12	2.1×10^{-5}	0.05	800
Ba	^{140}Ba	3	<0.1	0.9×10^{-5}	0.16	>300
Dy	^{165}Dy	200	5	3.7×10^{-7}	1200	2
Au	^{198}Au	0.03	0.001	1.9×10^{-1}	-0.53	0.01
Hg	^{203}Hg	0.03	0.2	1.4×10^{-2}	0.11	0.6

*Irradiated sample

Table 15

Experimentally determined interferences in copper analysis [Heydorn et al. 1976]

Interfering element	Radioactive tracer	Activity MBq	Mass µg	Separation factor K	Effective value f	mg of element ≡1 µg of Cu
Na	^{24}Na	*		1.4×10^{-5}	0.033	2000
Zn	^{65}Zn	0.4	1000	1.4×10^{-3}	-0.016	40
Ga	^{72}Ga	8	6	3.7×10^{-1}	-0.13	0.02
As	^{76}As	1	**	1.3×10^{-3}	0.93	0.8
Br	^{80m}Br	0.6	1.2	5.0×10^{-5}	0.32	60
Sb	^{122}Sb	0.1	**	2.6×10^{-2}	0.27	0.15
W	^{187}W	0.4	0.1	1.5×10^{-3}	0.96	0.7

* Irradiated sample

** Irradiated carrier

interfering element depends on several parameters. It is a function of irradiation time and decay time and has its minimum as calculated in [V] Section 2.4. Its value may be further reduced by the use of more selective counting equipment, but also by the use of more sophisticated data processing. All these things must therefore be precisely defined before meaningful measurements of interference can be made.

In some cases the effective values are so low that interference may be neglected even without any separation, and therefore the separation factor does not need to be determined but is assumed equal to unity. The interference values for V given in [XI] for instrumental neutron activation analysis are therefore the same as those used in [I] and presented here as Table 16.

Effective values are independent of neutron flux density, and it is easy to correct for changes in irradiation and decay times. However, differences in the neutron flux spectrum, particularly the epithermal to thermal ratio, may produce significant changes, which are made apparent by the differences in interference between the same separation method used with a TRIGA reactor [II] and with the DR 2 reactor [VII].

Reproducibility

When individual yield determination is employed, the contribution to the *a priori precision* from the radiochemical separation is limited to the contribution from the reproducibility of carrier addition. This can quite easily be kept below a relative standard deviation of 1 %, and further improvement is certainly possible if the precision of sampling and other sources of variation justify a special effort; this will rarely be the case for biological or environmental samples.

If constant chemical yield is assumed, the reproducibility of the entire procedure affects the a priori precision. With carrier added, this contribution is independent of the concentration of determinand, and its magnitude is therefore easily estimated from the variability of measured individual yields. For As, the relative standard deviation of yields from data presented in Section 2.7. is 15 %.

The cardinal effect of the radiochemical separation is to improve *counting statistics* and reduce interference. If the separation lasts longer than the selected decay period permits, a balance must be found between the improvements gained by increased radiochemical purity and the deterioration of precision and accuracy by increasing the decay time. This is an important point in a radiochemical separation procedure for V, but for the other elements chosen in this investigation, As, Se, Mn and Cu, the time of separation is sufficiently short.

Table 16

Experimentally determined interferences in vanadium analysis [I] without chemical separation (K=1)

Interfering element	Effective value f	mg of element ≡ 1 µg of V
Fe	8×10^{-7}	> 700
Mn	< 0.001	> 1
Ni	6×10^{-6}	> 80
Cu	8×10^{-4}	> 1
Zn	5×10^{-6}	> 100
Zr	1.3×10^{-5}	> 50
Mo	2×10^{-4}	> 4
Sn	2×10^{-4}	> 3
In	0.1	~ 0.01

Obviously, nuclear interference cannot be reduced by radiochemical separation, but it may influence the design of the procedure so that the detection of interfering quantities of otherwise unimportant elements is facilitated. An estimate of the Zn concentration for accurate measurement of Cu would be desirable in a TRIGA reactor, but was found unnecessary in DR 2 [Heydorn et al. 1976].

2.6. Measurement

The quantitative measurement of radioactivity is the most significant factor in the precise determination of trace elements by neutron activation analysis, and the plethora of detectors and electronic equipment provides the basis for the remarkable sensitivity with which this can be done.

Sensitivity and selectivity

High sensitivity is achieved by the counting of β-particles by means of simple GM counters, with which the activity of environmental samples was monitored at Risø [Heydorn et al. 1959] even before any activation in a nuclear reactor could have taken place.

Such high sensitivities are not required for neutron activation analysis, and the measurement of γ-radiation is always preferred, because preparation of counting samples is much less critical, self-absorption being negligible in most cases. In addition, the discrete γ-spectrum of a radionuclide offers much better possibilities of discrimination against interference than the continuous β-spectrum does.

Such increased specificity improves accuracy, if the composition of the counting sample is not quite certain. In the case of complete radiochemical separation, or in re-irradiation yield determination, no such uncertainty exists.

In samples of known composition, the ionization chamber is a useful instrument for the quantitative determination of a number of elements at and above the milligram level by neutron activation analysis, where the use of more sensitive detectors requires a special, low-level irradiation, or a reduction of sample size [V].

Low-level irradiation can be effected by irradiating in a special, low-flux facility, or for a very short period, but in many cases operation of the reactor at a reduced power level becomes necessary. Neutron activation analysis determination of trace elements relative to a major element in the same sample usually requires two different irradiations if the same detector is used throughout.

Reduction of sample size may not be possible because of a lack of homogeneity in the sample material; in other cases a sample must be analysed in its entirety, because subdivision might jeopardize the validity of the analytical results.

Measurement of ionization current yields a direct determination of the quantity of radioactive material in an irradiated sample at a given time, but rather little information on its composition. The accuracy of measurement is excellent over a wide range from the level of natural background radiation, but adequate precision usually requires activities in the μCi range. The ionization chamber thus appears to be an interesting alternative for the determination of major elements in neutron activation analysis.

Instantaneous readings from an ionization chamber are well suited for decay curve analysis, but in many cases proper selection of the irradiation and decay times will permit accurate determination of an element in a single measurement. Specifically in the determination of chemical yield by re-irradiation, where the composition of the sample is under strict control, a single reading will usually be adequate.

In all other cases energy dispersive detector systems are used to ascertain accuracy by their increased selectivity, which in turn affords the possibility of measuring more than one radionuclide at a time.

The most important detector of this type is the thallium-activated sodium-iodide crystal scintillation detector in the form of a 3" diameter

and 3" long cylinder, which has been the Standard Reference Detector in neutron activation analysis laboratories throughout the world for more than 15 years. The sensitivity of this detector is adequate for most trace elements in biological samples, and the selectivity does not warrant a complete radiochemical separation, only careful evaluation of potential interferences, see Section 2.5.

Increased sensitivity is obtained by using well-type crystals instead of the solid cylinder, but the summing of two or more γ-rays leads to more complicated spectra. In some cases this reduces interference from other elements, such as in the counting of the 0.41 MeV sum peak from ^{75}Se [Steinnes 1967]; however, in the measurement of ^{52}V in aqueous solution, the sum peak from ^{19}O at 1.56 MeV gave rise to an unexpected blank value of 1 ng V per ml H_2O. For single-element measurements of radiochemically separated samples, these objections are not important, and their convenient counting geometry made them the preferred type of detector for our determinations of As and Se [VII], as well as of Mn in serum [IX]. For purely instrumental determinations of V in air-filter samples [XI], the solid detector was preferred.

Far superior selectivity is found in semiconductor detectors, among which the lithium-drifted germanium crystal is the choice for instrumental neutron activation analysis, because its resolution is more than an order of magnitude superior to the scintillation detector, as shown in Fig. 17, whereas the efficiency can be less than an order of magnitude inferior, even for hard γ-rays like 1333 keV from ^{60}Co.

The lower sensitivity of these detectors calls for much longer counting times for radiochemically separated samples, in order to achieve the same precision as with the scintillation detector. In the case of As in serum [IX], 4 hours were found necessary to equal the precision obtained in 80 minutes with the scintillation detector; this is clearly inexpedient, but in other cases the use of a Ge(Li) detector may solve the analytical problem without radiochemical separation and thereby replace man hours by equipment hours.

Counting times

The cost of equipment hours together with the long counting period makes the choice of counting time more important than is the case for scintillation detectors; this subject was discussed from a somewhat different point of view in Section 2.4. It should be noted here that, even in the most favourable case of a constant background, no gain in the precision of low count-rates is produced by increasing counting time to more than 1.8 half-lives of the indicator nuclide.

When the duration of a measurement is no longer short in comparison with the half-life, several problems appear that are similar

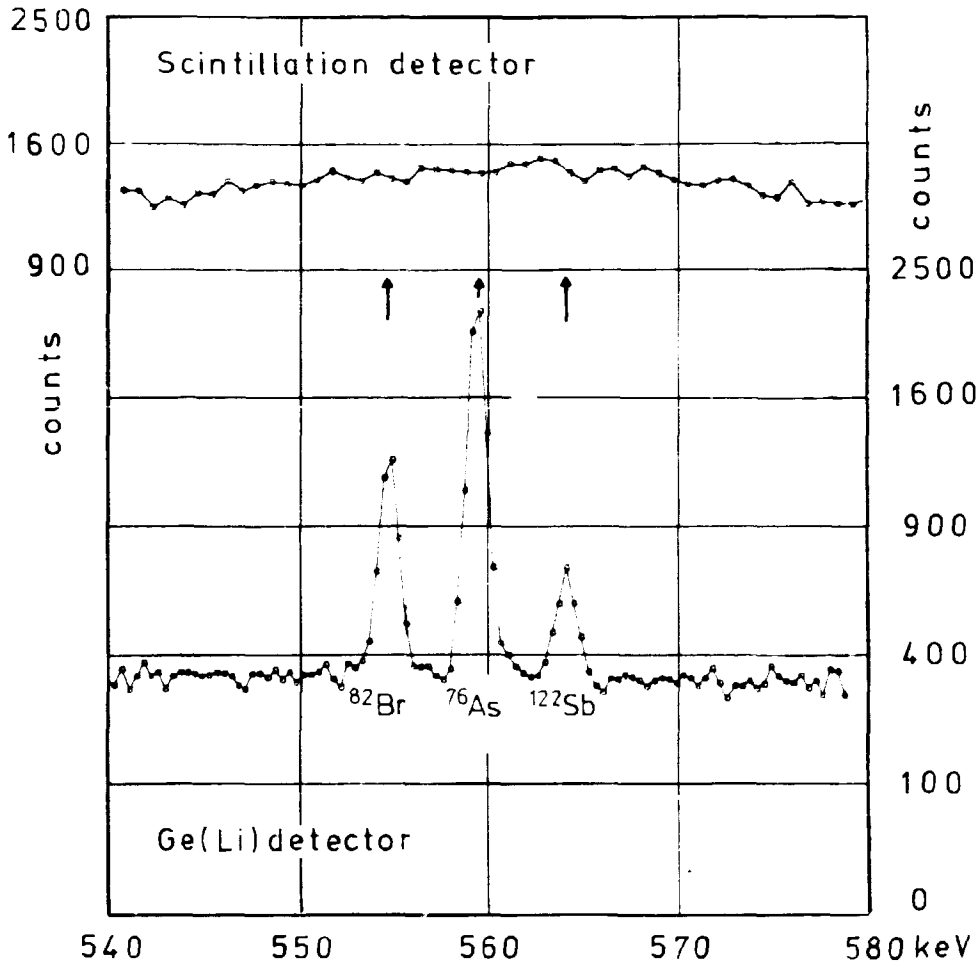


Fig. 17. The γ-spectra of two identical samples of SRM 1571 recorded with a good scintillation detector and a lithium-drifted germanium detector illustrate their difference in selectivity.

to the reduced accuracy and specificity of the ionization chamber measurements for half-lives shorter than 125 seconds [V]. These problems were studied by Junod [1974], but an exact calculation can only be made under simplifying assumptions.

In the present study we assume that average count-rates refer to the midpoint of the counting period, regardless of the half-life. A priori knowledge of sample composition is thus not required, and agreement with the formula recommended by Junod [1974] is within 1 % for counting times up to 0.7 half-lives.

Accuracy is best maintained when sample and comparator are measured for exactly the same period of time, and this is possible in most cases of short-lived indicators like ^{52}V [I] and ^{8}Li [Heydorn et al. 1977]. For ^{56}Mn and ^{76}As, counting times were short compared with

the respective half-lives, but for Se determinations based on the counting of ^{81m}Se [VII] with a 57-minute half-life for 80 minutes, a systematic error is introduced when the comparator standard is counted for only 4 minutes.

This case is most conveniently treated by calculation of a correction factor that is applied to the average count-rate during the counting period, in order to find the actual countrate at the midpoint of the counting time.

Let a radionuclide with half-life $T_{1/2}$ and decay constant λ be counted for a period T, which is not small as compared with $T_{1/2}$.

If the dead time is small and constant during the counting period, the rate of accumulation of counts is a constant fraction of the disintegration rate

$$\frac{dS}{dt} = f \times \lambda N \qquad N = N_0 e^{-\lambda t} \tag{36}$$

where t is the time from start of counting.

The total accumulated number of counts in the period T is

$$S = \int_0^T f \times \lambda N_0 e^{-\lambda t} dt = f \times N_0 (1 - e^{-\lambda T}) \tag{37}$$

and the average count rate

$$\bar{S} = f \times N_0 \frac{1 - e^{-\lambda T}}{T} \tag{38}$$

Now, the question is at what time t_1 does the average count rate equal the actual count rate

$$\frac{dS}{dt} = \bar{S} \qquad f \times \lambda N_0 e^{-\lambda t_1} = f \times N_0 \frac{1 - e^{-\lambda T}}{T}$$

$$e^{-\lambda t_1} = \frac{1 - e^{-\lambda T}}{\lambda T} \tag{39}$$

Using second-order approximations, the right side of the equation becomes

$$\frac{1 - e^{-\lambda T}}{\lambda T} \approx \frac{1}{\lambda T}\left[\lambda T - \frac{(\lambda T)^2}{2}\right] = 1 - \lambda \frac{T}{2} \tag{40}$$

which shows that, for λT small, the reference time becomes

$$t_1 \longrightarrow \frac{T}{2}$$

In all calculations of a routine nature the limiting reference time T/2 is used for decay corrections, and for λT not small a correction factor k must be applied. This factor is defined as the ratio between the actual count rate $s = \frac{dS}{dt}$ at $t = \frac{T}{2}$ and the average count rate $\bar{S}$

$$k = \frac{s}{\bar{S}} = \frac{f \times \lambda N_0 e^{-\lambda T/2}}{f \times N_0 \frac{1-e^{-\lambda T}}{T}} = \frac{\lambda T}{1-e^{-\lambda T}} e^{-\lambda T/2} = e^{\lambda(t_1 - T/2)} \quad (41)$$

For $T = T_{1/2}$ ln2[4]	$\lambda T = 1$	$k = 0.9595$
and $T = T_{1/2}$	$\lambda T = \ln 2$	$k = 0.9803$

The use of calculated correction factors to correct for differences in counting time is based on the assumption that the true clock time is known, or can be calculated with sufficient accuracy from *live time* and % dead time. The timer frequency is usually highly accurate, but the precision of the dead-time meter is often poor, and simultaneous measurement of *clock time* and *dead time* is always preferable; but it is only incorporated in one of the available counting systems (Nuclear Data 4410) mainly used for measuring short-lived indicators.

The use of difference counting, considered for the determination of V in Section 2.4, serves to reduce the interference from more long-lived activities that is caused by incomplete separation. The same applies to the measurement of 103 keV γ-rays from ^{81m}Se, which is determined from the difference between 2 spectra recorded at 200 minutes' time difference.

In this way the accuracy of the measurement is improved but with a simultaneous loss of precision. This is important to reduce interference problems when measurements are carried out with NaI(Tl) scintillation detectors. The superior selectivity of the Ge(Li) semiconductor detector does not call for this method of counting - whereas its inferior sensitivity may require at least the same total counting time for a single measurement.

Difference counting eliminates the need for background subtraction, just as the counting of a blank does, regardless of whether the blank is an empty irradiation container, a piece of unused filter paper, or an actual sample presumed to be without any detectable determinand.

Count-rate

Natural background radiation is usually no problem in activation analysis, and the reason for *shielding* the detector is to exclude

4. Very close to actual conditions for counting ^{81m}Se [VII].

unwanted contributions from activated samples and comparators present in the laboratory. If all such extraneous sources are properly kept in a shielded vault, no lead shield is needed for the detector, and in actual practice both Ge(Li) detectors in our Radioisotope Laboratory were successfully used entirely unshielded for several months while lead coffins were being manufactured.

Other types of shields may, however, be required to protect the detector from unwanted contributions to the measurements from the samples counted. The detector may be protected against contamination by a thin polyethylene foil, whereas the shielding of a γ-detector against β-particle emission from the sample requires about half an inch of perspex.

Shielding against soft γ-rays of about 30 keV from ^{125}I was achieved by means of a 0.5 mm Cu plate [III] in front of the scintillation detector. In the NPL re-entrant ionization chamber the source cavity is surrounded by a thick brass absorber, which improves the γ-energy response of the instrument; otherwise the chamber is used without any shielding whatsoever.

When several measurements are carried out at the same time, shielding of the detectors becomes inevitable, and the shield should be as large as practical. Size is important, not only because degradation of the spectrum is less pronounced, but also because particularly active samples, which must be counted at some distance from a sensitive detector, should be surrounded by the shield in order to eliminate the influence on the background of other detectors in the room.

The measurement of such *strong samples* is advantageous, because

subdivision of a sample may be avoided,
detectors with lower sensitivity may be used,
counting times may be reduced.

On the other hand, higher doses may be received during the radiochemical processing of highly active material, and the accuracy of measurement may be jeopardized by the increased count-rates to be processed by the counting equipment.

Both pile-up effects and analyser dead time are sources of systematic errors that increase rapidly with count-rate. Neither electronic nor mathematical corrections are perfect, and therefore the corrections should not only be limited to about 20 %, but the differences in count-rate between samples and comparators should be kept small. This was done in [III], and satisfactory accuracy was ascertained by running a check of the linearity of the counting system within the range of interest.

The much larger number of channels used for γ-spectroscopy with Ge(Li) detectors, compared with scintillation detectors, has stimulated

interest in the speed of analogue-to-digital conversion in order to keep the dead time at a low percentage of the elapsed time. With our latest 200 MHz ADC the correction required by the actual conversion process is no longer the most significant contribution to the total correction needed.

Count-rate-dependent errors are very annoying because they violate the superposition principle that is of fundamental importance in activation analysis and many other analytical methods. At the same time their effect starts to be felt already from count-rates of a few hundred counts per second. Count-rates lower than this are impractical, when Ge(Li) spectrometry in 4096 channels is carried out, because long counting times are required to get satisfactory precision.

The development of methods for accurate compensation for count-rate dependent errors has been the subject of a large number of publications, in which many different methods are suggested to improve accuracy over the live timer system built into the multi-channel analyzer.

Such improvements are particularly needed for the counting of shortlived indicators with high *dead times*, and both mathematical and electronic corrections have been successfully applied to specific samples.

Mathematical methods require an a priori knowledge of the number and identity of short-lived radionuclides present in the sample, because no general method is available. Exact formulas for systems with up to a maximum of two short-lived nuclides plus constant have been given by Sterlinski [1976], and for radiochemically separated samples the conditions are usually favourable for this approach. In a number of cases the approximate formula given by Junod [1974] gives completely satisfactory results with systematic errors not exceeding 1 %.

Electronic corrections for one particular radionuclide in an unspecified mixture may be performed with excellent accuracy by means of an exponentially decaying clock frequency, matching the half-life of interest [Görner 1970]. Simultaneous correction of all radionuclides in a mixture may be performed by keeping the dead-time constant and independent of count-rate during the whole counting period [Bartošek 1972 and De Bruin 1974].

With the increasing analogue-to-digital conversion rate dead-time is reduced, and another count-rate dependent effect, *pile-up*, assumes importance. As shown by Anders [1969], all peaks pile up to lose the same fraction of the counts, and a pulser with fixed frequency connected to the preamplifier can therefore be used to correct for this effect. The correction is only accurate for constant counting rates, but by introducing a count-rate-dependent pulser frequency Bolotin [1970] made accurate corrections also for short-lived radionuclides.

In samples subjected to a radiochemical separation the count-rate can easily be kept at such a low level that no pile-up problems are present, and for the analysis of trace elements in biological material errors of this kind may safely be neglected.

For samples like SRM 1571 Orchard Leaves, which were analysed both by INAA and by RNAA, the instrumental determination was made with a Ge(Li) detector at count-rates less than 500 counts/second [Damsgaard and Heydorn 1973].

Counting geometry

Variations in count-rate are brought about by changing the distance between the detector and the counting sample, and in order to achieve a satisfactory precision in the comparison between sample and comparator fixed counting *positions* are preferred.

Uncertainty of counting position gives rise to a random error in the analytical result, and for weakly radioactive samples, which are counted as close to the detector as possible, small variations in source-detector distance give relatively large errors. The use of well counters - re-entrant ionization chambers - reduces this uncertainty to 0.5 %/mm or less [Heydorn 1967], but for small, solid Ge(Li) detectors the effect is easily an order of magnitude greater.

It may be added that the precision of the counting position is also affected by fluctuations in the position of the Ge(Li) detector inside the cryostat, caused by variations in the height of liquid nitrogen in the Dewar bottle. This source of variability is reduced to a minimum by using a bottle with a capacity of more than a month and filling it up to the mark every week.

Variations across the face of a coaxially drifted Ge(Li) detector were reported by Haas [1974] and give rise to quite appreciable changes in efficiency. Therefore we work only with closed-end coaxially drifted detectors, mounted with the closed end facing the source.

With this configuration the relative efficiency slope was found to be independent of source-to-detector distances up to about 300 mm for γ-energies from 0.5 to 1.5 MeV, and only slightly dependent down to 200 keV. This is a very favourable situation for the use of the single comparator method.

The experimentally determined slope is not in agreement with the value expected for a detector with 5 % efficiency from the empirical function proposed by Vaño [1975]. This is perhaps connected with our use of a 12 mm β-shield of perspex, which affected the slope in such a way that the volume of the detector in Vaño's equation must be increased.

Although the strategy of counting is intended to reduce or eliminate most systematic errors, the actual quality of the results is determined by the performance characteristics of the counting *equipment*.

Several investigations have therefore been made to verify the absence of some errors and to measure the magnitude of other sources of variability. The remaining errors are kept within bounds by exercising some control over parameters such as dead time.

The most important parts of the equipment are the radiation detectors, which include several types of NaI(Tl) scintillation detectors, two Ge(Li) semiconductor detectors, several ionization chambers, GM-counters, and a home-made Čerenkov detector. Used properly they are all capable of producing highly accurate results, but additional equipment is required to make this possible. Hence it is this peripheral equipment rather than the detectors themselves that determines the quality of the outcome of a radionuclidic measurement.

Reproducible counting geometry is determined not only by the precision of the counting position, but also by the size and shape of the sample [Becker 1974]. The use of standard half-dram polyethylene vials for all countings ensures precise positioning, and the use of liquid samples ensures homogeneity and well-defined shape. Differences in geometry between samples and comparator are thus limited to variations in volume.

The usual counting assemblies with vertical source-detector orientation, such as described by e.g. Yule [1965], are quite sensitive to variations in volume, because the height of the liquid in the vial determines the effective distance from the detector.

With a horizontal *arrangement* of the detector-source assembly, such as is the case inside the Risø lead coffin, shown in Fig. 18, the height of the liquid does not affect the distance to the detector, and the influence of sample volume is greatly reduced. Similar arrangements are constructed for the Ge(Li) detectors employed at the laboratory as shown in Fig. 19, and satisfactory elimination of volume effect was also achieved in this case.

Figure 20 shows the relative count-rates for ^{137}Cs as a function of sample volume for 3″ × 3″ NaI(Tl) scintillation detectors mounted vertically and horizontally, respectively. Also shown is the volume effect for our Ge(Li) detector (both of them) mounted with horizontal source-detector assembly. All samples were measured in contact with a 0.5″ perspex absorber on the detector.

Uncertainty in the counting position from a slightly less than perfect fit of the sample holder in the grooves of the perspex box was investigated by measuring the 136 keV γ-ray from ^{75}Se in the same, innermost position with a Ge(Li) detector. The difference between the extreme positions was found to be 0.66 ± 0.20 %.

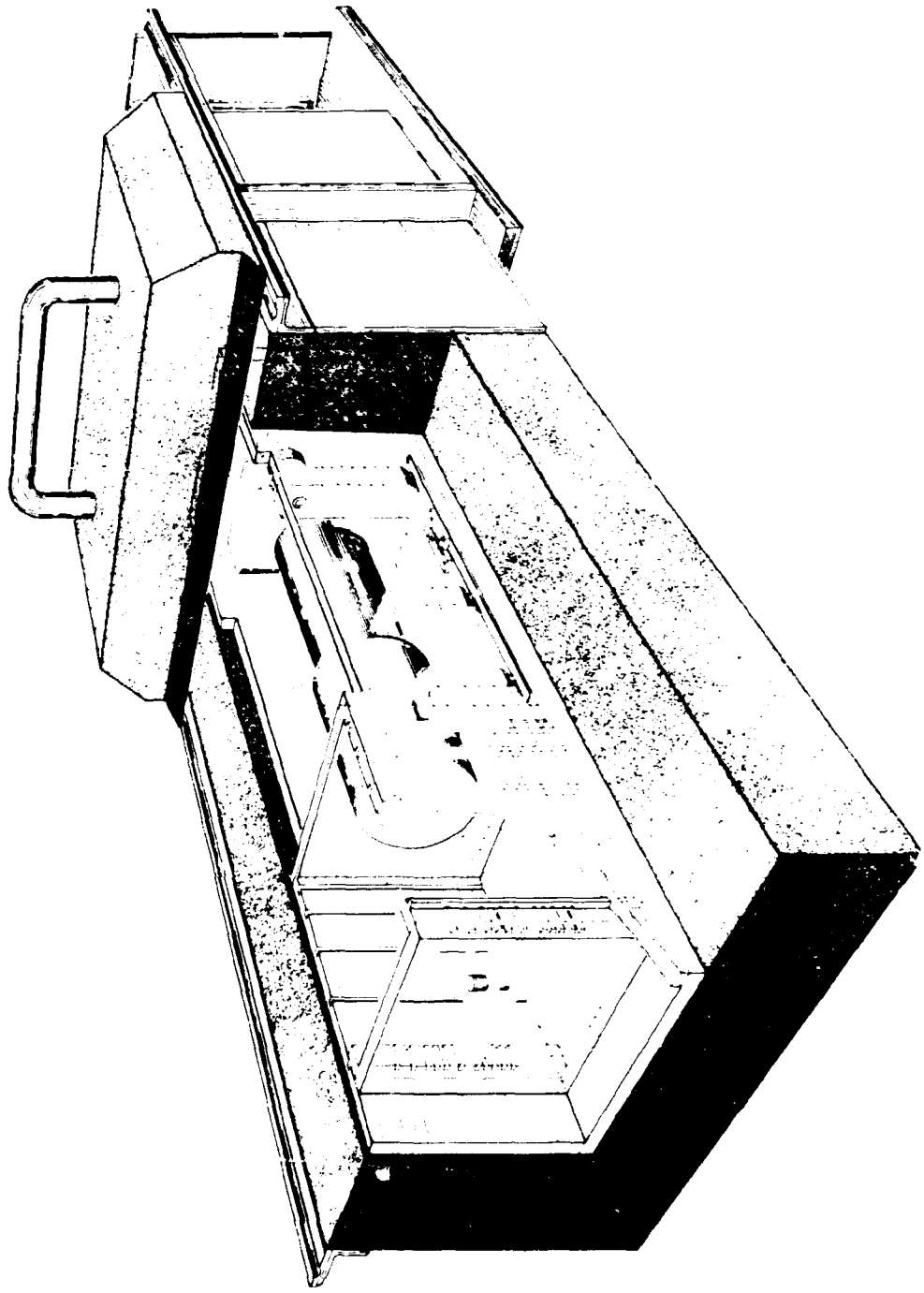


Fig. 18. Horizontal lead coffin for counting of liquid samples in neutron activation ...

A 0.8 m long perspex box supports a horizontal 3" × 3" NaI(Tl) scintillation detector centrally in the 150 × 150 mm^2 cross section cavity. The position of the detector relative to the source compartment is accurately controlled by means of a nonius scale. A sample holder with exchangeable shelves for vertical source positioning is placed at a well-defined location by means of grooves in the wall of the source compartment.

The perspex box is situated in a coffin with 100 mm thick lead walls, clad with Cu and Cd foils. It is covered by a three-part sliding shield of the same effective shielding thickness; the weight of the assembly is approximately 1200 kg.

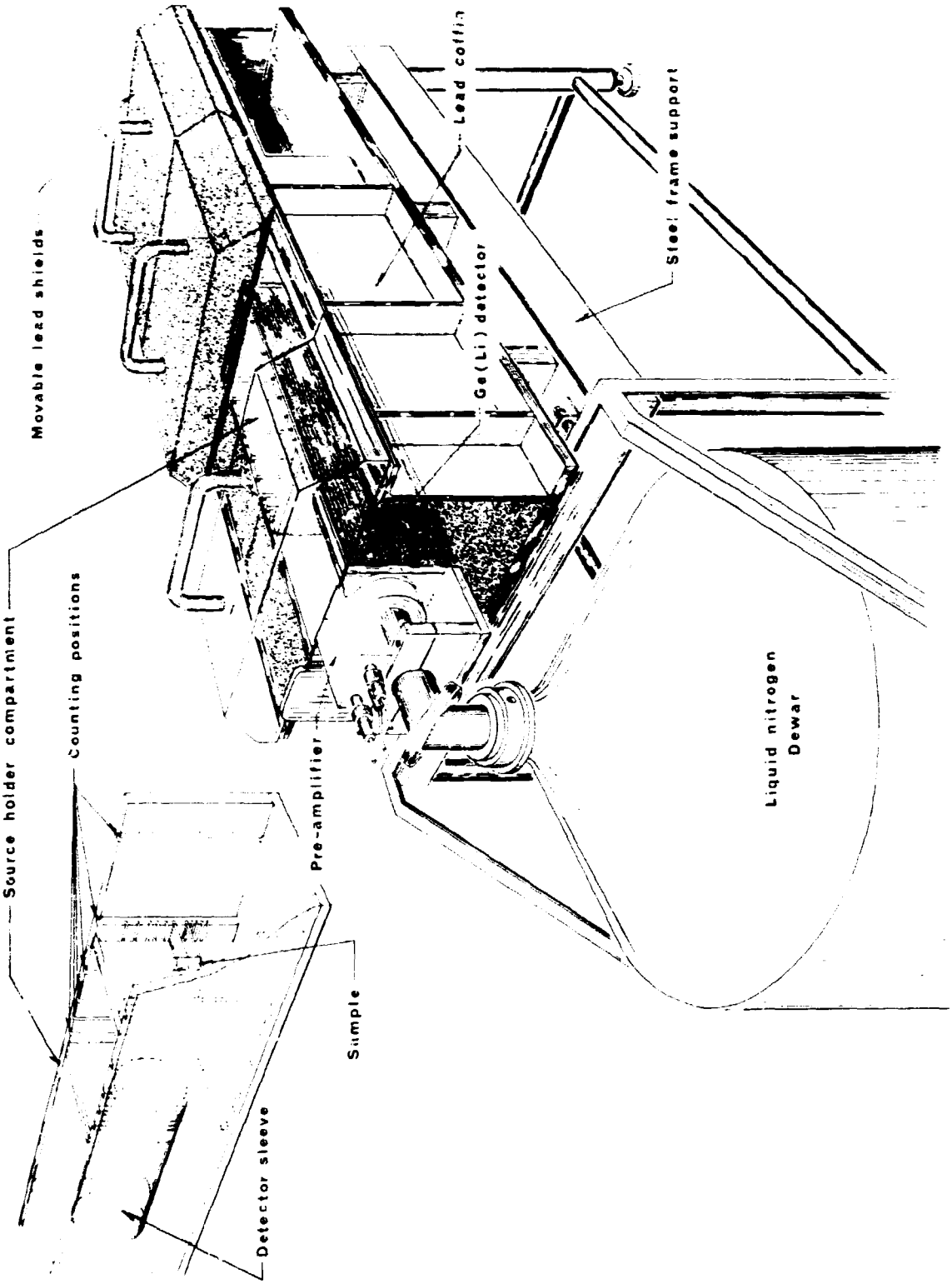


Fig. 19. Horizontal lead coffin for counting with a Ge(Li) semiconductor detector.

The perspex box shown as an insert illustrates that the modifications required by the different detectors do not affect the source compartment.

The lead coffin is open at the detector end but otherwise identical with the previous design in Fig. 18.

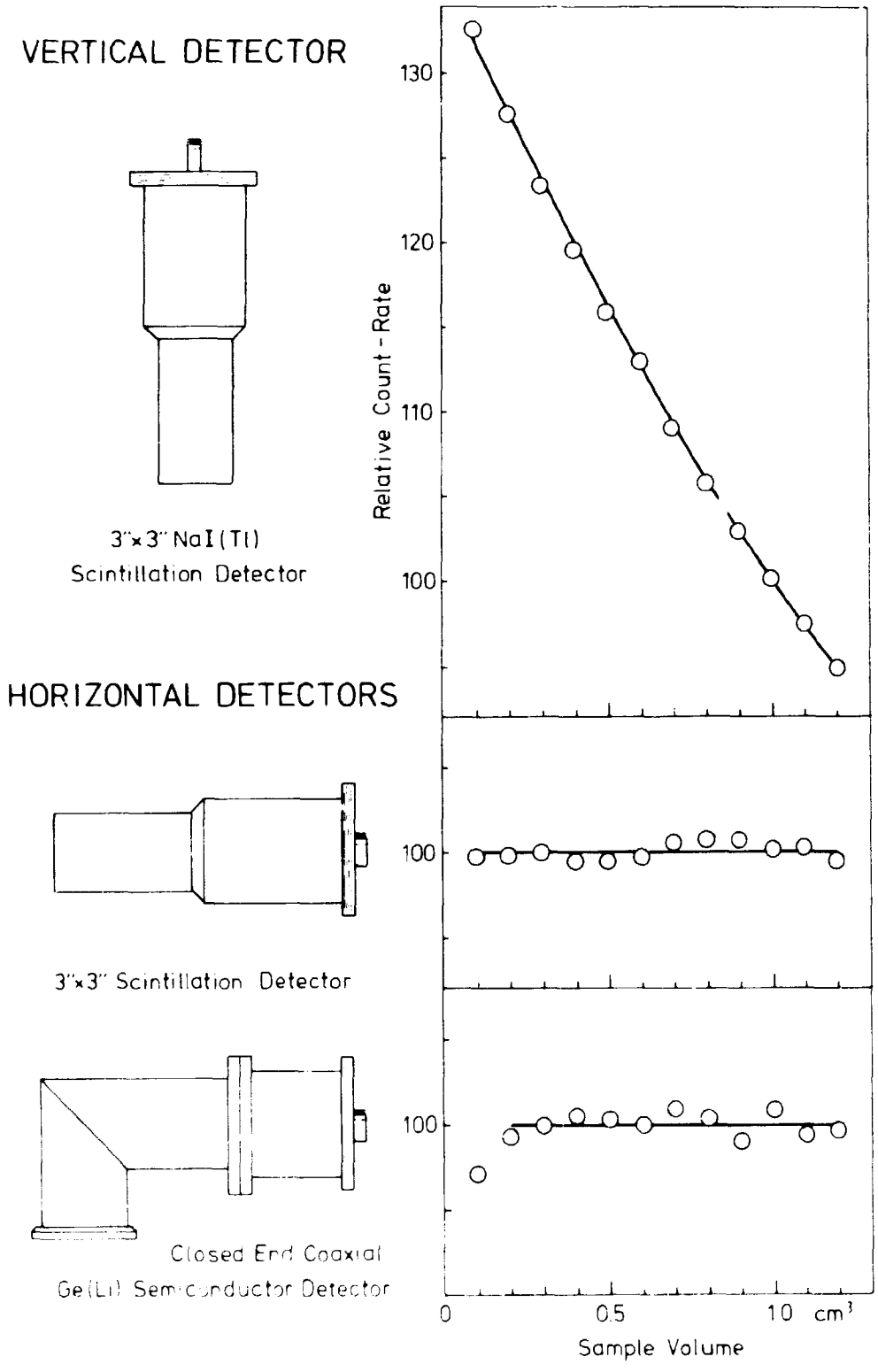


Fig. 20. The influence of sample volume on detector response for vertical and horizontal configurations.

A total of 7 discrete counting positions for accurate placing of sample holders is available in the perspex box; each step represents a change in counting geometry of close to a factor of 2. Thus, samples differing by more than two orders of magnitude in activity may be counted with the same detector under accurately reproducible counting conditions, i.e., with an error of considerably less than 1 %.

For our Ge(Li) detector, the same relative efficiency curve for γ-energies ≥ 0.2 MeV is applicable to all counting positions from close contact with the detector up to about 300 mm distance, within a standard error of estimate of ± 2 %. This means that a single calibration factor for each position suffices for a large part of the elements determined by INAA.

Live time control

For such measurements, the most important performance characteristic of the electronic equipment is the accuracy of the time control. The precision of the clock frequency is generally far better than needed, and the accuracy is usually better than 0.5 %. The crucial point, however, is the similarity between the rejection of clock-pulses and real pulses from the detector, on which the accurate control of live time depends.

This point was investigated by varying the count-rate to which the ADC was exposed, and thereby the fractional dead time, by means of the lower level discriminator. In this way the same source combination can be measured under different conditions without moving the source or changing the count-rate in the detector. Pile-up effects are constant under these circumstances, and the effectivity of dead-time compensation can be checked without interference.

Such measurements were carried out for a 3" x 3" NaI(Tl) scintillation detector coupled to a ND-160 F multichannel analyzer with combinations of ^{137}Cs and ^{88}Y, as well as with ^{57}Co and ^{137}Cs. Photo-peaks of 1837 keV, respectively 662 keV, were evaluated by the Total Peak Area method for dead times up to about 40 %, produced by a total count-rate of about 10 kcounts/s.

Both sets of measurements showed that the error from the built-in live timer could be limited to ~ 0.5 %, if only differences in dead time between sample and comparator did not exceed 20 %. As previously mentioned, the general policy is to keep the dead time below 20 % in order to avoid significant errors in clock time estimates; by the same token the dead-time errors are thus negligible.

Installation of a Ge(Li) detector prompted a repeat of the measurements with our two more advanced multichannel analyzers with much higher analogue-to-digital conversion rates. Large systematic errors were here detected [Heydorn 1973], also for dead

times less than 20 %, and Fig. 21 shows the relative errors observed for two different multichannel analyzers as a function of per cent dead time. Also included are the results of the previous measurements, shown in the same scale.

Similar results were reported later by Huysmans [1974], and apparently the problem existed in all commercially available multichannel analyzers at that time; altogether 7 different analyzers from 4 different manufacturers were investigated during 1973 at our laboratory at Risø, and none was satisfactory.

Actual results for the purely instrumental determination of As in SRM 1571 Orchard Leaves by the Ge(Li) detector coupled to the two different Nuclear Data multichannel analyzers are shown in Table 17 for two different count-rates and two different discriminator settings.

Results in the table show the significant difference between results obtained with different levels of discrimination, low dead times giving lowest results in agreement with the trends shown in Fig. 21. This trend is opposite to pile-up, which gives low values for high-count-rates, and thereby the two effects partly cancel each other so that the overall error that arises from disregarding both effects is comparatively smaller than can be estimated from Fig. 21. This is also brought out by the results in Table 17, where the choice of the most accurate result would therefore be that in the lower, right corner; in fact this value is in excellent agreement with the value reported by Damsgaard and Heydorn [1973] based on much lower count-rates.

As pointed out by Anders [1969], pile-up affects all peaks in a spectrum in the same way, just as dead time does; the ratios between photo-peak areas of different γ-lines are therefore independent of count-rate.

This effect was utilized in the accurate determination of As in Orchard Leaves, where an accurately known quantity of ^{74}As was added to the arsenic carrier, and As was determined by simultaneous counting of ^{76}As and ^{74}As [Damsgaard and Heydorn 1973] by the Ge(Li) detector.

For instrumental neutron activation analysis, the same principle is utilized by injecting pulses with an accurately known frequency of 50 Hz at the preamplifier stage. By choosing a suitable amplitude, a pulser peak is obtained at a convenient location in the spectrum, and with good approximation this peak loses the same fraction of the counts as the true photo-peaks. Although the intervals between successive pulses are not Poisson distributed but constant - which, i.a., means that 2 pulses never pile up - the accuracy of such a system is very satisfactory as long as no appreciable decay takes place during the counting period. However, not all methods of peak evaluation are equally suitable, because the shapes of the full energy peaks and the

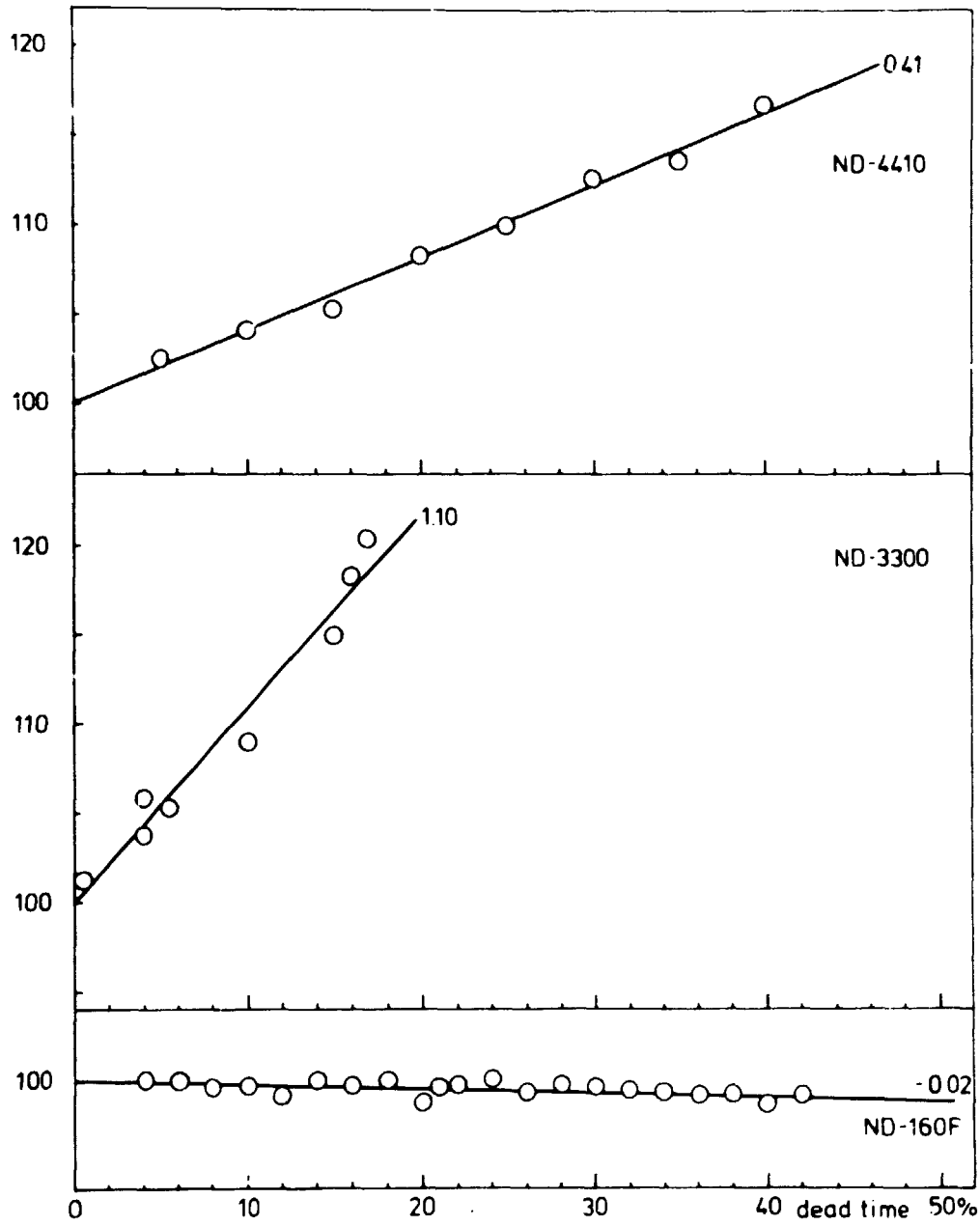


Fig. 21. Experimental investigation of the built-in dead-time compensation for three different multi-channel analyzers acquired in the period 1963-1971.

pulser peak are different. Peak area calculation methods are expected to fare better in this respect than methods based on the fitting of a Gaussian or similar function, and this was confirmed by Debertin [1977].

Results for the measurement of ^{137}Cs photo-peak areas relative to the 50 Hz pulser peak as a function of dead time produced by the

Table 17

Results for As in SRM 1571 Orchard Leaves by INAA [Heydorn 1973]

Total count-rate Analyzer model	Level of discrimination Low		High
4900 count/s	15	% dead time	10
ND 3300	9.65 ± 0.10	As mg/kg	9.09 ± 0.10
1700 count/s	16	% dead time	7
ND 4410	10.40 ± 0.12	As mg/kg	10.08 ± 0.13
1700 count/s	5	% dead time	3
ND 3300	10.31 ± 0.13	As mg/kg	9.97 ± 0.13

addition of ^{75}Se activity are plotted in Fig. 22. These measurements were carried out with a Histomat-N multichannel-analyzer by means of the built-in peak location and area calculation program [Philippot 1970].

This instrument is also equipped with a pile-up correction circuit designed by L. Nolkrantz [1974], and the adequacy of the system was checked in the same way as above by counting without pulser, but with pre-set live time, and the results are included in Fig. 22.

Both systems are satisfactory with average systematic errors of <0.5 % for dead times up to 20 % when counting long-lived isotopes. For isotopes which decay appreciably during counting, the pulser is useful for the simultaneous determination of live time, when counting is terminated by the end of a pre-set clock time, controlled by the built-in timer.

The contribution to the total systematic error of the analytical result from the measurement of the radioactive sample may be kept below about 1 % by careful consideration of a number of factors that may affect the sample and the comparator differently.

The influence of this measurement on the precision of the results is usually taken into account by counting statistics, and only counting geometry variations may affect the a priori precision. Too little attention to this point may easily result in relative standard deviations of about 5 % [Heydorn et al. 1977]. However, the careful design of detector assembly and source compartment may reduce this contribution to a standard deviation of less than 1 %.

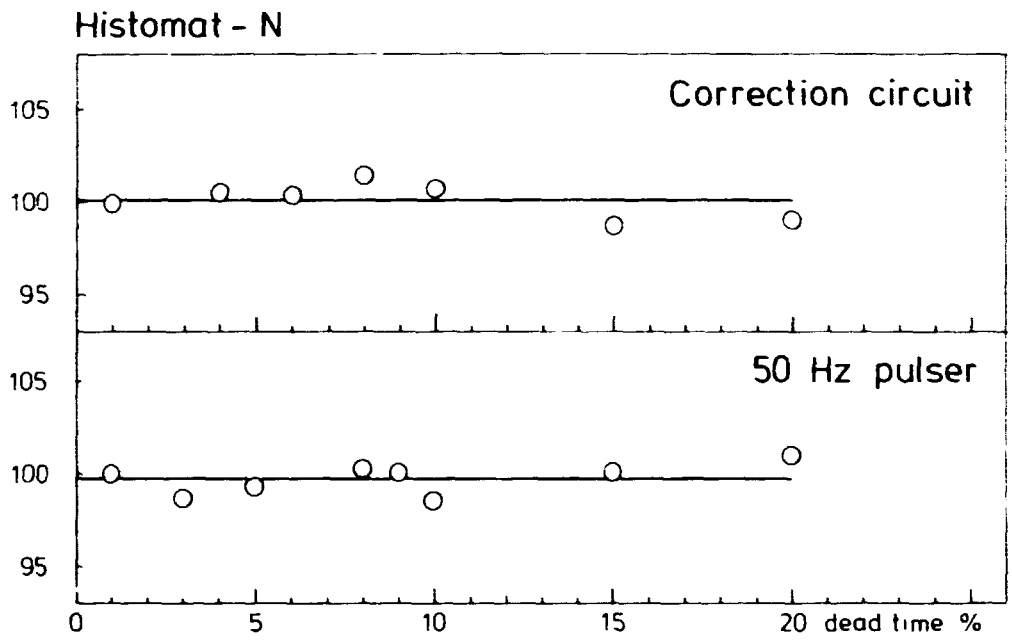


Fig. 22. Experimental verification of two different methods of dead time compensation used in the present work:
Compensation circuit by Nolkrantz and an external 50 Hz pulser.

2.7. Yield Correction

The determination of chemical yield is one of the outstanding possibilities in radioanalytical methodology, and originally the only purpose was to correct for possible losses of determinand during a radiochemical separation.

This is still the main purpose, but other systematic differences between samples and comparator may be compensated for or randomized at the same time by means of an appropriate yield correction factor. The actual chemical recovery of added carrier is included in this correction factor, but may not be calculated separately; factors compounded with recovery may include the effects of neutron flux density gradients, Section 2.4., pile-up at high count-rates [Damsgaard and Heydorn 1973], and interference from other elements in the sample [II].

Assumed yields

The actual yield correction factor for a particular sample may be derived from *previously* analyzed samples, or from investigations of the recovery of the analytical *method* by means of radioactive tracers

[Samsahl 1968]. Only when the recovery is practically quantitative in all cases investigated can the accuracy of a single determination be trusted. In the case of disagreement between duplicates, the highest result should be chosen rather than the mean value. From other points of view, such as possible sample contamination, however, the lowest results would be considered most reliable, and therefore duplicate values based on assumed yield correction factor are likely to lead to confusion.

Yield correction based on the *simultaneous* analysis of samples with *standard addition* was used in the method for the determination of vanadium in biological materials by pre-irradiation separation [I]. Here, a slow decrease in vanadium recovery is caused by the degradation of the interior surface of the quartz crucibles, accompanied by increasing retention of vanadium at the digestion stage. This effect is compensated for by including a sample with added standard in each batch, while making sure that all crucibles in a batch have been used the same number of times.[5] The accuracy of the results depends on the reproducibility of yields, which is not directly observable; tracer experiments were therefore used to establish a maximum permissible number of re-uses of quartz crucibles [I].

In the end *individual* yield correction determined for each sample was introduced in the method for determination of V, just as for all other elements for which chemical processing of the samples is required.

Only in this way can one control potential sources of variability, such as accidental losses of material, experience of the analyst, individual differences between the technique of the different analysts, as well as a host of other more or less well defined factors. Hereby, the accuracy of a single determination is ascertained so that expensive or unique sample material is not wasted, but utilized efficiently.

Only by individual yield correction determination can the a priori precision be estimated with sufficient accuracy, and only then are the differences between duplicate results representative of the actual precision of the analytical method.

The methods available for individual determination of yield correction require the addition of either carrier or tracer to all samples, comparator standards, and blanks.

Tracer addition

The use of a *radioactive tracer* for yield determination provides several advantages and was used in determinations of Mn [VII] and As [Damsgaard and Heydorn 1973] in preference to other methods. Obviously, only radionuclides that are not normally produced in the

5. Randomization of this factor would lead to considerably poorer precision of individual results.

irradiated sample and that have suitable decay characteristics can be used. When available in carrier-free form they can be used indiscriminately, even in pre-irradiation chemical separations, [Damsgaard et al. 1972], or for example in substoichiometric separations where carrier addition is not possible.

When the γ-ray energy of the tracer differs from that of the indicator isotope, the two radionuclides may be counted simultaneously, such as was done by Damsgaard and Heydorn [1973] with ^{74}As as tracer and ^{76}As as indicator. In this way errors from differences in pile-up, dead-time, or counting geometry are automatically compensated for with a resulting gain in overall accuracy. More recently, the same method was used by Iwashima [1976] for the determination of As in aerosols.

With ^{54}Mn as tracer and ^{56}Mn as indicator [VII], the γ-energies are too close for simultaneous determination by means of the scintillation detector, and the yield correction is determined in a separate counting after decay of the ^{56}Mn. In this case, compensation for differences in selfabsorption is just as complete as with re-irradiation, but pile-up errors are not eliminated.

In spite of their unquestionable advantage, the use of radioactive tracers for yield determination is very seldom reported, and no reference to the method is given by Norton [1967] in her monograph on chemical yield determinations.

Carrier addition

The addition of carriers to the sample before the radiochemical separation makes the determination of chemical yield a trivial task. Even when very small quantities of carrier are added, their determination can usually be carried out by many different analytical techniques with good accuracy, because interference from other elements is usually absent. Norton [1967] presents reliable methods for about 75 elements, most of them based on spectrophotometry.

Instrumental neutron activation analysis, or rather *re-irradiation yield determination*, was first reported by Kamemoto [1964] and has several advantages over other methods of yield determination. There is no loss by transfer from the counting vial, and when identical indicators are used for both analysis and yield, differences in self-absorption between sample and standard are automatically compensated for.

In addition, positional differences in neutron flux during the simultaneous irradiation of sample and comparator may be neutralized, and the influence of interfering elements may be reduced by multiple carrier addition, Section 2.5.

A practical example of the improvement of accuracy that can be obtained in the determination of As in biological material by our usual method was reported in [II].

The addition of antimony, copper and possibly gold carriers to the arsenic carrier in the ratio in which these elements are expected to occur in the sample will result in improved accuracy of the arsenic determination.

The degree of improvement can be calculated if data are available on the distribution of these trace elements in the sample material.

Such data for human hair have been published by Coleman et al. [1967], who found an approximate log-normal distribution for a number of trace elements, including Cu, Sb and Au. Table 18 lists the results of these authors for the male population of England and Wales.

A median value of 0.62 μg of arsenic per gram hair of males was taken from Smith [1962].

On the assumption of a complete absence of any correlation between the concentrations of these elements and arsenic, Fig. 23 shows the distribution of errors in the determination of arsenic in hair resulting from interference by Cu, Sb and Au. The effect of multiple carrier addition is to superimpose this error distribution on to the distribution of arsenic concentrations so that its mean value is not changed.

The resulting improvement in accuracy depends on the logarithmic standard deviation of the arsenic concentrations, and only very slightly on the mean arsenic concentration. The fraction of analyses with improved relative accuracy is calculated from the distributions of the relative errors for various standard deviations, and the results are presented in the second column of Table 11.

A positive correlation between arsenic and interfering elements increases the fraction of analyses with improved accuracy. Such correlation between As and Sb was reported by Perkons and Jervis [1965], and will significantly increase the percentages quoted since Sb is the element of dominating interference.

On the assumption of a complete correlation, i.e. constant ratio of concentrations of Sb and As, the fraction of analyses with improved relative accuracy is shown to be essentially 100 %.

In addition to the randomization of interference, re-irradiation yield determination also permits randomization of systematic neutron flux density differences between sample and comparator, as described in Section 2.4. When yield comparators and comparator standards are of the same physical and chemical form, the form of the counting sample is immaterial. This means that the possible self-absorption of γ-rays of ^{76}As in the concentrated ammonium-sulfide solution used in [IV] should be disregarded.

Table 18

Logarithmic distribution of trace element concentrations in hair in the male population of England and Wales [Coleman et al.1967]

Element	Mean		Standard deviation	
	Logarithm	mg/kg	Factor	Logarithm
Cu	2.555	12.9	2.6	0.952
Sb	-0.153	0.86	2.7	0.986
Au	-3.061	0.05	2.8	1.043

The large amount of carrier in a separated sample compared to other elements usually makes interference from other radio-nuclides quite unimportant. At the same time, some elements in carrier amounts give rise to considerable activities, even when irradiated for a short time. For some elements, the use of re-irradiation yield determination is not easily compatible with the use of the same decay time and the same counting conditions as in the measurement of the indicator activity.

In such cases a far less discriminating and much less sensitive detector such as the ionization chamber was found to be an attractive possibility, and in [V] 14 elements were selected as first choices for a practical test.

Among the elements of interest in the present studies, only As was of potential use; a modification of the original method reported in [IV] was therefore investigated, and it was found that yield determination by counting with a NaI(Tl) scintillation detector could be replaced by measuring ionization currents in the National Physical Laboratory ionization chamber.

The counting samples contain about 1 mg of arsenic carrier dissolved in ammonium sulfide solution.

Interfering elements give rise to an error, which can be expressed as the percentage of the ionization current i from the element to be determined at a concentration c, produced by other elements present in the sample.

The contribution from a particular element with the concentration c_i is

$$100 \frac{c_i}{c} \times \frac{\sum_j i_{ij}^* q_{ij}}{i^0} \leq 100 \frac{c_i}{c} \times \frac{i_i^0}{i^0} \sum_j q_{ij} \qquad (42)$$

where summation with respect to j takes into account the various

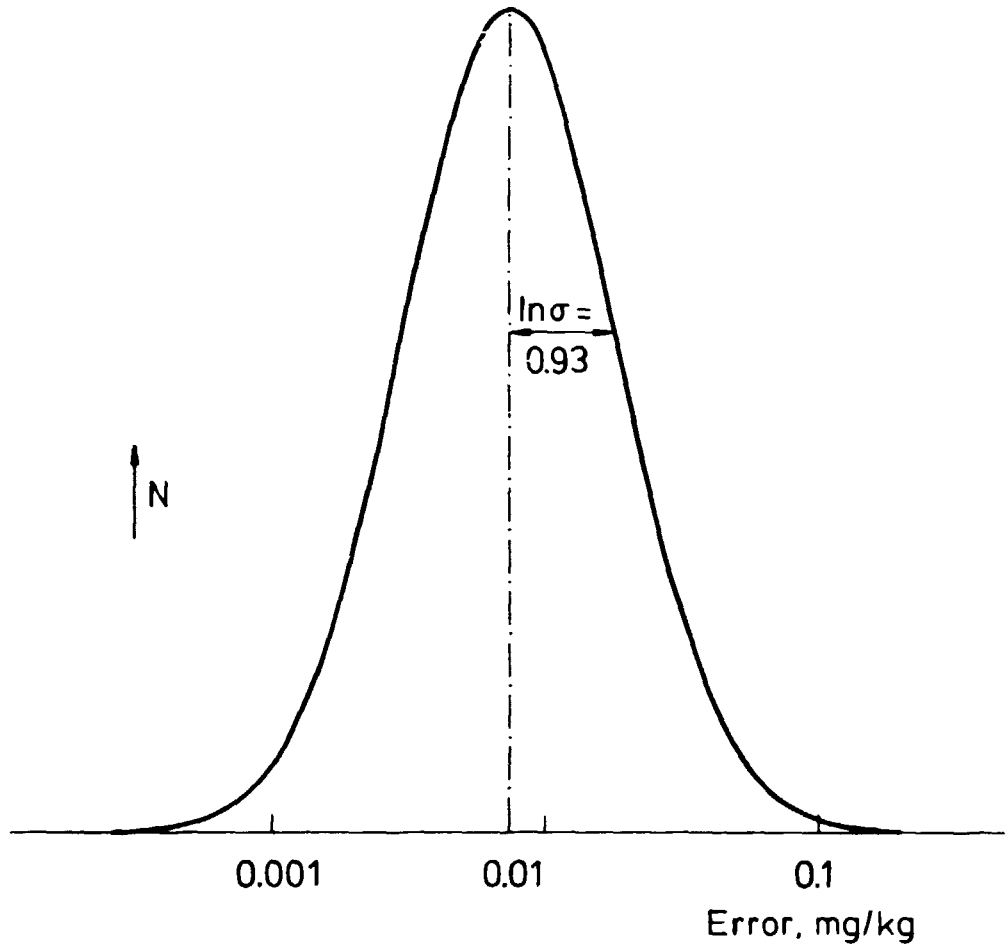


Fig. 23. Distribution of errors in the determination of arsenic in hair, based on chemical yield measurement [11].

radionuclides produced from the same element, and q_{ij} is the appropriate discrimination factor, shown in Fig. 10.

In practice, $\sum_j q_{ij} < 1$, and interference from elements with $i_i^0 / i^0 < 0.01$

may be neglected, unless these elements are present in higher concentrations than the element to be determined. Similarly, elements present in concentrations $c_i < 0.01 \cdot c$ may be disregarded unless their tabulated sensitivity i_i^0 is higher than i^0.

Moreover, q_i is easily estimated from the graph in Fig. 10, and if necessary $i\bigstar_i$ can be calculated for the most interfering nuclides.

In this way a reasonable estimate of the total error is obtained, and justification for the use of the ionization chamber for determination of a particular element can be assumed, or correction can be made.

Carriers of copper and antimony are added in the chemical separation, and from experimental separation factors [II] their concentrations relative to arsenic can be estimated at well below 1 %, while other impurities would be present only at the trace level. The estimation of interference from the elements in question made by means of calculated sensitivities [V] indicates that no significant error should be expected in the determination of arsenic by ionization chamber measurement of the irradiated samples.

The samples were irradiated together with a 1 mg arsenic comparator sample and an equal volume of ammonium sulfide blank in sealed, half-dram polyethylene vials for 20 min in the pneumatic tube system of the Danish reactor DR 2 to produce an ionization current of about 0.2 pA. The irradiated samples were measured in the ionization chamber at $1/\lambda - t_i \sim 38$ h after the end of irradiation, and yields were calculated after correction for blank and background. With less than 10 min between measurement of sample and comparator, decay corrections could be neglected together with corrections for temperature and pressure.

Measurements on 16 blank samples gave an average of $0.5 \cdot 10^{-14}$A, 5 fA, and blank variation was insignificant compared with the background fluctuation; a constant value could therefore be assigned to the blank, equivalent to 0.02 mg of arsenic at the time of measurement.

Neither Se nor Cu is particularly suitable for ionization chamber measurement as shown in [V], and both could be counted under exactly the same conditions as in the determination of indicator activity after re-irradiation for 10 seconds in the pneumatic tube system in DR 2.

Precision and accuracy

Both the radioactive tracer and re-irradiation yield determination permit the use of very small amounts of added carrier, although only the tracer method is applicable to methods without any carrier at all.

For the determination of trace amounts of determinand near the limit of detection, however, re-irradiation is superior to the tracer method for yield determination. A modification of the procedure for determination of Mn in serum [IX] was therefore introduced for the analysis of redistilled water blanks.

The limit of detection for manganese is determined by the concentration of ^{54}Mn tracer required for a satisfactory measurement of chemical yield. In the standard method a ^{54}Mn concentration was chosen to ensure a precision of better than 1 % for the chemical yield, and the

resulting limit of detection is practically the same as the manganese blank value.

The limit of detection for manganese is inversely proportional to the relative standard deviation of the chemical yield, so that a lower concentration of ^{54}Mn could be useful in the analysis of redistilled water blanks. However, if a limit of detection below 5 $\mu g/m^3$ is desired, yield determination by re-irradiation gives superior precision, and no ^{54}Mn tracer is added.

The same might be said of the use of ^{48}V as tracer in our analysis of biological materials. However, in this case no alternative exists for individual yield determination, and moreover the limit of detection is determined by actual variations in the blank value rather than by counting statistics.

The contribution of a yield correction to the analytical results consists in the main of an improvement to their accuracy, but at the same time its precision must be taken into account when estimating their overall precision.

Yield determination by counting a radioactive tracer or the re-irradiated carrier is taken into account by calculating its precision by counting statistics, and no special contribution is therefore included in the a priori precision. Only measurements by ionization chamber add to the a priori precision, but with a relative standard deviation at 1 % for a single reading [V], a total of 10 readings reduces the contribution to insignificance.

At the usual level of carrier addition, the accuracy of yield measurement is generally extremely good and requires no blank correction from impurities in the reagents. In the case of ionization chamber measurements of re-irradiated As-carrier, however, a blank value of 0.02 mg of As was found to be contributed from the irradiation container and was used as a constant blank correction in all cases. An accidental contamination of the ammonium sulfide with a small amount of sodium produces a much higher blank value, and routine batch control of even very small blanks should thus be performed in order to avoid significantly biased yield corrections.

Errors of this type may sometimes be detected by ascertaining that the yield, R_i, and the observed quantity of determinand, q_i, are structurally related. Their correlation coefficient should be close to *one*, and their regression lines should pass through the origin [0,0].

An application of a quality control of this type is shown in Table 19 for a set of 9 results for the concentration of As in SRM 1571 Orchard Leaves.

No significant correlation was found between the arsenic recovered and the chemical yield, and the best result was therefore calculated from the overall means of the two columns. Although this value is in

Table 19

Corresponding values of observed quantity and measured recovery of As radiochemically separated from SRM 1571 Orchard Leaves

Arsenic recovered mg/kg	Chemical yield %
7.57	77.7
7.72	81.1
7.71	82.0
7.68	85.0
7.60	81.5
8.17	85.1
8.25	77.6
8.16	76.0
8.29	70.2
7.90 ± 0.10	mean value 79.6 ± 1.6
Correlation coefficient	r = -0.49
Concentration of As	9.93 ± 0.23 mg/kg

good agreement with that recommended in [Damsgaard and Heydorn 1973], the regression line gives a highly significant positive value for recovered arsenic at zero chemical yield. Individual results are therefore not valid; the error could be ascribed to the presence of traces of Na in the ammonium-sulfide, the comparator as well as the samples; the utilization of peak boundary selection by Yule [1965] and not by Heydorn [VI] has the consequence that the contribution to the Total Peak Area of 559 keV ^{76}As by the annihilation peak of ^{24}Na decreases with increasing yield.

The use of individual yield determinations, combined with corrections for differences between sample and comparator, randomization of interference, etc., is essential for the accuracy of single results in neutron activation analysis with radiochemical separation or similar processing of the sample.

The detection of unexpected sources of variability by the Analysis of Precision of results from duplicate analysis of a number of different samples, however, also depends on the individual yields and their precision. Replicate analysis is sometimes assumed to substitute for individual yield determinations and an average yield based on previous

results is used. The use of the Analysis of Precision might here be carried out on the basis of a contribution to the *a priori* precision from the variability of yield corrections.

The applicability of this method and the magnitude of the actual variation of yields was investigated for arsenic by means of 170 individual determinations from the study of serum, plasma and whole blood [IV]. A histogram of the distribution of chemical yields determined by re-irradiation is shown in Fig. 24. All separations were carried out by the same person within a period of 12 months. Here the occurrence of 4 outliers is clearly seen; these cannot be corrected by an average yield and would thus produce meaningless results.

Even when these outliers are omitted, the distribution is strongly negatively skewed. A test for normality by the chi-squared distribution of the agreement between observed and expected frequencies gave a highly significant value of 31.85 for 5 degrees of freedom.

The replacement of individual yields by an average yield with a standard deviation, pooled with other sources of variation for the a priori precision, is therefore unlikely to produce satisfactory results of an Analysis of Precision of duplicate samples. Neither is in the actual case a relative standard deviation of 15 % of the chemical yield a satisfactory precision of the results.

A yield which is considerably lower than the average may be assumed to indicate gross deviations from the prescribed analytical procedure, and the result - although corrected for loss - may very well be erroneous or at least doubtful, unless substantiated by a replicate determination.

Particularly when a blank correction is necessary, such as for V in biological materials [Damsgaard et al. 1972] and [I], abnormal yields invalidate the assumptions normally underlying the subtraction of a blank value. The chemical yield of the blank itself is useful for the regular quality control, but it does not lend itself to individualization of blank corrections *per se*.

In the present study individual yield corrections for chemically separated samples have been applied throughout, and their contribution to the overall precision of individual results is in most cases estimated by the calculation of counting statistics.

2.8. Calculation

No other identifiable step in activation analysis has been treated in such detail and from so many different points of view as the processing of data from detectors of the indicator nuclides. With artificial data, all methods give true results but they differ with respect to precision.

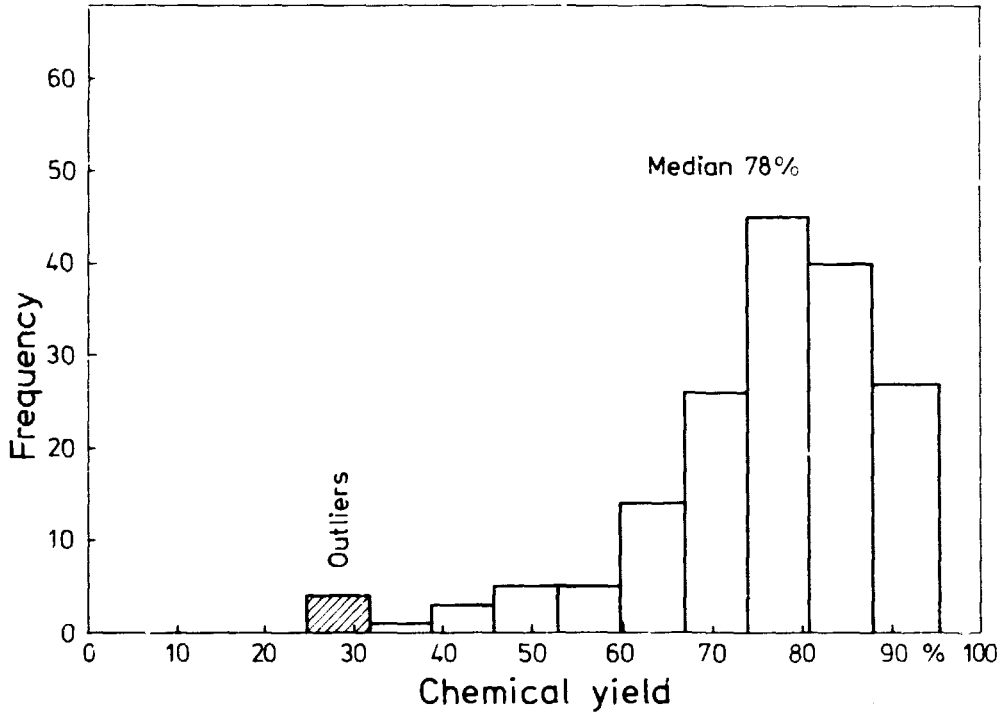


Fig. 24. Distribution of 170 chemical yields of arsenic obtained by a single analyst over a period of 12 months.

With actual data, differences in sensitivity towards imperfections or unsolicited contributions make the various methods differ also with respect to accuracy. There is no simple way of checking this important performance characteristic, but in 1976 the IAEA initiated an intercomparison of data processing methods for the calculation of results from unknown multichannel analyzer data.

The treatment of counting data from a multichannel analyzer, operated in the multiscaling mode, by decay curve resolution is described by O'Kelley [1962] and has been used in the measurement of very short-lived indicators by Heydorn et al. [1977]. In the present work only methods for the calculation of results from multichannel analyzer data for γ-ray spectra will be discussed.

Total count methods

The first point to consider is whether the *entire spectrum* is to be processed, or only those parts of it that are characteristic of the particular indicators. In instrumental neutron activation analysis, where no information on sample composition is available, the whole spectrum should be scanned by means of a peak location programme, such as described by Yule [1966] or Philippot [1970]. When all significant

peaks have been identified, the entire spectrum can be resolved into its indicator components by means of a weighted, least-squares procedure. This utilizes the spectral information better than all other methods, but the requirements to indicator spectra are difficult to fulfil. Corrections for even small changes in peak position or shapes must be provided [Eckhoff 1968, Yule 1969], and no safe method has been devised - not even a significantly negative result - for excluding possibly contributing isotopes [Yule 1971].

This means that there is no guarantee against occasional, significant systematic errors in the results caused by the absence of an appropriate indicator in the comparator spectra or in the sample.

Less sensitive to extraneous influence is the calculation of a result from a *selected part* of the spectrum where the determinand indicator predominates and the contribution from other radionuclides is at a minimum. Results based on the required indicator spectra may be found by means of a set of simultaneous equations, and in this case the accuracy of results may be checked by determining the interference from other elements, such as was done for V in [XI]. A similar procedure was used for the determination of Pb and Cl in air-filter samples by means of a single-channel analyzer with 7 scalers and at 2 different discriminator levels [Heydorn and Wacks 1973].

Removal of the most interfering elements by chemical separation before counting further improves the accuracy of a determination based on total counts in a selected part of the spectrum. In this case the remaining interference may be eliminated by computer stripping without undue deterioration of precision. This method was used in [I] for the determination of V in human serum, for which no discernible photo-peak could be envisaged.

Samples subjected to a radiochemical separation usually give rise to spectra with well-defined radionuclidic purity, and here an actual indicator spectrum of the determinand may be recognized and compared with a comparator spectrum. This permits matching of characteristic features such as photopeaks, resulting in improved discrimination towards other radionuclides.

Peak area methods

Quantitative results were mainly based on the comparison of peak-heights, until the introduction of digital methods of *photo-peak integration* by Covell [1959] increased the accuracy as well as the precision of activation analysis, as shown by Bowen and Gibbons [1963]. This method is still the most widely used method for photo-peak area determination. Its precision and accuracy are entirely satisfactory when the peak-to-base ratio is much greater than unity.

At lower peak heights, the contents of boundary channels become crucial, and a number of modifications of the method have been proposed over the years in order to reduce their effect on the precision and accuracy of small peak areas.

The Total Peak Area method as described by Yule [1966] is generally considered the most accurate method [Hoste 1971], because it is least sensitive to gain and base-line shifts [Yule 1969], as well as to variation in count-rate between samples and comparator standards [Yule 1968].

In subsequent years several other methods were introduced to improve the precision, and attempts were made to evaluate results from several methods [Yule 1968, Turkstra 1970, Baedecker 1971]. The outcome of such comparisons depends, however, to a large extent on the choice of experimental conditions.

Methods should always be compared under optimum conditions, and in this case Hertogen [1974] found no single technique superior among 10 different methods compared with the method of Covell. Only one additional method was amenable to exact calculation of the standard deviation, and its precision was generally better than that of Covell. This method of Sterlinski [1968] was therefore investigated more closely.

None of these methods is suitable for the evaluation of two or more photo-peaks that are not completely resolved, and this situation should be avoided by suitable choice of counting conditions or improved radiochemical separation. In cases where this is not possible, overlapping peaks can be resolved by fitting comparator peaks with known widths by means of a weighted, non-linear least squares fit based on a Gaussian peak with exponential tail, e.g. Routti and Prussin [1969].

Let a γ-spectrum recorded with a detector of resolution R, connected to a multi-channel analyzer at a gain g, contain a photo-peak of energy E and a total peak area P standing on a sloping continuum.

The pulse-height distribution N(i) is illustrated in Fig. 25, where the peak is centered in channel $I = E/g$ with a peak height a and a full width at half maximum $w = R \cdot I$.

The height of the continuum in channel I is b and its slope $\alpha = -c/w$ where

$$\frac{b}{a} = \beta \qquad\qquad \frac{c}{a} = \gamma$$

A given source and detector combination fixes E, R, β and γ, while P is determined by counting time, and w and I are determined by the gain of the system.

Calculation of the peak area by the method of Covell, using h channels on either side of the peak channel, gives

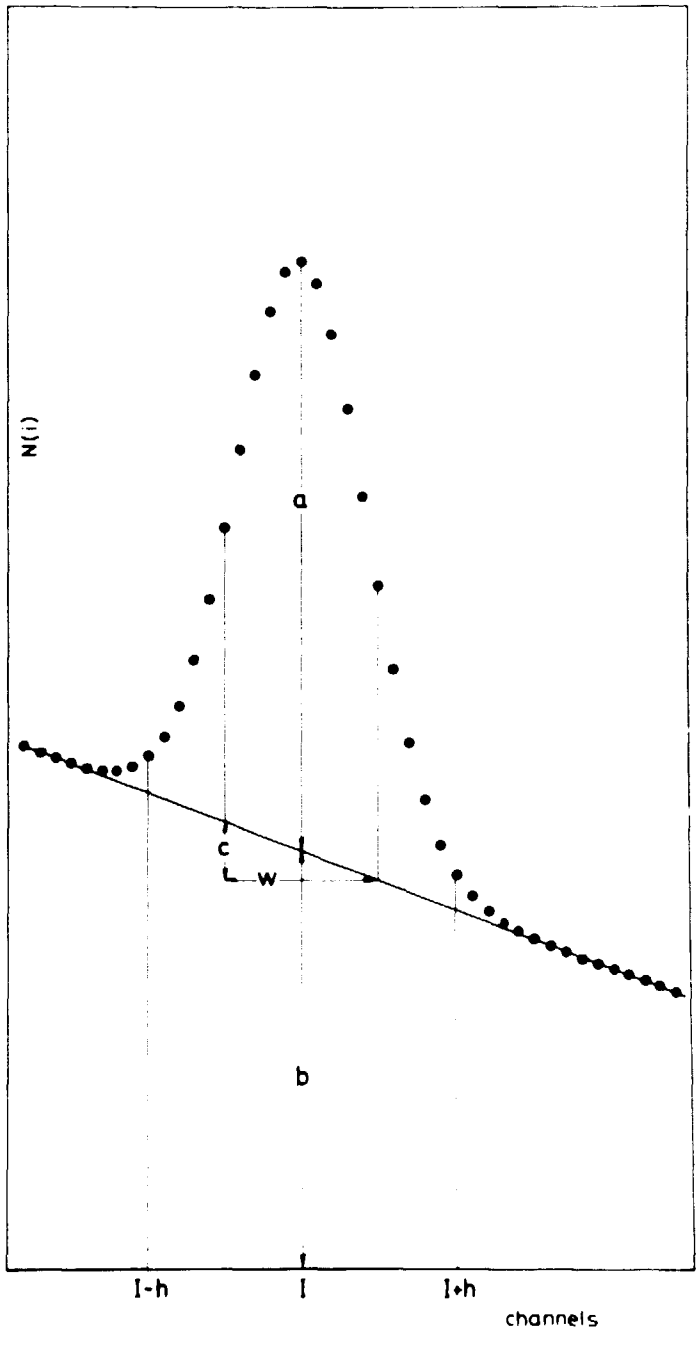


Fig. 25. Gaussian peak on a linear continuum [VI].[6]

$$A = \sum_{I-h+1}^{I+h-1} N(i) - (2h-1)\frac{N(I-h)+N(I+h)}{2} \qquad (43)$$

6. Reprinted with permission from Analytical Chemistry, Vol. 44, No. 14, Page 2313, December 1972. Copyright by the American Chemical Society.

The method of Sterlinski differs slightly according to whether an odd or an even number of channels is included in the peak

$$S_o = \sum_{1}^{h} (h - 2i + \tfrac{1}{2}) \left[N(I-i) + N(I+i)\right] + h \times N(I) \quad (44)$$

$$S_e = \sum_{o}^{h} (h - 2i) \left[N(I-i) + N(I+i)\right] \quad (45)$$

A similar method was developed at the Risø Radioisotope Laboratory shortly after the publication by Sterlinski [1968]. This does not require knowledge of the peak-channel and is therefore useful for very small peaks. Summation is over a total of (n+1) successive channels.

$$H = \sum_{o}^{n} N(i) \times \left[6i(n-i) - n(n-1)\right] \quad (46)$$

This method also allows the exact calculation of precision from the chosen weighting factors and counting statistics, and a direct comparison of the calculated relative standard deviations for the method of Covell, Sterlinski and the present one has been made by means of synthetic spectra. For a purely Gaussian photo-peak (β=0), the last method gives the best precision, but the difference from Sterlinski's method is negligible as can be seen from Fig. 26.

Both methods, however, are definitely more precise than Covell's under these conditions. On the other hand, differences in gain and zero shift between sample and comparator will introduce unaccountable systematic errors in these methods.

Covell's method, which is only distinguishable from the Total Peak Area (TPA) method by the choice of boundary channels, expresses the actual number of events within a specified energy range. This figure is not altered by changing amplifier settings.

Improvement of the precision of Covell's photo-peak integration may also be brought about by *smoothing*, which is exactly the opposite of the deconvolution used for resolving overlapping peaks. Smoothing increases interference from other radioisotopes and may therefore result in reduced accuracy; when interference is reduced to a negligible level by radiochemical separation, the improved precision of peak area determination for a single peak is worth while.

Smoothing by data convolution according to Savitzky and Golay [1964] was recommended by Yule [1967], who used an odd number of

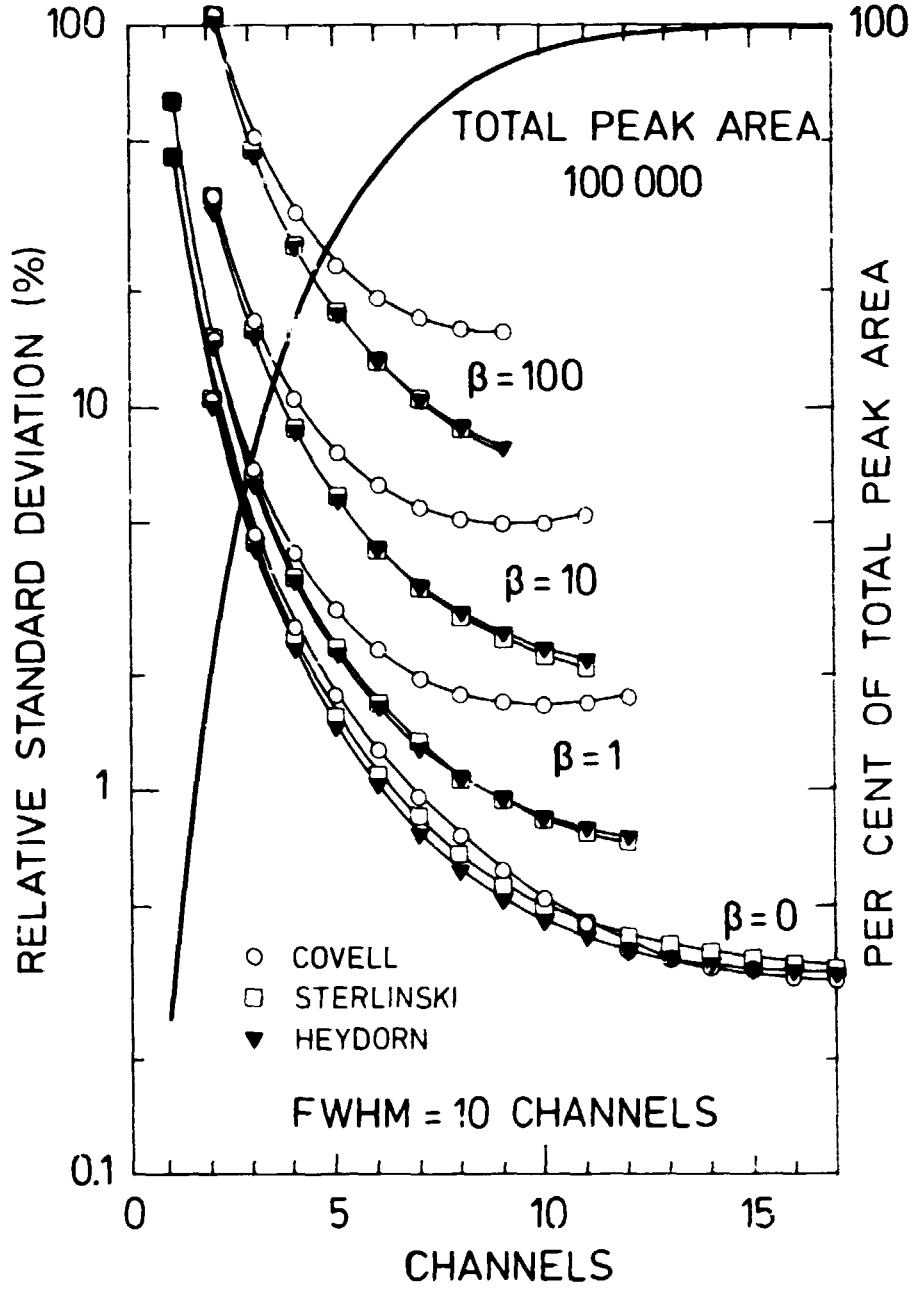


Fig. 26. Calculated precision as a function of integration half-width for 3 different methods of peak area evaluation for a Gaussian peak on a linear continuum with an average height β relative to the peak height.
Also shown is the peak fraction included in the area evaluations.

points equal to or less than the full width at half maximum (FWHM) of the photo-peak. Under these conditions, the peak area is not affected, and the reduction of peakheight is less than with a Gaussian filter [Tominaga 1972].

The weighting factors given in the original paper [Savitzky 1964] contain several erroneous values, but may be calculated by moment analysis [Sterlinsky 1975]. With FWHM > 5 the variance of a very small peak is reduced by a factor >2, and with an approximately linear base-line the Covell method with smoothing is superior to least-squares analysis [Tominaga 1972].

Actual methods

The most important characteristic of a peak together with width is its height relative to the height of the base-line, and no single method is the best to cope with all possible cases. In the present studies, therefore, several different methods of determining the intensity ratio of a γ-ray from two samples were used.

The choice of method was in each case made with two principal objects in mind, the absence of bias and the knowledge of precision. Only then can simultaneous improvement of accuracy and precision be achieved by pooling a number of analytical results.

The use of integral counts near the ultimate detection limit in [I] requires careful selection of energy range and accurate knowledge of contributions from all other radionuclides present in the sample. A comprehensive list of components in the γ-spectrum of a vanadium blank is presented in Table 20 together with their probable origin, cf. also Table 41.

The *energy range* selected for the determination of the ^{52}vanadium photo-peak at 1.44 MeV is restricted by the 1.28 MeV photo-peak of ^{29}aluminium and by the ^{28}aluminium Compton edge at 1.555 MeV, since too steep a variation of the count rate at the upper or lower limit of the energy range would tend to invalidate the blank in the case of even minor base-line shifts or variations in gain from sample to sample in the multichannel analyser.

The adopted energy range of 75 keV on either side of the 1.44 MeV photo-peak represents 10 channels at the usual multi-channel analyzer setting of 15 keV/channel.

It has a variation in blank value of less than one standard deviation for a ±0.5 channel shift, while still accomodating more than 95 % of the ^{52}vanadium photo-peak counts when a sodium-iodide scintillation detector of good resolution is used.

The only identified radioisotopes contributing to the count-rate in the selected energy range, apart from ^{52}V, are ^{28}Al, ^{29}Al, ^{38}Cl, and perhaps ^{101}Mo. As they all originate from trace elements in the matrix or the polyvial, their abundance is likely to vary from sample to sample, as is actually found to be the case.

Table 20

Components of γ-spectrum of V-blank [I]
irradiated in a TRIGA Mk I nuclear reactor*

Radio-nuclide	Half-life $T_{\frac{1}{2}}$	γ-energies MeV	Matrix elements major	Matrix elements trace	Polyvial trace
^{19}O	27 s	0.20, 1.38	x		
^{13}N	10 min	0.51	x		
^{28}Al	2.3 min	1.78		x	x
^{41}Ar	1.8 h	1.29		x	x
^{38}Cl	37 min	1.64,2.17		x	x
^{24}Na	15 h	1.37,2.75		x	x
^{80}Br	18 min	0.51,0.62		x	
^{56}Mn	2.6 h	0.85,1.81, 2.11		x	x
^{66}Cu	5.1 min	1.04		x	
^{128}I	25 min	0.44,0.53		x	
^{52}V	3.76 min	1.43		x	
^{29}Al	6.5 min	1.27,2.03, 2.43			x
^{101}Mo	15 min	0.19,0.59, 0.70,1.01		(x)	
^{65}Ni	2.6 h	0.37,1.11, 1.48		(x)	

*thermal to fast neutron flux density ratio ~ 1.2

The contributions from ^{29}Al and ^{101}Mo are so small, however, that even large variations in abundance would not significantly change the blank, whereas the influence of variations in ^{28}Al and ^{38}Cl has to be eliminated by stripping the difference spectra of the individual samples by means of standard spectra of the two isotopes. The stripping is carried on until the 1.78 MeV and 2.16 MeV photo-peaks disappear.

The same method of calculation was used in [XI] for the instrumental neutron activation analysis of air-filter samples for vanadium. Here the pooling of large numbers of blank values for filters of the fibrous type, as well as for membrane filters in solution or in a polyethylene bag, resulted in the accurate determination of a blank correction without any significant contribution to the uncertainty of results for actual samples.

In the majority of cases where complete knowledge of the components of a γ-spectrum is not available, the use of Covell's method

of peak area determination (43) was preferred. The precision of Covell's method also depends on the energy range used in the calculation, and particularly, when no peak is detected, the choice of boundary channels giving the lowest upper limit at a particular level of confidence assumes importance.

Peak boundary selection

Assuming the absence of correlation between different channels in the spectrum [Eckhoff 1969], the variance may be calculated as

$$V = \sum_{I-h+1}^{I+h-1} N(i) + (2h-1)^2 \frac{N(I-h)+N(I+h)}{4}$$

$$= A + (2h-1)(2h+1)\frac{N(I-h)+N(I+h)}{4} \qquad (47)$$

Maximum relative precision in peak area evaluation by means of Covell's method is achieved when h is selected so that $A/\sqrt{V}$ is maximized.

The pulse-height distribution shown in Fig. 25 can be approximated by a Gaussian curve superimposed on a straight line, expressed as

$$y = \frac{P}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{x-x_0}{\sigma}\right)^2} + \alpha(x-x_0) + y_0 \qquad (48)$$

where

$$\sigma = \frac{w}{2\sqrt{2\ln 2}} \qquad \text{and} \qquad (x_0, y_0) = (I, b)$$

Since

$$a = \frac{P}{\sigma\sqrt{2\pi}} \qquad \text{and} \qquad \alpha = -\frac{c}{w}$$

the introduction of

$$t = \frac{x-x_0}{\sigma} \qquad \text{and} \qquad \alpha^* = \frac{\gamma}{2\sqrt{2\ln 2}}$$

transforms expression (48) into

$$y = a\left(e^{-\frac{1}{2}t^2} + \beta - \alpha^* t\right) \qquad (49)$$

Calculation of the peak area by

$$A^* = \int_{x_0-h}^{x_0+h} y\,dx - 2h\,\frac{y_{x_0-h} + y_{x_0-h}}{2} \qquad (50)$$

is strictly equivalent to the calculation by Covell's method, see formula (43), and the corresponding variance given in eq. (47) is equivalent to

$$V^* = A^* + h^2 \times (y_{x_0-h} + y_{x_0+h}) \qquad (51)$$

provided $4h^2 >> 1$.

Determination of $h\star$ corresponding to

maximum of the function $A^*/\sqrt{V^*}$ (52)

is therefore equivalent to achieving maximum relative precision by the method of Covell.

The integration half-width $h\star$ is calculated by differentiating expression (52) with respect to τ where $h = \sigma \cdot \tau$

$$\delta\left(A^*/\sqrt{V^*}\right)/\delta\tau = \frac{V^*\delta A^*/\delta\tau - \frac{1}{2}A^*\,\delta V^*/\delta\tau}{V^*\sqrt{V^*}} \qquad (53)$$

which equals zero for

$$\frac{\delta V^*/\delta\tau}{\delta A^*/\delta\tau} = 2\,\frac{V^*}{A^*} \qquad (54)$$

With τ as the independent variable, eq. (50) is transformed into

$$A^* = \frac{P}{\sqrt{2\pi}}\left[\int_{-\tau}^{\tau}\left(e^{-\frac{1}{2}t^2} + \beta - \alpha^* t\right)dt - \tau\left(2e^{-\frac{1}{2}\tau^2} + 2\beta\right)\right]$$

$$= \frac{2P}{\sqrt{2\pi}}\left[\int_{0}^{\tau} e^{-\frac{1}{2}t^2}dt - \tau \times e^{-\frac{1}{2}\tau^2}\right] \qquad (55)$$

and eq. (51) becomes

$$V^* = \frac{2P}{\sqrt{2\pi}}\left[\int_{0}^{\tau} e^{-\frac{1}{2}t^2}dt - \tau \times e^{-\frac{1}{2}\tau^2} + \sigma\tau^2\left(e^{-\frac{1}{2}\tau^2} + \beta\right)\right] \qquad (56)$$

By means of the auxiliary variables

$$F = \frac{\int_0^{\tau} e^{-\frac{1}{2}t^2} dt}{\tau \times e^{-\frac{1}{2}\tau^2}} \qquad \text{and} \qquad B = \beta \cdot e^{\frac{1}{2}\tau^2}$$

and the derivatives of eqs. (55) and (56)

$$\delta A^*/\delta\tau = \frac{2P}{\sqrt{2\pi}} \tau^2 \times e^{-\frac{1}{2}\tau^2} \tag{57}$$

$$\delta V^*/\delta\tau = \frac{2P}{\sqrt{2\pi}} \tau \times e^{-\frac{1}{2}\tau^2} [\tau(1-\sigma\tau) + 2\sigma(1+B)] \tag{58}$$

eq. (54) can be expressed as

$$\frac{\tau(1-\sigma\tau)+2\sigma(1+B)}{\tau} = 2\,\frac{F-1+\sigma\tau(1+B)}{F-1} \tag{59}$$

Separation of B gives

$$1+B = \frac{1-\sigma\tau}{2\sigma} \times \frac{(F-1)\times\tau}{F-1-\tau^2} \tag{60}$$

Equation (60) shows that the optimum value $h\bigstar = \sigma \times \tau$ is independent of P, the absolute number of counts in the photo-peak, and of α, the slope of the continuum.

This value is determined solely by the relative height of the continuum, β, and the full width at half maximum $w = 2.355\ \sigma$.

Equation (60) determines the relationship between the optimum halfwidth $h\bigstar$ and the parameters β and w, but it is not applicable for explicit calculation of $h\bigstar$.

The range of values assumed by β is virtually unlimited, while w is restricted in practice to the interval [1;25]; a graphical representation of $h\bigstar$ as a function of β for selected values of w is therefore a convenient solution of eq. (60).

Interpolation between discrete values of w then becomes necessary, and this is greatly facilitated by expressing $h\bigstar$ in units of w.

In Fig. 27 the optimum half-width $\frac{\tau}{2\sqrt{2\ln 2}} = \frac{h^*}{w}$ in

units of full width at half maximum is shown as a function of β in the range [0.1;1000] for selected values of *w*. The β scale is supplemented by a scale of 1/β showing the corresponding peak-to-base ratios.

For $\beta \to 0$, the optimum half-width $h\star \to \infty$ for all *w*, but more interesting is it that for $\beta \to \infty$, the value of $h\star/w$ becomes independent of *w*. This limiting value is determined by the denominator in eq. (60),

$$F - 1 - \tau^2 = 0 \qquad (61)$$

which is satisfied for $h\star = 0.90758\ w$.

The maximum signal-to-noise ratio or asymptotic ratio [Currie 1968] is found for F=2 with 0.59452 *w*. This concept refers to the situation where the mean value of the base is known with infinite precision, a case treated by Fedoroff [1971]. This is not assumed in the application of the Covell method, which therefore has its optimum at a greater integration width. The previous calculation of optimum channel width by Reber [1963] for a single-channel analyzer assumes that the base-line is accessible for direct measurement. These results would be more applicable to the method of Wasson, reported by Baedecker [1971].

The value of 0.90758 *w* is of particular importance when no peak has been detected, because it results in the lowest upper limit at a given level of confidence.

Discussion

In spite of deviations from the Gaussian shape, particularly for the photo-peak in the Ge(Li) detector spectrum, and in spite of a pronounced non-linear shape of the base in the scintillation detector spectrum the mathematical calculation of the point of maximum precision was verified under experimental conditions [VI].

This means that the graphs in Figure 27 can be used to predict the best boundaries for peak area calculation by the method of Covell, also for spectra where no definite photo-peak is observed. Selection of boundaries nearest to $I \pm h\star$ will in many cases be tantamount to choosing 1 full width at half maximum at either side of the peak channel.

The observed decrease in precision with increasing deviation from the optimum half-width $h\star$ is largely independent of the relative peak height, and it was quite similar for the two different detectors investigated. The proper choice of *h* is most important for measurements near the limit of detection with Ge(Li) semiconductor detectors, where long counting times are involved; the per cent increase in counting time equivalent to a deviation of *h* from $h\star$ is shown in

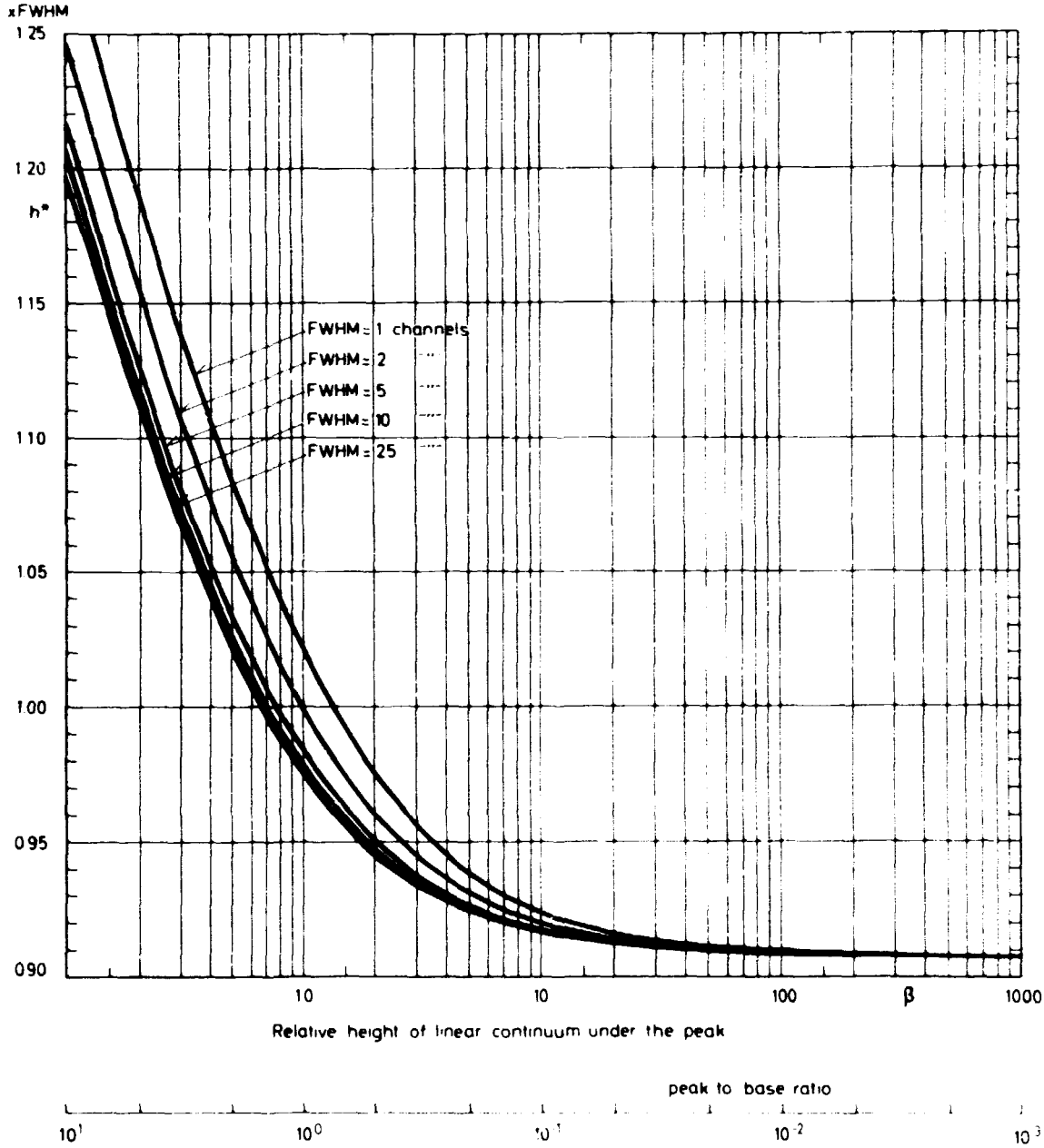


Fig. 27. Optimum half-width of Gaussian peak fraction for peak area evaluation by the method of Covell as a function of height of linear continuum under the peak for selected values of full width at half maximum [VI].[7]

Fig. 28, which represents the generalized shape of the curves for a detector with $h\bigstar \sim 5$ channels.

The method yields accurate results provided that the peak is free from interference from extraneous γ-rays, and its base may be accurately represented by a straight line - or is accurately known from a

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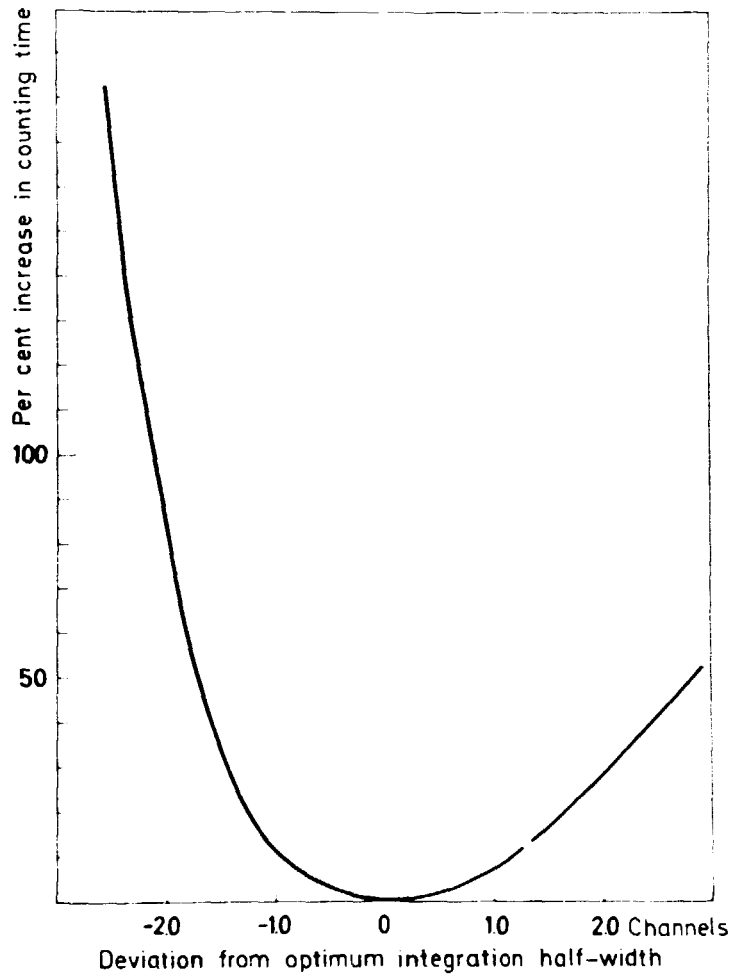

Fig. 28. Increase in counting time required to compensate for loss of precision caused by deviation from optimum integration half-width for a Ge(Li) detector [VI].[8]

reference spectrum. Calculation of peak areas and their standard deviations is easily done manually or with a simple desk calculator, which facilitates the implementation of the method in the analytical laboratory.

A comparison has been made with the method of total peak area calculation, using peak boundaries selected by means of the criteria introduced by Yule [1966]. The accuracy of this method is less influenced by variations in gain and resolution [Yule 1968] but rather more by base-line curvature, because of the larger number of channels involved.

Calculations of total peak areas were made by using a 5-point smoothing of data, and it was found that in spite of the improvement in precision resulting from the use of smoothed boundary values, the

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relative standard deviation exceeded that of the Covell method in all cases.

For large relative peak heights, the difference was negligible, but for small peaks where the boundaries are determined solely by the shape of the Compton continuum, the equivalent increase in counting time was 40 % for the Ge(Li) detector and 140 % for the scintillation detector.

For peaks with a height less than the base, results reported by Turkstra [1970] agree with [VI] that the method of Covell gives better results. The difference between the results is basically caused by the difference between the selection of peak boundaries: a priori selection for Covell according to [VI], but a posteriori for the Total Peak Area method according to Yule [1966].

The use of amplifiers with pole-zero correction together with base-line restoration and pulse pile-up rejection has greatly reduced shifts and resolution changes in connection with semiconductor detectors. For such counting systems, the classical method of Covell using optimum boundary channels appears preferable to the total peak area method with respect to precision as well as accuracy, when relative peak heights are less than unity.

If the number of channels in a peak becomes very small, the difference between various methods disappears, and if practically all photo-peak counts are found in only 1 channel, good precision for small peaks is found when using the mean of the 2 nearest channels as base-line. This situation was described by Taczanowski [1975], who recommended a channel width of 1.5 FWHM for optimum precision for very small peaks. The accuracy when using such a crude energy-discrimination is very poor, and interference from other radionuclides will be severe. However, the operation of a Ge(Li) detector in combination with, e.g., a 400-channel analyzer may necessitate this type of operation in order to cover a reasonable energy range.

Proper utilization of the detector resolution by the least-squares method requires at least 3 channels per FWHM [Sasamoto 1975], and for photo-peak integration methods >5 channels is definitely preferable, such as was the case in the experimental part of our work [VI].

With the resolution of better than 2.0 keV that is easily obtained with Ge(Li) detectors, the counting of an entire spectrum for instrumental neutron activation analysis requires a large number of channels.

In the present work a channel width of 0.4 keV/channel was found to be a satisfactory compromise when using a multi-channel analyzer of up to 7168 channels, and the instrumental determination of V in SRM 1571 Orchard Leaves was attempted from such spectra.

Pooled results from 11 spectra are shown in Table 21 for an integration half-width of h=5 channels, and for comparison purposes results calculated by the method of Sterlinski and our own method are

Table 21

Concentrations of V in SRM 1571 Orchard Leaves
determined by instrumental neutron activation analysis

Integration formula	mg/kg	Standard deviation
Covell (43)	420	25 %
Sterlinski (44)	337	20 %
Heydorn (46)	334	20 %
Damsgaard et al. 1972	41[illegible]	10 %

also included. No peak could be detected by the TPA method according to Yule [1966] in any of the spectra.

No significant difference is found between the 3 values; but it should be noted that the agreement with the value reported in [Damsgaard et al. 1972], which is based on a separated sample, does not support the notion that Covell's method gives large systematic errors with very small peaks [Hertogen 1974].

The use of NaI(Tl) scintillation detectors usually guarantees a considerable number of channels in a peak, even when operated under our usual conditions of 6.67 keV/channel. Again, the difference between our Covell method and the total peak area method is a matter of choosing peak boundaries *a priori* or *a posteriori*.

This feature assumes importance when correction for interference has to be made; interference is determined in a separate run with suitably chosen peak boundaries. If these are not the same as those in the actual analysis, a systematic error is introduced [Damsgaard 1975].

In addition, it was observed that the peak boundary selection method of Yule [1966] introduces a non-random component into the evaluation of very small photo-peak areas. This is characteristic of a technique incapable of yielding negative results, and a positive bias results from the abnormal distribution of peak areas near the limit of detection. This factor manifested itself by the appearance of an abnormally small variability of results for As in human serum after correction for Br interference [X].

As a consequence of the above investigations, we found that the *a priori* boundary selection method described in [VI] was preferable for our evaluation of peaks with a peak-to-base ratio of unity or less.

For large, well-shaped photo-peaks, the original method of Yule continues to be used, because of its slightly better precision and lack of sensitivity to minor spectral variations.

3. Precision of the Analytical Method

The set of rules that constitutes the precision of an analytical method must be based on an evaluation of the magnitude of all known or suspected random errors, such as made in the preceding chapter.

This approach is not limited to activation analysis, and may well be adapted to all other analytical methods when the appropriate factors are considered [Rothman 1975]. Interactions between factors, however, may require very extensive investigations, and the overall effect on the precision of an analytical result may be very difficult to estimate for some analytical techniques.

In such cases the precision of the method may have to be based on purely empirical, a posteriori evaluations of the precision of replicate results for actual or selected types of samples.

Classical methods

Such a posteriori evaluation has been the tradition for many classical analytical methods with an incomplete theoretical basis. The overall random errors are determined - along with possible systematic errors - by repeated analysis of samples to which known quantities of determinand are added [Linnig 1954], or of Standard Reference Materials with certified concentrations of determinand.

It is usually assumed that the relative standard deviation is a constant, and that the analytical variation closely follows a normal distribution. These assumptions do not hold for concentrations less than 2 orders of magnitude above the detection limit [Thompson 1976], and estimates of the precision of results at the trace or ultratrace level are consequently unreliable.

Thompson [1973] therefore introduced an absolute standard deviation, σ_o, which was added to the relative contribution

$$\sigma = \sigma_0 + k \times c \qquad (62)$$

where the constants were determined experimentally from duplicate results with actual samples by linear regression.

The range of these methods is limited to the range of concentrations for which experimental tests of precision and accuracy have been made [Wilson 1973]. Once established, eq. (62) or simpler expressions may be used as a priori precision for new samples analysed [Gottschalk

1976], and the validity may be tested by the analysis of precision on duplicate results with a chi-squared test [VIII].

The empirical nature of eq. (62) makes extrapolation into the ultratrace level of concentration, <10 μg/kg, inacceptable.

Contemporary methods

For analytical methods with a precisely known theoretical basis, the individual sources of variability are well defined, and their corresponding contribution to the overall precision may be determined by specific measurement, instead of implicitly by actual analysis of samples.

Here the analysis of precision serves to verify the algorithm for the estimation of overall precision from knowledge of individual variance components. This is again most properly done by repeated analysis of Standard Reference Materials at different concentrations and in different matrices.

If good agreement is achieved in all cases between actual and estimated variability of results, then the precision of the method is well established. Hence it can be expected to give accurate estimates of *a priori* precision also outside the range of materials and concentrations for which experimental tests were made.

For *nuclear activation analysis* with carrier addition and radiochemical separation, the *a priori* precision is entirely independent of the concentration of the determinand. The precision of a single, analytical result must, however, be supplemented by an estimate of the precision of the counting process based on Poisson statistics.

With the inclusion of this so-called counting statistics in the overall precision, the analysis of precision based on duplicate analysis of actual samples may again be performed with the chi-squared test [VIII].

The inherently constant a priori precision is particularly advantageous for neutron activation analysis in relation to its tremendous range. Verification of precision may thus be carried out by repeated analysis of Standard Reference Materials at the trace level, or even higher concentrations, where the contribution from counting statistics is negligible. The adequacy of estimates of counting precision may then be tested separately if necessary.

The extension of precision to the ultratrace level, or to applications where no suitable reference materials are available, should therefore be a matter of course; this is probably the most important distinguishing characteristic in the comparison of RNAA with other contemporary methods of analysis.

Neutron activation analysis

With special reference to neutron activation analysis, the present **Thesis** of correct *estimation* of the precision of an individual, analytical result is discussed in the first part of this chapter. The second part describes its experimental verification by the *analysis of precision,* as well as its **Antithesis** that the estimated precision is not always in acceptable agreement with the observed variability of results.

In the **Synthesis** this is interpreted as the detection of unexpected sources of variation that may affect the reliability of results. Oppositely, the statistical *control of precision* assures the quality of analytical results by the absence of unexpected errors.

3.1. Estimation

The precision of a single analytical result represents the overall effect of a number of independent, random errors, associated with different stages of the analysis.

$$\sigma^2 = \sigma_a^2 + \sigma_b^2 + \sigma_c^2 \ldots\ldots\ldots\ldots\ldots \sigma_n^2 \qquad (63)$$

The individual contributions are determined by the actual experimental conditions under which a particular result is obtained, and they will depend on the type and quantity of material analysed, as well as on the result itself. In a rigidly standardized procedure for routine analysis within a limited range of concentrations, the precision of the analytical method may be expressed simply as a constant absolute or relative standard deviation.

In activation analysis the useful range of an analytical method may cover many orders of magnitude, and different components of the total variance will dominate at different levels. In addition, the statistics of the counting process introduce a variance component into equation (63), and this may well be different even for identical samples. The precision of an activation analysis method must therefore specify the estimation of individual variance components, and the precision of a single result is estimated as the overall effect of counting statistics, and of relative and absolute random errors.

The contributions to the overall precision from all sources other than the counting process may be pooled to represent the *a priori precision* [Heydorn and Parr 1973], in contrast to the contributions from counting statistics estimated a posteriori by means of instructions included in the precision of the analytical method.

Distribution of results

The following *mathematical development* corroborates the analogy between the known, a priori precision, and the estimated precision based on the actual measurement of the sample.

Let a number, M, of different materials be subjected to repeated analysis, and let the total number of analytical results be N, where $N \geq 2M$. Let the mth material be analyzed I_m times, and let Y_{im} represent the ith result. Thus we have

$$m = 1, \ldots\ldots\ldots\ldots\ldots\ldots M$$

$$i = 1, \ldots\ldots\ldots\ldots\ldots\ldots I_m \quad \text{for material } m$$

and

$$\sum_{\bullet}^{M} I_m = N \tag{64}$$

With an estimated variance of the results $\hat{\sigma}^2_{im}$ their absolute weight is given by

$$\frac{1}{\hat{\sigma}^2_{im}} = w_{im} \tag{65}$$

and their mean value is estimated by

$$\hat{\mu}_m = \frac{\sum_{i}^{I_m} w_{im} y_{im}}{\sum_{i} w_{im}} \tag{66}$$

in strict analogy with the case of known precision.

For all materials together, the residual sum of squares, T, is given by

$$T = \sum_{m}^{M} \sum_{i}^{I_m} w_{im} (y_{im} - \hat{\mu}_m)^2 \tag{67}$$

which can be shown to approximate asymptotically to the χ^2-distribution with

$$\sum_{m}^{M} (I_m - 1) = N - M \quad \text{degrees of freedom}$$

as in the case of known precision.

This does not mean, however, that such an approximation is justified for the analysis of precision of a limited number of actual analytical results.

If each material is analyzed in duplicate, equation (67) is reduced to

$$T = \sum_{m}^{M} \frac{(y_{1m} - y_{2m})^2}{\hat{\sigma}_{1m}^2 + \hat{\sigma}_{2m}^2} \qquad (68)$$

Provided $(Y_{1m} - Y_{2m})$ has a normal distribution with zero mean and variance $(\sigma_{1m}^2 + \sigma_{2m}^2)$, and $(\hat{\sigma}_{1m}^2 + \hat{\sigma}_{2m}^2)$ is a central estimate of $(\sigma_{1m}^2 + \sigma_{2m}^2)$, the individual terms in the expression

$$T = \sum_{m}^{M} \frac{(y_{1m} - y_{2m})^2 / (\sigma_{1m}^2 + \sigma_{2m}^2)}{(\hat{\sigma}_{1m}^2 + \hat{\sigma}_{2m}^2) / (\sigma_{1m}^2 + \sigma_{2m}^2)} \qquad (69)$$

will have a χ^2-distribution with 1 degree of freedom in the numerator, whereas the estimate of the denominator is unity.

If the relative standard error of the estimated precision is sufficiently small, then the values of T have a χ^2-distribution with M degrees of freedom; if in addition $(\hat{\sigma}_{1m}^2 + \hat{\sigma}_{2m}^2)$ is independent of $(Y_{1m} - Y_{2m})$, the degree of approximation is directly expressed by the deviations from unity of the denominator in equation (69).

Under such conditions the statistic T in equation (68) is suitable for testing the adequacy of the estimated precision to account for the observed variability of duplicate results.

For analytical results based on the measurement of radioactivity, estimates of precision based on the *Poisson distribution* are of paramount importance.

Let the analytical results be calculated as

$$y_{im} = S_{im} \times P_{im} \qquad \text{and} \qquad \hat{\sigma}_{im}^2 = S_{im}^2 \times P_{im}$$

where the factor *S* converts the recorded number of counts, *P*, into the final result.

The individual terms in equation (68) now become

$$T_m = \frac{(y_{1m} - y_{2m})^2}{\hat{\sigma}_{1m}^2 + \hat{\sigma}_{2m}^2} = \frac{(P_{1m} - \varkappa_m P_{2m})^2}{P_{1m} + \varkappa_m^2 P_{2m}} \qquad (70)$$

where

$$\varkappa_m = S_{2m} / S_{1m}$$

For $\kappa=1$, all conditions specified in the preceding section are fulfilled. [Fisher 1922].

For κ in the neighbourhood of 1, the correlation between the terms $(P_{1m} + \kappa_m^2 P_{2m})$ and $(P_{1m} - \kappa_m P_{2m})$ is small, so that deviations from the χ^2-distribution are caused only by deviations from unity of the denominator in equation (69).

Application of the χ^2-distribution to test the adequacy of estimates of precision based on Poisson distributions by means of equation (68) is therefore justified.

The applicability of Poisson distributions to the estimation of counting statistics is a fundamental problem in all types of radioanalytical work; the estimation of a priori precision is a practical problem with bearings on analytical work of any type.

A priori precision

The estimation of *a priori* precision may be based on two principally different methods, both of which were used in the present study.

The traditional method determines the precision of a routine analytical method by replicate analysis of a number of samples within the appropriate range of the method. This was done in [IV] for the method described in [II] over the concentration range 2-200 mg/m^3 by means of a total of 39 determinations on 18 different samples.

Even though the contribution from counting statistics could be disregarded, the precision of results within the range investigated could not be expressed by a single parameter. Decomposition of the a priori precision into contributions from absolute and relative, random errors was therefore carried out by successive approximations [IV]. An a priori standard deviation was found to be the combination of 5 % relative and 2 mg/m^3 absolute, random errors; calculations were performed by means of a manual, but computer-aided, iterative procedure, similar to the approach later reported by Currie [1972].

The estimated standard deviation $\hat{\sigma}$ of a single result $\hat{\mu}$ in ng is here expressed as

$$\hat{\sigma}^2 = s_a^2 + s_r^2 + \sigma_c^2 \qquad (71)$$

with $s_a = 2$ ng, $s_r = 0.05 \times \hat{\mu}$, and σ_c^2 derived from counting statistics, which in these cases gave <0.5 ng.

The quality of the estimate depends on the number of replicate samples analyzed, and for a routine method a satisfactory number of degrees of freedom may be reached within a relatively short time.

For analytical methods at the stage of development, or during a process of modification, however, this approach is of little use. The

effect on precision of even minor changes in the procedure is not known, and if improved precision is required the point of attack must be decided by guesswork.

Much more useful information is available when the a priori precision is estimated from the individual contributions from a number of sources known to be present in the analytical method. For activation analysis methods, a number of such sources of variation are discussed in the preceding chapter; however, conventional sources of variation, such as volumetric dispensing, transfer operations, etc., should not be overlooked as potential contributors to the overall precision of the analytical result.

The variability of some of these factors may be known from past experience, such as the standard error of addition of 100 mm^3 portions of carrier for subsequent re-irradiation yield determination [VII]. The addition of 10 mm^3 portions of carrier-free ^{48}V tracer for yield determination, however, deserved a special investigation [Damsgaard et al. 1972], not only because of the smaller volume, but also to ascertain the absence of adsorption problems with this highly dilute solution.

If individual yield determinations are not carried out [I], tracer experiments must provide not only the average yields of samples and comparators, but also their variability; this was found to increase with the number of dry ashings carried out in quartz crucibles for the determination of V in human serum, and the crucibles had to be discarded after a maximum of 5 consecutive ashings to keep the standard deviation below 4 %.

Also in instrumental neutron activation analysis, where the sample is transferred to an unirradiated container, the variation in transfer efficiency was investigated and found to be <1 %

The same precision was obtained for yield determination by means of an ionization chamber [V] read by the aid of a digital voltmeter. Human reading of the usual analogue instrument was found by Gysel [1953] to produce highly abnormal distributions of results, because of personal digit preferences.

The vertical flux density gradient in the neutron irradiation facility, which is compensated by the re-irradiation yield correction principle, also gives rise to a random error, corresponding to a standard deviation of ~0.6 %.

The contributions to the overall standard deviation of the results are not only relative, but also absolute. To this category belong variations in blank value that are caused by the variable addition of reagents to the sample before irradiation, [V] and [Damsgaard et al. 1972]. Contamination of the sample during conditioning, such as by ashing in porcelain crucibles [I], is *not* a legitimate part of the a priori precision, because randomization of this systematic error is not possible.

The determination of the standard deviations of different steps in the analytical method is often conveniently carried out by radioactive methods, but should not be affected by counting statistics. Activity at levels higher than those usually encountered may therefore be needed, and it is added in the form of a suitable tracer.

Alternatively, the analysis of reference materials with higher than normal concentrations of the determinand may be used for measurement of the a priori precision. However, such samples tend to get preferential treatment, so that the variability of results for reference materials is significantly lower than for real samples [Byrne 1976].

Counting statistics

The a priori precision is affected by the quality of the analytical work, which is determined not only by the equipment used, but also by the dexterity and competence of the staff involved, some of whom may be outside the control of the analytical chemist.

In contrast, *counting statistics* is determined by the nature of the counting process, being of course the result of radioactive decay. At low levels of the determinand, the contribution from counting statistics to the overall precision may completely dominate the a priori precision.

Calculation of the precision of a peak area or similar parameter used for the determination of an element by neutron activation analysis, is usually referred to as counting statistics. It is based on the assumption that radioactive decay follows a Poisson distribution that can be approximated by a Gaussian distribution with the same mean and variance for expected values above 10.

With large counting efficiencies and counting times of several half-lives, Rogers [1972] found smaller variances in agreement with the Bernoulli distribution [Stevenson 1966] with binomial statistics. However, Currie [1971] as well as Taczanowski [1974] showed that in activation analysis the generalised counting efficiency must include the fraction of nuclei activated during irradiation, which means that the Poisson distribution applies even for infinite counting time.

Identity of mean and variance was verified by Plesch [1973] in repeated countings to ascertain the proper functioning of his counting equipment. A more thorough experimental control of the actual distribution of radioactive decay events was attempted by Berkson [1975], who found significant deviations in an examination of 250 000 disintegration time intervals. The type of discrepancy, however, did not exclude instrumental error.

The calculation of counting statistics from multi-channel analyzer data is based on one additional assumption, namely the absence of covariance between channels.

This assumption was checked by Eckhoff [1969] by means of 696 spectra of ^{137}Cs, each with 64 channels. His results justify the assumption of zero covariance between channels, as well as the normal distribution of counts in the individual channels, for all practical purposes.

This assumption is no longer true when data convolution or spectrum smoothing is applied in order to improve precision. The statistical scatter of data smoothed according to Savitzky and Golay [1964] may be reduced by a factor of 2 or more [Quittner 1972], but the covariance between channels can no longer be disregarded.

It is therefore necessary to incorporate the smoothing process in the method for calculating peak area or similar parameters, so that the standard deviation of the final result is determined from the original, unmodified data by means of the generally accepted procedures for the propagation of independent errors. This is done in all our computer programs used for the calculation of precision at Risø, because it is a prerequisite for the successful control of observed precision.

Complete agreement with the observed variability of results is only achieved by correct calculation of the variance according to the formulas given in Section 2.8. [Heydorn 1978]. The most common error is an overestimation of the calculated variance, which then conceals other unexpected sources of variation.

Detection of such errors usually necessitates a detailed investigation of the actual computer program, which has only been possible in a few cases, such as the DERAN program [Jacobi 1977] and the GRETEL program [Guzzi 1974]. In the last case, the calculated variance V^* exceeds the true variance V by

$$V^*-V=(2h+1)\times\left[N(I-h)+N(I+h)\right] \qquad (72)$$

This difference represents a positive, random error, which assumes importance when counting statistics dominates the overall uncertainty of results.

The agreement between calculated and observed variation of results was tested by Covell [1959] in his method for peak-area determination. Measurements of 3 different ^{137}Cs sources were repeated 10 times, and one was counted 10 times at each of 3 gain settings. Integration widths from 25 to 250 keV were included in the comparison, and reasonable agreement was found in most cases.

Very good agreement was later found by Baedecker [1971] in a similar investigation of the method for photo-peak integration attributed to Wasson.

Poor agreement was, however, reported by Covell [1959], when a ^{65}Zn spectrum was superimposed on the pure ^{137}Cs spectrum.

Although no real explanation is offered by the author, his description of the experimental conditions leads to the assumption that the countings of the pure ^{137}Cs sources were all made in succession so that the source was not moved between the individual countings; for the mixed spectra, the ^{137}Cs source and the ^{65}Zn source were counted alternately.

The reproducibility of the counting geometry is a potential source of variation, which affected neither of the previously mentioned investigations, but must be taken into account in practical work with verification of the calculated precision of analytical results. Thus, in a set of data reported by Mandel [Merten et al. 1975] of replicate countings of a ^{137}Cs source for 10 successive days, lack of statistical control can probably be attributed to uncertain counting geometry.

The *limit of detection* of an analytical method is determined from the standard deviation of the result obtained with a blank sample. In neutron activation analysis this is the standard deviation based on counting statistics alone, and it may therefore be calculated without knowledge of the a priori precision.

Selection of peak boundaries according to [VI], together with a comparator standard, permits calculation of a detection limit from the actual spectrum of a sample in which the element in question was not found. This is equivalent to the calculation of upper limits for elements not detected, which was carried out automatically for 68 elements in the computer program SCOTCH used at General Atomic.[1]

This program was used to investigate the possibility of determining other elements of interest in the separated sample for the determination of V in human serum [I]. Of all these elements, only Cu and In had detection limits substantially lower than their expected concentration in the serum, when a separated sample was irradiated for 30 minutes in the TRIGA reactor and counted with a 3" × 3" NaI(Tl) scintillation detector.

Such calculations are useful when developing new procedures or improving existing ones, and also for judging the applicability of a method for a planned investigation. For the reporting of results, upper limits are of limited use because they escape statistical treatment.

Overall precision

The a priori precision discussed in chapter 2 is subdivided in such a way that the individual components are independent of each other and can be pooled by the addition of their variances to form the total a priori precision. When this is combined with the contribution of counting statistics, an overall estimate of the standard deviation of the final analytical result is obtained.

1. H.P. Yule 1965.

In some cases a reduced precision is more correct when comparisons between results have to be made. Thus, samples processed simultaneously in a single batch and having the same blank value and the same comparator standard, may be compared with each other with better precision than with samples in a different batch. This reduced precision is sometimes referred to as *differential precision* [Damsgaard et al. 1972] and may be considerably better than the total precision under special circumstances. However, in order to avoid confusion in subsequent data treatment, the analytical method should be designed so that the difference between differential and overall precision is negligible. This was achieved in the determination of V by pre-irradiation separation by introducing ^{48}V into all samples for individual yield determination [Damsgaard et al. 1972], instead of using the original method [I] with batch-wise yield monitoring.

The randomization of factors affecting the accuracy of results may give an additional contribution to the a priori precision. The multiple carrier addition followed by re-irradiation yield determination [II] randomizes interference from other elements, but the actual contribution to the variability of results is only a second-order effect of the concentration of the interfering element and may be assumed to be insignificant.

The adequacy of the estimated analytical precision is tested by the repeated analysis of homogeneous samples, representative of materials and concentrations for which the method is claimed suitable. It is tempting to rely on the use of established reference materials, for which the true concentration may be known in addition. However, the preferential treatment of such samples seems unavoidable, and not all sources of variation may be adequately represented in these experiments. Real samples are therefore to be preferred, although the problem of homogeneity may be of concern.

Anyway, the statistical analysis of such data cannot be made with conventional analysis of variance [Hirsch 1977], which assumes the same precision for all data, nor with weighted analysis of variance, which requires all standard deviations to have known ratios. The analysis of precision of activation analysis results therefore requires a somewhat different approach.

3.2. Analysis of Precision

Serious attempts to demonstrate agreement between estimated and observed standard deviations of analytical results are very few, and only Biloen [1973] tested this over his useful range of analytical precision. Overall precision was estimated from all known sources of variation

combined with counting statistics, exactly as described in the preceding chapter. The observed standard deviation was determined from replicate measurements by instrumental neutron activation analysis, and correlation analysis showed very satisfactory agreement between observed and calculated precision, as shown in Fig. 29, which is reproduced from Biloen [1973].

Failure to achieve satisfactory agreement was found in a study by Salbu [1975] and is illustrated in Fig. 30. Here again the observed standard deviation for a number of elements is shown as a function of the standard deviation from counting statistics. The full, curved line shows the expected overall relative standard deviation, assuming an *a priori* contribution of 5 %. Clearly, the variability of actual results is much smaller than expected - which is most likely attributable to erroneous calculation of counting statistics.

Comparison between the experimentally determined precision of the analytical method for the determination of As in human serum [IV], and the observed variation of concentrations in blood from normal, Danish subjects showed no significant differences between individual samples. All results could therefore be pooled, and the standard error of the mean be calculated from the analytical precision, rather than from the actual data.

Comparison between the experimentally determined precision and the estimated, a priori precision based on the contributions from known sources of variation, however, reveals a considerable difference between the two.

Precision of irradiation

The total a priori relative standard deviation is estimated at 1-1.5 %, whereas the experimentally determined relative standard deviation was 5 % [IV]. It was found that the simultaneous analysis of 3 samples with the same comparator gave greater variability than the successive analysis of single kale samples each with their own comparator. This could be interpreted as an effect of a horizontal, neutron flux density gradient, which was randomized because of the uncontrollable horizontal positioning of individual containers in the rabbit.

In the thermal column in the DR 2 reactor the flux density gradient is very nearly purely exponential with a relaxation length of L. A horizontal displacement l of a polyvial at an angle θ with the flux gradient gives rise to a flux density of

$$\Phi = \Phi_0 e^{\frac{l \cos\theta}{L}} \qquad (73)$$

and rotation of the sample causes a variance of

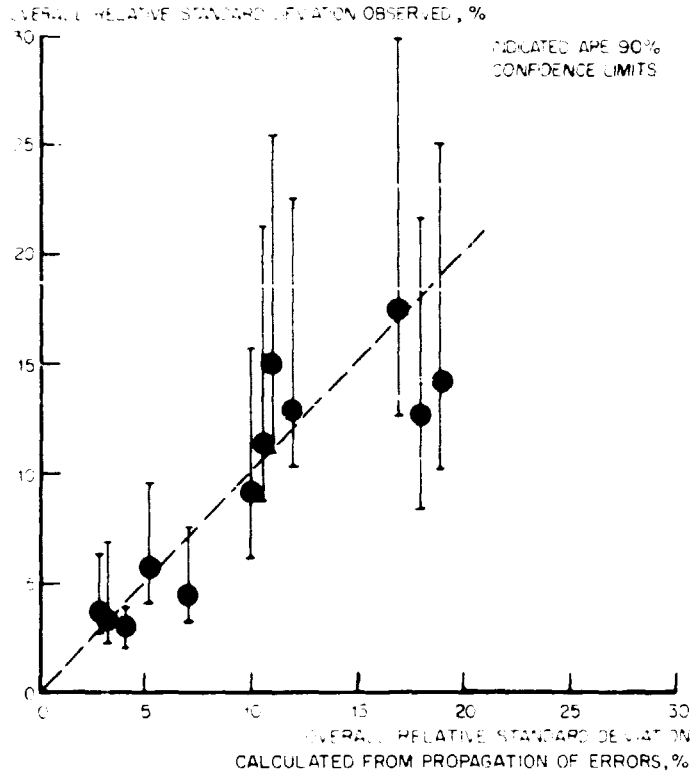

Fig. 29. Correlation between observed and calculated standard deviations [Biloen 1973].[2)]

$$\sigma_{log}^2 = \frac{\int_0^{2\pi}\left(\frac{\delta \ln\Phi}{\delta\theta}\right)^2 d\theta}{\int_0^{2\pi} d\theta} = \frac{(l/L)^2}{2\pi}\int_0^{2\pi}\sin^2\theta d\theta = \frac{1}{2}(l/L)^2 \quad (74)$$

With a displacement of l=9 mm and a relaxation length of 257.5 mm [Stærkind 1970], a relative standard deviation of the neutron flux density becomes

$$\sigma_{log} = 2.47\ \%$$

The exponential decline of neutron flux density may not be strictly valid at the position of the pneumatic tube, where only about 15 cm of graphite is passed. An experimental verification of the calculated coefficient of variation was therefore warranted.

The experiment was carried out in the 2 different pneumatic tube systems accessible from the radioisotope laboratory, R2 in DR 2 and R4 in DR 3, both of which use the same irradiation container for transport. Simultaneous irradiations of 9 samples of 1 cm^3 La-comparator standard were carried out as shown in Fig. 31 (c), and a few minutes'

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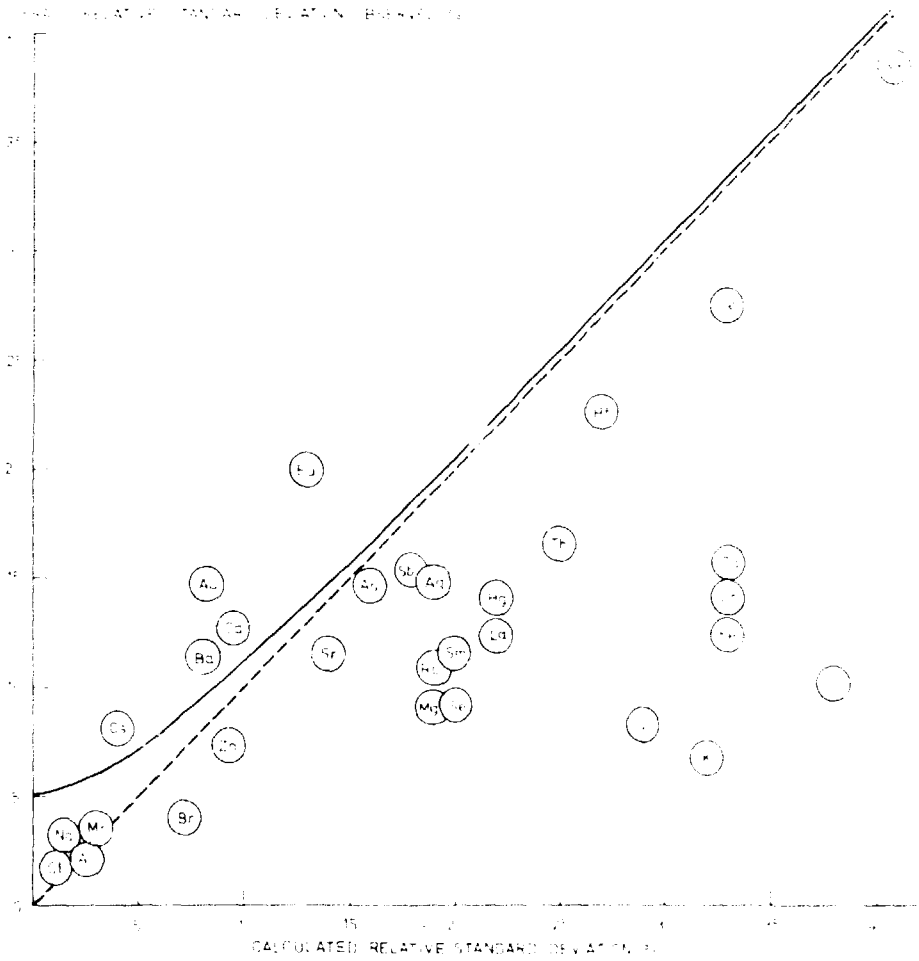

Fig. 30. Relation between observed and estimated standard deviation [Salbu 1975].

irradiation produced about 0.4 MBq (10 μCi) of ^{140}La suitable for measurement in a N.P.L. ionization chamber [V].

After a logarithmic transformation, the observed specific activities were subjected to a three-way analysis of variance without replication. With the alternative vertical positions and the separate irradiations as fixed effects, A and C, and taking the residual, triple interaction for the experimental error, σ, the mean square for factor B, the horizontal position, has an expectation value of

$$\sigma^2 + (a \times c)\sigma_B^2 \qquad (75)$$

where a and c are the numbers of - fixed - levels for factors A and C [Brøndum 1972].

The ratio between the neutron flux densities in the top and the bottom layers is expressed by the antilogarithm of the difference between their means; in both reactors the flux density was highest in

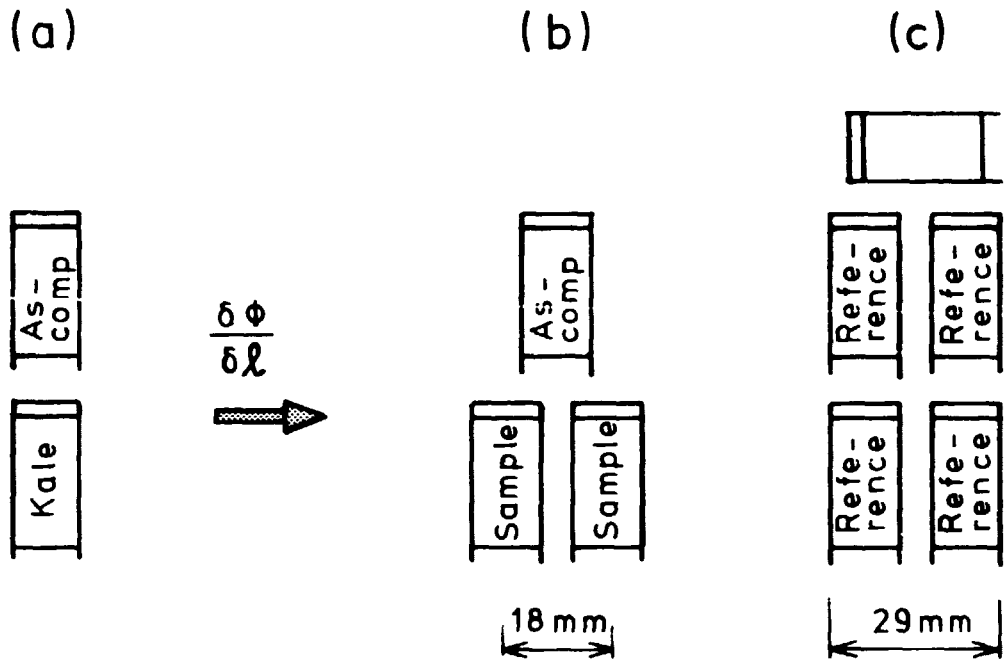

Fig. 31. Minimum (a) and maximum (b) influence of horizontal neutron flux density gradient.

the lower position. No significant interaction between vertical and horizontal flux variations was found.

The results from 4 irradiations in DR 2 and 5 in DR 3 are presented in Table 22. A similar investigation was made by Bruninx [1972] in different channels of the BR-1 reactor, in which standard deviations for the irradiation of small vials ranged from 0.4-2.2 % at neutron flux densities considerably lower than those present in DR 2 and DR 3.

The agreement between the calculated standard deviation from eq. (74) and the measured horizontal flux variability in Table 22 is very satisfactory, when the uncertainties involved and the limited number of degrees of freedom are considered.

Verification of precision

Hence, the a priori precision may be based on the calculation of variations in neutron fluence according to eq. (74). With sample and comparator standard in the same vertical position, their ratio has a standard deviation of 3.5 %, which represents the *limiting precision* for single determinations of manganese [VII]. This was experimentally confirmed by analysis of the dried animal blood supplied by the International Atomic Energy Agency [Heinonen 1972]. The concentration of manganese in this material is more than two orders of

Table 22

Experimentally determined neutron flux density gradients in alternative pneumatic tube facilities

Nuclear reactor	DR 2	DR 3
Irradiation facility	R 2	R 4
Number of samples	4 × 8	5 × 8
Vertical flux factor	0.969	0.945
Experimental error σ	0.85 %	0.50 %
Horizontal flux variation σ_{log}	2.0 %	2.3 %

magnitude higher than that in human serum, and counting statistics is therefore almost completely insignificant compared with an a priori precision of about 3.5 %.

Results of an intercomparison reported in [IX] are presented in Table 23 and show complete agreement between the observed and the calculated standard deviation of our data for manganese in dried animal blood.

For arsenic and selenium, where a single determination requires 2 irradiations, the limiting precision corresponds to a standard deviation of 5 % in good agreement with the experimentally determined precision for As in [IV].

The concentrations needed to confirm the adequacy of the estimated, relative a priori precision must be high enough to avoid significant contributions from absolute sources of variation and counting statistics. Results for Standard Reference Materials, which are known to be homogeneous, and which are used for ascertaining the accuracy of the analytical method, may well be used for this purpose. However, the special care often accorded such samples will defeat this application by reducing the normal sources of variation. A characteristic example is the analysis of such materials one by one, whereby the important contribution from horizontal displacements between several samples and their comparator is not observed [Heydorn et al. 1976].

Counting statistics is the dominant factor in the precision of analytical results in the grey zone between the limit of detection and the limit of determination [Currie 1968]. Here the a priori relative standard deviation is unimportant, and even when pooling a considerable number of results the resulting improved precision is determined by counting statistics only.

An example of this is the determination of V in human serum [I], where all results are very close to the detection limit. The variance of

Table 23

Analytical results for manganese in IAEA dried animal blood [IX]

Number of results	Standard deviation calculated	observed	Mean value µg/kg
6	6	5	150 ± 2
16*	-	16	151 ± 4

* Pooled results from 3 different laboratories [Merten 1971]

the distribution of results from 36 samples did not exceed the contribution from counting statistics, and all results were therefore pooled to give a grand mean of vanadium of 4.6 ± 0.8 mg/m^3 serum.

Similar results from repeated analysis of identical samples close to the detection limit include the blank values for V carried out with each batch of analyses. These are summarized in Table 24 together with results from the instrumental determination of V in SRM 1571 Orchard Leaves, presented in Table 21 and calculated by 3 different methods.

It is seen that even with calculated standard deviations in excess of 50 % the actual variation of results is in satisfactory agreement.

The separate verification of a priori precision and counting statistics lends support to the estimation of precision of individual results by adding their contributions to the overall variance. However, samples with concentrations above or below the appropriate range of interest are not representative of actual sample material, and the precision of the analytical method must be checked by the repeated analysis of real samples covering the proper range of interest.

This was done in [VII], and a comparison between the observed and calculated precision was based on the weighted *average standard deviation* $\bar{\sigma}$

$$\frac{1}{\bar{\sigma}^2} = \frac{1}{m} \sum^{m} \frac{1}{\sigma_i^2} \qquad (76)$$

With the useful range of an analytical method based on neutron activation often covering several orders of magnitude, the simple comparison of one or more observed and calculated standard deviations is not sufficient, and it does not permit conclusions to be made with well-defined statistical significance.

Table 24

Agreement between observed and calculated precision of results for V based on counting statistics

Matrix	Number of samples	Relative standard deviation observed %	calculated	Calculation method
Oxine blank	25	103	87	Stripping [I]
NBS	11	56	82	Covell (47)
SRM	11	55	65	Sterlinski*
1571	11	61	67	Heydorn (46)

*Cf. Table 21

Test of precision

In the precision of a method of activation analysis contributions from the counting process to the precision of the analytical result should be estimated on the basis of a Poisson distribution, rather than a binomial distribution [Currie 1971]. In addition, the number of counts recorded in an actual analysis will always be large enough to justify an approximation to the normal distribution.

The situation where a duplicate analysis is based on a single counting of the comparator standard is therefore accurately depicted in the preceding Section 3.1 with $\kappa_m \neq 1$ representing the decay correction.

For the analysis of precision of the analytical method, however, each sample should be activated separately together with its own comparator standard so that all sources of variation are allowed to influence the results. In this case κ_m becomes a variable, but in return its value will be close to $\kappa_m = 1$, and its precision will usually be much higher than that of the analytical result. The χ^2-distribution of equation (70) in the preceding section is therefore retained with good approximation. Results of duplicate analysis of M different materials are thus much more efficiently utilized in the analysis of precision, where the statistic T is known to be approximated by a chi-squared distribution,

$$T = \sum_{1}^{M} \frac{(y_{1m} - y_{2m})^2}{\hat{\sigma}^2_{1m} + \hat{\sigma}^2_{2m}} \qquad (77)$$

with M degrees of freedom [VIII].

Here Y_{1m} and Y_{2m} are the duplicate results for material *m*, and $\hat{\sigma}_{1m}$ and $\hat{\sigma}_{2m}$ their estimated standard deviation, calculated as the combined effect of *a priori* precision and counting statistics. The preferred experimental design is to analyze in duplicate, which also gives the most efficient coverage of range and materials. If more than two results are available for the same material, randomly selected, independent pairs may be included in the calculation of T to increase the number of degrees of freedom.

In order to eliminate the resulting ambiguity in the value of T, a suitable computer program draws all possible pairs and averages their contributions to T, which is then calculated for the number of degrees of freedom corresponding to the number of independent pairs.

For normally distributed results, this is equivalent to the statistic calculated for N-M degrees of freedom from equation (67) in Section 3.1., the latter presumably giving a slightly stronger test because of the increased number of degrees of freedom.

Even for actual results with pronounced heteroscedasticity the difference is, however, quite insignificant. As an example, the As results reported in [Damsgaard and Heydorn 1973] were pooled with 11 other results by activation analysis from the period 1974 - 1976 presented in Table 54, and calculation of a statistic T was made in both ways. Even though the maximum variance ratio was 100, the percentiles for T matched very well, as shown in Table 25.

If the materials analyzed are known to be homogeneous, high values of T indicate lack of control of some significant parameter, and improvements in the analytical procedure should be introduced.

Low values of T indicate lack of independence of different contributions to the precision, or failure of the experimental design to encompass all expected sources of variation.

These explanations, however, do not seem compatible with the conditions under which duplicate results for F, Sc, Se and Hf by INAA were produced by Dams [1975]. The Analysis of Precision with 27 pairs shows that the observed variability of results is significantly lower than indicated by their estimated precision. Although no calculation method is described, the low value of T seems explicable only by assuming an erroneous method of calculating variance estimates.

Statistical control

According to Heraclitus,[3] it is not possible to step twice into the same river - which may here be interpreted that duplicate samples are not always easy to obtain. On the other hand, samples that cannot be duplicated are probably not worth analyzing; the analytical effort is then

3. Fragment 91

Table 25

Analysis of Precision of published results for As in SRM 1571 by activation analysis*

Method	d.f.	T	$P(\chi^2 \geq T)$
Paired	6	4.4	0.62
Pooled	12	10.53	0.57

*See Table 54

not only halved, but wasted. It should be emphasized that, for activation analysis employing radiochemical separation with carrier addition, the a priori precision is *independent* of the concentration of the determinand. It is therefore possible to verify the precision of an activation analytical method without access to homogeneous reference materials or samples with concentrations covering the complete range of the method.

Thus, replicate analysis must be made at the development stage to make sure that all sources of variation are taken into account in the estimation of a priori precision. When that has been demonstrated, the precision of the method becomes an established performance characteristic, and observed deviations from the expected variability of results must be attributed to other sources of variation, such as heterogeneity of sample material.

The number of biological samples analyzed in duplicate is therefore not a certain fraction of the total number, but rather a function of the number of potential new sources of variation introduced over the years. A summary of the analysis of precision for the methods used to determine As, Se, Mn, Cu and V in biological material by neutron activation analysis is presented in the accompanying tables.

The agreement between the precision of the method and the precision of the results is very satisfactory, as seen by the probabilities given at the bottom of the tables. The importance of this is not so much connected with the considerable number of degrees of freedom with which the χ^2-distribution of the test parameter T is obeyed, but rather with the wide concentration range to which the precision of the analytical method applies.

This confirms the absence of concentration effect on the a priori precision for these methods, as well as the adequacy of the calculation of counting statistics; the methods are said to be in statistical control, and conclusions based on the precision of individual results can be made at well-defined levels of confidence.

Table 26

Analysis of Precision of As in biological material

Samples analyzed in replicate				Analysis of Precision			Source material	
Type of material	Concentration		Number	A priori	T	d.f.	References	Year
Reference materials								
Kale	0.1	mg/kg	4	5 %	0.55	2	[VII]	1973
Orchard Leaves	10	-	8	5 %	2.83	7	[X]	1975
Bovine Liver	0.05	-	6					
Oyster Homogenate	10	-	3	3½ %	0.66	1	Heydorn et al.	1976
Animal Muscle	0.005	-	4	3½ %	2.24	3	Damsgaard et al.	1976
Serum	1-13	mg/m^3	26	5 %	15.99	13	Heydorn et al.	1978
Tissue								
Autopsy	4-20	µg/kg	30	5 %	23.98	15	[VII]	1973
Biopsy	~ 5	-	16		7.11	8	Heydorn	1978
Biological material			Range	Test	53.36	49	Analytical method	
Orders of magnitude mass fraction			4	$P(\chi^2 \geq T) = 0.31$			[II]	1967

Table 27

Analysis of Precision of Se in biological material

Samples analyzed in replicate				Analysis of Precision			Source material	
Type of material	Concentration		Number	A priori	T	d.f.	References	Year
Reference materials								
Kale	0.1	mg/kg	4	-	1.15	2	[VII]	1973
Bovine Liver	1	-	6	5 %	3.19	3	Heydorn et al.	1976
Oyster Homogenate	2	-	4	3½ %	2.11	2		
Animal Muscle	0.3	-	4	3½ %	2.81	3	Damsgaard et al.	1976
Serum	50-120	mg/m³	24	5 %	16.60	12	[IX]	1973
			24	5 %	16.94	12	Heydorn et al.	1978
			12	5 %	8.13	6	Larsen et al.	1978
Tissue								
Autopsy	50-500	µg/kg	19	5 %	6.41	9	[VII]	1973
Biological material			Range	Test	57.34	49	Analytical method	
Orders of magnitude mass fraction			1½	$P(\chi^2 \geq T)$ =		0.19	[VII]	1973

Table 28

Analysis of Precision of Mn in biological material

Samples analyzed in replicate Type of material	Concentration		Number	Analysis of Precision A priori	T	d.f.	Source material References	Year
Reference materials								
Kale	15	mg/kg	4	3½ %	1.65	2	[VII]	1973
Animal Blood	0.15	-	6	3½ %	2.34	3	[IX]	1973
Bovine Liver	10	-	6	3½ %	3.47	3	Heydorn et al.	1976
Oyster Homogenate	70	-	3	3½ %	0.08	1		
Animal Muscle	0.5	-	4	3½ %	3.25	3	Damsgaard et al.	1976
Tissue								
Parenchymatous	30-170	µg/kg	16	3½ %	4.34	8	[VII]	1973
Nervous	180-300	-	10	3½ %	2.69	5	Larsen et al.	1978
Biological material			Range	Test	17.82	25	Analytical method	
Orders of magnitude mass fraction			3½	$P(X^2 \geq T) = 0.85$			[IX]	1973

Table 29

Analysis of Precision of Cu in biological material

Samples analyzed in replicate				Analysis of Precision			Source material	
Type of material	Concentration		Number	A priori	T	d.f.	References	Year
Reference materials								
Orchard Leaves	12	mg/kg	4	-	1.44	2		
Oyster Homogenate	330	-	4	3½ %	2.75	2	Heydorn et al.	1976
Water	4	mg/m³	10	-	9.91	9	Damsgaard et al.	1976
Tissue								
Muscle	4	mg/kg	15	3½ %	13.75	14	ibid.	1976
Biological material			Range	Test	27.85	27	Analytical method	
Orders of magnitude mass fraction			5	$P(\chi^2 \geq T) =$	0.42		Heydorn et al.	1976

Table 30

Analysis of Precision of V in biological material

Samples analyzed in replicate				Analysis of Precision			Source material	
Type of material	Concentration		Number	A priori	T	d.f.	References	Year
Reference materials								
Kale	0.3	mg/kg	8	-	3.8	6	Damsgaard et al.	1972
Oxine	0.01	-	25	-	33.9	24	[I]	1966
Tissue								
Autopsy	4-12	µg/kg	15	-	13.3	13	Rietz	1972
Biological material			Range	Test	51.0	43	Analytical method	
Orders of magnitude mass fraction			2	$P(\chi^2 \geq T) =$		0.19	[I]	1966

This technique was used in [X] to demonstrate the lack of personal bias in the determination of As in reference materials by the present method. At the same time, however, a series of duplicate samples of human serum taken under good, standard hospital conditions in 1971 were also analyzed for As, and here the Analysis of Precision clearly indicated the presence of additional sources of variability not accounted for in the precision of the analytical method.

A correction for ^{82}Br interference was now introduced into the analytical method, and a new set of duplicate serum samples was taken in 1972 under far more stringent conditions than previously. The results are presented in the left part of Table 31, and it is seen that the variation between duplicate samples has now reached the point where it is significantly smaller than prescribed by the precision of the analytical method.

It was found that the significantly low value of T could be attributed to an abnormal distribution of results after the correction for ^{82}Br interference.

This was caused by the peak boundary selection method then used [Yule 1966], which introduces a non-random component into the evaluation of very small photo-peak areas, such as the 777 keV ^{82}Br peaks in the present spectra.

All results were therefore recalculated using the peak boundary selection method described in [VI], and the revised results are presented in the right part of Table 31; it is noted that excellent agreement is now achieved between the precision of the analytical method and of the analytical results.

Subsequent investigations reported in Table 60 confirm that the analytical method is now in complete statistical control.

3.3. Control of Precision

When the precision of the analytical method has been brought under statistical control, and all sources of variation have been taken into account, reliable estimates of the precision of single results are made from the *a priori* precision and the counting statistics.

The development of the analytical method is then completed, and the practical applications may begin. This, however, does not mean that unexpected sources of variation no longer turn up.

Modifications

First of all, no method remains unchanged indefinitely; although *intentional* modifications might be expected to be inconsequential, this

Table 31

Duplicate results for arsenic in human serum of the 1972 series [X]

Results, corrected by previous method		Results, corrected by alternative method	
mg/m³	mg/m³	mg/m³	mg/m³
0.67 ± 0.12	0.62 ± 0.17	0.79 ± 0.11	0.66 ± 0.11
0.91 ± 0.14	0.8 ± 0.11	0.91 ± 0.13	0.84 ± 0.11
1.70 ± 0.12	1.81 ± 0.13	1.77 ± 0.12	1.90 ± 0.14
0.34 ± 0.13	0.36 ± 0.07	0.49 ± 0.09	0.44 ± 0.07
1.62 ± 0.12	1.48 ± 0.12	1.75 ± 0.13	1.57 ± 0.11
0.70 ± 0.11	0.76 ± 0.09	0.81 ± 0.10	0.76 ± 0.09
1.25 ± 0.15	1.16 ± 0.21	1.54 ± 0.16	1.48 ± 0.16
0.67 ± 0.20	0.36 ± 0.39	0.83 ± 0.15	0.98 ± 0.22
0.61 ± 0.19	0.58 ± 0.09	0.97 ± 0.16	0.65 ± 0.08
1.09 ± 0.15	1.08 ± 0.11	1.11 ± 0.14	0.75 ± 0.14

10	degrees of freedom	10
T = 2.35	test of precision	T = 9.71
P = 0.007	$\chi^2 \leq T$	P = 0.53

must be demonstrated in each case. This was done when instrumental determination of V in air filter samples had to be carried out in the DR 3 reactor instead of in DR 2. Here, thermal neutron irradiation for 2 minutes at 115 nmol/(m^2·s) was replaced by 30 seconds at ~400 nmol/(m^2·s), and 15 samples of Whatman No. 1 were analyzed in both reactors.

The 15 duplicate results were subjected to the analysis of precision, for which results are presented in Table 32, and no additional source of variation was required to account for the observed variability between duplicate runs; cf. Section 4.2.

Another such change was the transition from plasma to serum samples for the determination of As in human blood [IV], which was necessary in order to eliminate the citrate blank value. Both serum and plasma were prepared from a number of blood samples, and results are reported in [IV] without standard deviation. By means of the experimentally determined precision of the analytical method, the results could be subjected as duplicates to the analysis of precision, for which results are presented in Table 32.

The conclusion reached in the original paper is here substantiated by the probability stated in the table that the observed differences between serum and plasma results can easily be explained by their estimated standard deviations.

Changes of sample substrate or sample composition are, of course, quite common, and neutron activation analysis is less sensitive to such variations than most other analytical methods. These changes may, however, quite often be accompanied by *unintentional* effects, which may contribute considerably to the variability of results.

Duplicate samples are the only generally applicable method for the detection of such unexpected sources of variability, but where each sample is unique, other methods may have to be applied.

Air filter samples are in some respects unique, although fibrous filter samples like Whatman No. 1 may be subdivided. The use of instrumental neutron activation analysis with short-lived indicators like ^{52}V and ^{28}Al, however, permits repeated analysis of the same sample.

Switching from fibrous filters to membrane filters was checked by duplicate analysis, and the second result for both V and Al was always lower for cellulose-acetate membrane filters, when they were treated in the same way as the Whatman filters.

This was found to be significant by a conventional *analysis of variance* which at the same time failed to demonstrate any difference between the Al to V ratio of first and second measurements. Much higher levels of significance were found by the analysis of precision, by which method a highly significant - but random - difference in the Al to V ratios of the samples was also demonstrated [XI]. Losses of these two

Table 32

Examples of intentional changes of methodology tested by the Analysis of Precision

Analytical method	INAA	RNNA
Element and reference	V [XI]	As [IV]
Original	DR 2	Plasma
Alternative	DR 3	Serum
Analysis of Precision		
Range in ng	7 to 85	15 to 70
Number of comparisons	15	7
Test	T = 16.27	T = 3.44
$X^2 \geq T$	P = 0.37	P = 0.84

elements are therefore independent of each other, which agrees with the tendency of Al to be found in the larger particles, while V belongs to the small particulate matter.

An absence of *correlation* between factors affecting the precision of an analytical result is assumed in the analysis of precision and should be checked with data for actual samples. An example is the lack of significant correlation between storage time and result for Se concentrations in serum reported in [IX].

Replicate analysis

Regardless of the statistical methods employed, the control of precision is dependent upon the availability of redundant information, which is usually supplied by repeated analysis.

The use of replicate measurements in analytical *quality control* is as old as analysis itself, and until very recently it served the purpose of determining the overall precision of the analytical results, *a posteriori*.

In a long series of similar analytical determinations with replicates their precision then becomes known *a priori*, and control of precision is now possible by comparing expected variation with observed variation. Significant disagreement signals the appearance of additional factors affecting the results.

A continuous quality control system based on successive duplicates was investigated by Payne [1972], but the mathematical basis is incorrect. A more comprehensive system was devised by Thompson [1973], who took into account the variation of precision with concentration and found good agreement between actual and estimated

parameters when experimental data were subjected to linear regression. The system, however, has no satisfactory theoretical basis, and the significance of deviations cannot be gauged.

The precision of counting can be estimated from the property of the Poisson distribution that mean and variance are equal, and Plesch [1973] used this information to control the stability of the counting equipment. For $N > 30$ replicates, he used the normal distribution of $s/\sqrt{\hat{\mu}}$ with variance $1/2(N-1)$ to evaluate the significance of observed deviations.

Continuous quality control

The statistic T, which was introduced to verify the precision of an analytical method at the development stage [VIII], lends itself readily to the continuous monitoring of results by the *analysis of precision.*

The initial fraction of duplicate samples is normally close to 100 %, but when statistical control is ascertained the fraction of duplicates may be reduced considerably. The analytical quality control procedure for the WHO/IAEA Research Programme on Trace Elements in Cardiovascular Diseases [Heydorn and Parr 1973] requires that at least 20 % of the samples are analyzed in duplicate for instrumental analysis and for radiochemical separation methods without yield determination. For radiochemical separations with individual yield determinations, this fraction may be reduced to 10 %, because of the additional quality control, discussed in Section 2.7, which is based on actual yield data.

The practical implementation of a continuous, analytical quality control programme in the instrumental determination of V in air filter samples is described in [XI].

The a priori precision without regard to sampling problems was here estimated to be about 2.5 %, and counting statistics were based on the use of fixed integration widths. Duplicate results refer to the repeated analysis of the same sample, and the control system is based on the variability of actual analytical results. Highly redundant information from blanks and comparator standards is also included in the quality control programme.

Continuous control is maintained by the analysis of 10 - 15 % of the samples in two different batches. The M duplicate results $Y_{.m}$ and their calculated standard deviations $\hat{\sigma}_{.m}$ are tested by the statistic

$$T = \sum_{m=1}^{m=M} \frac{(y_{1m} - y_{2m})^2}{\hat{\sigma}_{1m}^2 + \hat{\sigma}_{2m}^2} \qquad (78)$$

approximated by a χ^2-distribution [VIII] with M degrees of freedom.

Agreement of observed differences between duplicates with the variability expected from the calculation of precision was achieved on a routine basis for three different types of material, presented in Table 33.

The duplicate control is illustrated in Fig. 32. for the period March 1973 to April 1975. The black points show the release of results taking place in groups of approx. 7 batches, and the dotted lines indicate 1 % and 99 % levels of significance for deviations of the χ^2-distribution. It is noted that statistical control is maintained over levels of precision varying by an order of magnitude.

Vanadium comparator standards irradiated in each batch are continuously controlled by comparing the recorded counts with a previous comparator standard.

Their variability represents the overall effect of the a priori precision, counting statistics of about 1 %, and the reproducibility of reactor irradiation conditions.

Over the period 1973 - 1975 the total standard deviation of comparator standards was 3.5 %, so that the contributions to variability from changing control rod positions and from several changes in core configurations were only 2 - 2.5 %.

This makes the detection of significant comparator errors quite easy, and the same situation applies to blank values, which were all within statistical control by counting statistics.

Sampling conditions

The effect of sampling conditions was not included in the previous quality control system, but it assumes an important position in the analysis of biological materials, including blood.

Here, the agreement between serum and plasma results for As, shown in Table 32, indicate that sampling conditions are under control and included in the experimentally determined precision of the analytical method. The change in sampling conditions from the field in Taiwan to the hospital in Copenhagen, however, had a significant influence on the quality of results.

This was tested by the analysis of precision of a new set of duplicates with the established estimation of their standard deviation, as shown in Table 34. The statistic T assumes a value that is significantly *lower* than normal and hereby demonstrates the significant improvement in sampling conditions achieved [X].

Table 34 also presents the results of an analysis of variance in its last column; a residual, random error of 0.84 mg/m^3 indicates that the observed differences between samples may be real. This is a slight improvement in comparison with the previous results for domestic samples, reported in [IV].

Table 33

Analysis of Precision of results for V in air filter samples [XI]

Filter type Range in ng	Whatman 2 to 200	Cellulose acetate 40 to 350	Cellulose nitrate 80 to 3000
Precision			
Average $\bar{\sigma}$	1.6	11	15
Duplicates	134	30	34
Test	T = 128.1	T = 33.5	T = 37.1
$\chi^2 \geq T$	P = 0.63	P = 0.30	P = 0.33

Further improvement was achieved when the As results were corrected for interference from ^{82}Br [IX], which reduced the residual, random error to 0.27 mg/m^3. This is a reduction of the standard error of almost an order of magnitude compared with the original conditions from [IV], and the precision of a single result is now sufficient to detect actual differences between samples with high significance.

The analysis of precision presented in Table 35, however, shows that good, standard hospital conditions are entirely adequate for the analysis of serum for Se, but still leave room for significant improvement in the case of As.

Such improvement was in fact achieved in connection with attempts to avoid contamination with Mn, and excellent agreement between estimated and observed variability of results for As in human serum is shown in Table 31.

No such agreement was reached for Mn in spite of considerable efforts to improve sampling conditions, but the residual error was sufficiently small to affect neither the mean value nor the standard deviation of the distribution of manganese concentrations in serum [VIII].

The sensitivity for the detection of unknown sources of variation by the analysis of precision far exceeds that obtained from the mere comparison of mean values. It may be applied to materials with unknown concentrations, so long as their homogeneity can be trusted.

The types of error detected may be random, as well as non-random, and they may have a bearing on the accuracy of the results too. On the other hand, a constant bias will usually escape detection, but proof of its existence requires knowledge of the analytical precision.

The localization of errors is performed by stepwise application of the method to establish the stage of the entire procedure at which uncontrolled variation appears.

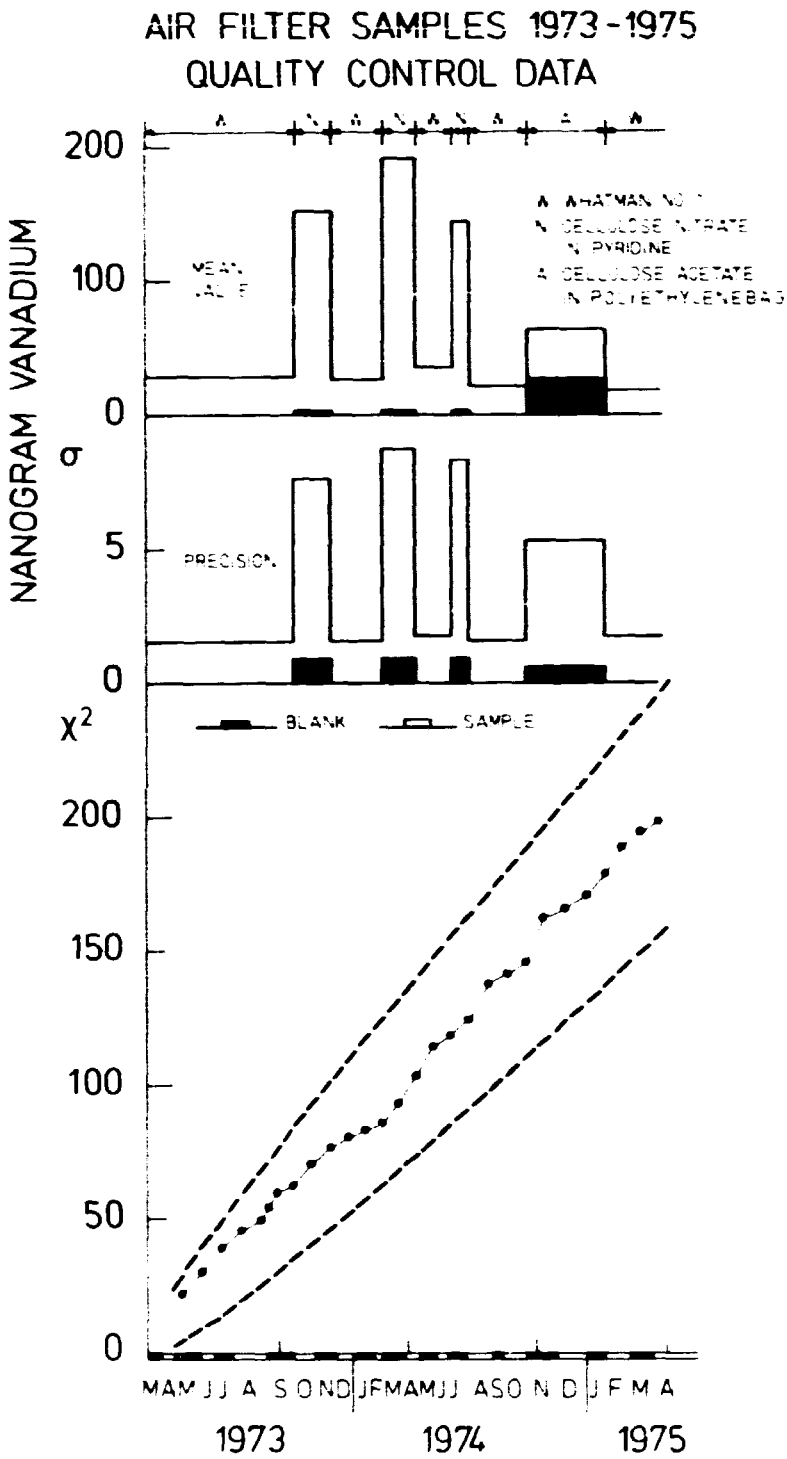

Fig. 32. Continuous quality control by means of the χ^2-distributed statistic T, plotted as a function of the number of duplicates during the period March 1973 to April 1975.

• marks release of results (in groups of approx. 7 batches).

The dotted lines indicate 1 % and 99 % levels of significance, and the histograms indicate average precision and mean values for samples and blanks.

Table 34

Comparison of field sampling conditions for As in human serum with good, standard hospital conditions [X]

Sampling conditions Standard error	Field s_o = 2 mg/m³	Standard hospital $\hat{\sigma}_o$ = 2 mg/m³	 s_o = 0.84 mg/m³
Variation between	Duplicates	Duplicates	Samples
Number of materials	18	11	1
Number of results	39	22	11
Degrees of freedom		11	10
Test parameter		T = 2.00	F* = 2.48
Probability		≤ 0.0015	< 0.08

* Estimated population variance relative to the a posteriori precision of a single determination with 11 degrees of freedom

The origin of the errors, however, must be left to the imagination of the analyst. Once the probable origin has been decided, the source of error may be eliminated; alternatively its effect may be measured separately, as was the case with the effect of a horizontal neutron flux density gradient between sample and comparator in Section 3.2.

With proper control of the analytical procedure, unknown errors must be referred to the sampling procedure - or to actual heterogeneity of the material. The variance contribution from these sources has to be estimated from the same results as used in the analysis of precision.

The problems involved in such estimation have been studied by Currie [1972], who showed that the determination of a small residual error with good precision requires a very large number of experimental results. A priori knowledge of precision is thus much to be preferred, also for judging the accuracy of the analytical results.

The great range covered by methods of activation analysis makes the analysis of precision particularly appropriate; at the same time the techniques employed are not very susceptible to human interference, and hence the result is less subject to unpredictable sources of error. Conditions for reliable estimates of precision in activation analysis are therefore very favourable.

Table 35

Analysis of Precision of duplicate results for As and Se in human serum under good, standard hospital conditions [IX]

Element	Arsenic	Selenium
Precision $\hat{s}_{log}$	23 %	8 %
Index of determination*	0.88	0.67
Analysis of Precision		
Number of duplicates	12	12
Test	T = 37.2	T = 16.6
$\chi^2 \geq T$	P = 0.0002	P = 0.17

4. Accuracy of Analytical Results

Like precision, accuracy is difficult to express quantitatively. Choosing the definition as the difference between the mean value of the analytical results and the true value, it is clearly independent of precision, and the two concepts need not be confused. It is therefore quite possible to have poor precision and excellent accuracy at one and the same time.

Accuracy is thus determined by the difference between two unknown numbers, one of which is not accessible for measurement at all, the other requiring an infinity of measurements. When systematic errors are absent, this difference is zero and accuracy is excellent.

All analytical methods worthy of consideration should account and correct for the presence of possible systematic errors, so that the expectation value of analytical results is the true value.

$$\bar{y}_{im} = y_m \tag{79}$$

This means that the residual systematic error η has an expectation value of zero

$$\bar{\eta} = \bar{y}_{im} - y_m \tag{80}$$

The closest one can get to ascertaining the accuracy of analytical results is to demonstrate that under the prevailing conditions $\hat{\eta}$ is not significantly different from zero.

The sensitivity for the detection of a residual systematic error different from zero depends on precision, but also upon the origin and types of error involved.

Types of systematic errors

If we disregard the *gross error* that affects only a small fraction of the samples, systematic errors contribute to the value of η in three ways:

(a) A *bias*, which is independent of the concentration of determinand - but may depend on many other factors.
(b) A *calibration error*, which increases with concentration, approximately as a proportional error - but may depend on other factors.
(c) A *variable error* of unknown origin, but caused by factors no longer under control or unintentionally being changed.

An evaluation of these contributions is only possible when the analytical method is in *statistical control*. Although unknown, the true value Y_m is known to be without variability, and the standard deviation of η is therefore equal to the standard deviation of Y_{im}, $\hat{\sigma}_{im}$.

Even without knowing the true value of $\hat{\eta}$, it is therefore possible to detect variable errors (c) by the Analysis of Precision; when the expectation value of η is no longer constant, this will be detected by the T-test as an additional source of variation between replicate results.

This type of error will therefore be detected in a routine quality control schedule based on the Analysis of Precision of duplicate results.

Depending on the number of duplicates, gross errors will be detected in the same way when their frequency exceeds an upper limit determined by the total number of results.

Tests for accuracy

The detection of (a) a bias or (b) a calibration error is only possible when the true concentration is known. Since the bias is assumed to be independent of concentration, it can be determined most readily by the analysis of materials not containing the determinand.

$$y_m \equiv 0 \tag{81}$$

The value of $\hat{\eta}$ under these circumstances is usually referred to as a *blank* value, and it is determined by replicate analysis of pure materials or by extrapolation of results from the analysis of samples with known ratios of determinand concentrations.

The significance of the blank value is tested by the statistic

$$U = \frac{\bar{\eta}}{\bar{\sigma}_0\sqrt{N}} \tag{82}$$

where N is the number of replicates and $\bar{\sigma}_0$ is the average standard deviation of a single result for zero concentration.

The true value is a useful concept, but it can only be approximated by actual measurement. The expectation value of all analytical results is the true value, regardless of the analytical method:

$$\bar{y}_{im}^{(1)} = y_m \qquad \bar{y}_{jm}^{(2)} = y_m \tag{83}$$

This means that, when the analytical methods considered are in statistical control, the mean values of replicate determinations are not significantly different

$$\eta = \bar{y}_m^{(1)} - \bar{y}_m^{(2)} \tag{84}$$

If η is not significantly different from zero, all results from the two methods may be pooled to yield an overall *consensus value*. When the two methods are based on different analytical principles and carried out completely independently with comparable precision, their consensus value is assumed to be a practical representation of the true value.

Such considerations form the basis for the certification of reference materials by institutions like the National Bureau of Standards; but uncertified reference materials with a wide, international circulation may be treated in the same way without the official stamp.

These materials are therefore suited for the investigation and control of (b) calibration errors,

$$\bar{\eta} = \bar{y}_m - y_m^* \tag{85}$$

but the uncertainty of the certified value $\bar{y}\star_m$ is often considerable. Reference materials are therefore not very suitable for the determination of bias. In the absence of suitable certified reference materials (CRM), calibration errors may be controlled by standard addition or other, alternative methods discussed by Malissa [1976].

Referee methods

An analytical method which gives results with insignificant systematic errors is sometimes called a referee method [Cali 1972]. It is the object of the present chapter to show that the methods employed in the present study qualify as referee methods.

These methods were at the same time selected for their superior precision at very low trace element levels. Comparison with other methods is therefore limited to the detection of calibration errors. Other errors must be determined by the Analysis of Precision or other statistical techniques which may be adapted to the detection of specific errors.

Both approaches are used in the internal quality control, where *verification* is based on the use of alternative methodology, and *detection of systematic errors* is based on the monitoring of the variability of duplicate results by the Analysis of Precision.

The problem of *blank values* is discussed at some length, because of its importance in ultratrace analysis. Like the true value it is inaccessible to direct measurement, but its importance can be inferred from actual results near the limit of detection.

International validation of analytical results is based on the analysis of *reference materials*, and the most important of these have been

analyzed to ascertain the absence of calibration errors. These investigations conclude that the methods applied in this work have no significant systematic errors and may be used as referee methods. At the same time, however, it is found that no analytical method, including neutron activation analysis, is infallible, even in experienced hands.

Only by careful verification and continuous control can analytical results with paramount precision and accuracy be produced at the trace or ultratrace level of concentration.

4.1. Verification of Analytical Results

Internal measures to verify the accuracy of analytical results require the availability of independent control methods.

For major elements, a simple, but effective control is based on the summing of all element concentrations to give 100 %. This was actually used by Bruninx [1973] and Miller [1975] to verify results by neutron activation analysis.

For trace elements, this method can only be used in extreme cases [Meinke 1973], and for ultratrace levels it is, of course, out of the question. In these cases alternative methods must be used.

In the same way as the *Precision* of the analytical method is verified by means of duplicate results, its *Accuracy* is verified by using duplicate methods. These methods should preferably be based on entirely different principles, and they should be applied separately on identical samples. The precision of the duplicate results produced by either method may therefore be quite different; but if the precision of both methods is well known, results may be analyzed exactly as if they were produced by the same method. If no significant difference between the results of the two methods is detected by the Analysis of Precision, the accuracy of the appropriate results is verified.

Although perhaps a dozen different analytical techniques can claim sensitivities high enough to measure elemental concentrations at the trace and ultratrace level (<10 µg/kg), their precision and accuracy at such low levels are not comparable with neutron activation analysis, except in a few cases.

Internal control

Verification may often have to take the form of demonstrating the absence of some specific errors by means of a modification of analytical methods based on neutron activation analysis, rather than by means of independent analytical methods.

Table 36

Comparison of results of neutron activation analysis with and without chemical separation

Matrix	Element	INAA	RNAA	Source
		mg/kg		
Oil	V	2.00 ± 0.05	1.95 ± 0.04	[I]
Plant	As	9.93 ± 0.13	9.7 ± 0.2	Damsgaard 1973
		µg/kg		
Water A	Cu	4.0 ± 1.2	4.6 ± 0.3	Damsgaard 1976
Water B		3.1 ± 1.0	2.9 ± 0.3	

The absence of *interference* may be verified by changing the conditions of measurement significantly without getting significant changes of the analytical results.

Such changes result from the introduction of a chemical separation procedure before measurement, and this may be used as a control for samples that can also be analyzed by instrumental neutron activation analysis. Actual examples for 3 elements from the present study, which are presented in Table 36, confirm the accuracy of results determined without chemical separation.

Most biological materials cannot be analyzed by neutron activation analysis without chemical separation, but significantly different measurement conditions may also be realised in such cases, by counting

(a) different radionuclides of the same element, Se, Zn, etc.,
(b) different photo-peaks of the same element, e.g. Mn,
(c) at different decay times,
(d) with different detectors.

The first 3 choices are usually made at the development stage of the analytical method from exactly the point of view of minimizing interference, and these factors are therefore not readily applicable to the verification of the success of these choices. Instead, the actual magnitude of interference from other elements is determined experimentally under the exact conditions of the analytical procedure.

The last option of using a different detector with improved or different discrimination towards interfering radionuclides may well be used to verify the absence of significant interference. This is particularly important in the case of counting - radiochemically separated - samples for ^{64}Cu; here the use of a Ge(Li) semiconductor detector instead of a NaI(Tl) scintillation detector limits the interference to contributions from high-energy γ-emitters.

Table 37

Duplicate countings of ^{64}Cu samples from the analysis of SRM 1577 [Heydorn et al. 1976]

Sample weight (freeze dried) mg	Counting on NaI(Tl) detector mg/kg	Ge(Li) detector mg/kg
230.3	184.3 ± 2.9	189.3 ± 1.6
289.5	176.4 ± 2.6	176.1 ± 1.4
336.2	194.7 ± 2.5	191.8 ± 1.5
301.7	184.1 ± 4.1	188.4 ± 1.5
285.0	178.0 ± 2.6	180.6 ± 1.9
282.3	188.0 ± 2.8	189.9 ± 1.4
289.3	192.1 ± 3.0	186.0 ± 1.3
Test of precision	$T = 8.75$ for 7 d.f.	
Cumulative probability	$P(\chi^2 \geq T) = 0.27$	

Results for 7 different samples of bovine liver counted on both detectors are presented in Table 37 together with their standard deviation based on counting statistics only [Heydorn et al. 1976].

An Analysis of Precision of these results does not indicate the presence of sources of variation other than counting statistics; the great difference in counting geometry also affects interference from high-energy γ-emission, so this type of error would also be detected.

Usually the Ge(Li) detector requires counting times much longer than the scintillation detector, and therefore it has been very little used in connection with the determination of low levels in biological materials. A single comparison reported in [IX] showed that a counting time of 4 hours was required for a single determination of As in human serum.

The agreement between the 3.5 ± 0.4 ng of As found by the scintillation detector and the 3.2 ± 0.7 ng found by the Ge(Li) detector supports the assumption that interference is negligible even below the 1 mg/m^3 level of As in serum, when the method described in [IX] is used.

Another possible detector is the ionization chamber, the use of which in neutron activation analysis is in many respects analogue to the use of a balance in gravimetric analysis, cf., e.g., Campion [1975]. In both types of analysis the chemical composition of the sample must be known in order to avoid interference from unwanted elements. In the determination of chemical yield of As after re-irradiation of the

separated sample [V], the absence of interference was verified by comparison of the ionization chamber results with the yields determined by counting with a 3" x 3" NaI(Tl) scintillation detector.

The difference in chemical yields determined by the 2 methods averaged 0.6 % absolute for 20 different samples covering the range of 80 to 100 %. Although this difference is statistically significant at the 1 % level, its average effect on tne analytical results is trivial.

It should be recalled, however, that the improvement of accuracy by multiple carrier addition with re-irradiation yield determination proposed in [II] is only achieved when the same detector is used for counting the sample both before and after re-irradiation.

This does not give rise to any conflict of interests, because either:

(a) the absence of interference is verified by comparison between ionization chamber and scintillation detector; no improvement of accuracy can thus be obtained by using the scintillation detector, and the ionization chamber gives reliable results,

or:

(b) the presence of interference is observed by comparison between ionization chamber and scintillation detector; the ionization chamber does not give reliable results, whereas some improvement of accuracy may be achieved by using the scintillation detector, preferably in connection with multiple carrier addition.

The most serious errors encountered in analysis at ultratrace levels (<10 μg/kg) stem from the risks of contaminating the sample before irradiation, i.e. during conditioning or at the actual sampling stage. Absence of *contamination* during conditioning of the sample may be verified by changing the type or amount of conditioning performed between duplicate samples.

This was done in [I] by the use of wet ashing as an alternative to dry ashing, but the interpretation of results is hampered by the simultaneous introduction of different blank values. The distinction between a blank value, for which correction can be made, and an unknown contamination of the sample is very difficult and will be discussed in a separate chapter.

At the sampling stage, contamination cannot be confused with blank value, and absence of contamination is verified by agreement between samples taken in duplicate. At the ultratrace level sampling conditions are extremely difficult to control, and it is advisable to take all samples in duplicate. This was done in the studies of human serum reported in [IX], as well as in all subsequent investigations of this type.

Although sampling conditions for Mn and As developed in [IX] were proven satisfactory with Indexes[1] of determination ≥ 0.90, subsequent experience proved that occasional, significant contamination could not be excluded, even when conditions were apparently completely unchanged. A method for the elimination of individual, erroneous results is therefore still greatly needed.

Such methods are also required in the statistical treatment of data from international intercomparisons of analytical results, such as those organised by the International Atomic Energy Agency. Here, the methods of choice are rejection of outliers by Chauvenet's criterion or by Dixon's test.

With the small number of results usually available in our investigations only the most tangible errors are detected by these methods, and the mere absence of outliers does not ensure adequate control of accuracy. Such control is only verified when all samples are duplicates, and no unexpected sources of variation are detected by the Analysis of precision.

When this desirable situation cannot be reached, a reduction of the influence of contamination can be achieved by systematic rejection of the highest of all duplicate results. This *Method of least duplicates* was used by Damsgaard [1971] when interpreting results for Mn in human serum, thereby presenting the first confirmation of the results of Cotzias [1966].

The distribution of the lower of 2 independent results may be estimated from the parent distribution, and if this is normally distributed with a variance of σ_x^2 and a mean value μ, the reduced parameters are

$$\hat{\sigma}_{low}^2 = \sigma_x^2\left(1 - \frac{1}{\pi}\right) \tag{86}$$

$$\hat{\mu}_{low} = \mu - \frac{\sigma_x}{\sqrt{\pi}} \tag{87}$$

which means that the mean and variance of the parent population can be calculated from the distribution of the lower duplicate results [Kendall 1969].

This method does not eliminate the problems originating from the contamination of samples at the ultratrace level, but it helps to improve the accuracy of results for which contamination cannot be excluded. It is applicable to small series of samples, and it does not jeopardize statistical inference based on the expurgated data material.

1. See Section 5.1.

Specific activity

The verification of the accuracy of neutron activation analytical results by completely independent methods of comparable precision and accuracy was found possible in 2 cases, where the specific activity of radioisotope preparations was determined.

In a case of ^{125}I [III] *absolute accuracy* at the nanogram level was verified by comparison of activation analysis and ionization chamber measurement; in a case of ^{36}Cl, a *relative accuracy* of within 1 % was verified by comparison with mass spectrometry.

The determination of ^{125}I by ionization chamber measurement, followed by neutron activation analysis with ^{126}I as indicator, permits analysis by two different radiometric methods of the same nuclide in a particular sample. Such measurements were made in [III] on CO_2-purged polyvials in connection with a study of the specific activity of ^{125}I preparations. Results for the two methods are illustrated in Fig. 33 for 13 different samples.

The uncertainty of the ionization chamber measurements was previously found [Heydorn 1967] to be <0.2 % full scale, and with a ^{125}I to ^{126}I ratio $>10^4$ at the time of measurement, the systematic error is also <0.2 %.

Counting statistics for the NAA results reflect the correction of ^{126}I counts for the activity present before irradiation, and standard deviations range from 0.5 % to almost 3 %.

A linear regression of the 386 keV γ-radiation, produced by neutron activation, on the ionization current, produced by the same sample before irradiation, yielded a zero intercept that was smaller than the standard error of estimate. The presence of a significant bias between the two methods is therefore completely unwarranted, and the results are consequently proportional.

The ratios between ionization currents and NAA results show a standard deviation of 8 %, indicating the presence of sources of variation not included in the estimated precision of the measurements.

One such source of variation might be incomplete correction for day-to-day differences in reactor operation and neutron flux density; however, this factor was later determined by the V-comparator measurements reported in [XI] and it contributes only 2-2.5 %. The major source of variability is probably connected with the problems involved in ^{125}I measurements by the 1383 A Ionization Chamber, as reported by Woods [1975].

An absolute ^{125}I standard from the Radiochemical Centre was used as a comparator to convert results from both methods into nanograms of ^{125}I; the comparison of the two methods, however, is not dependent on this calibration.

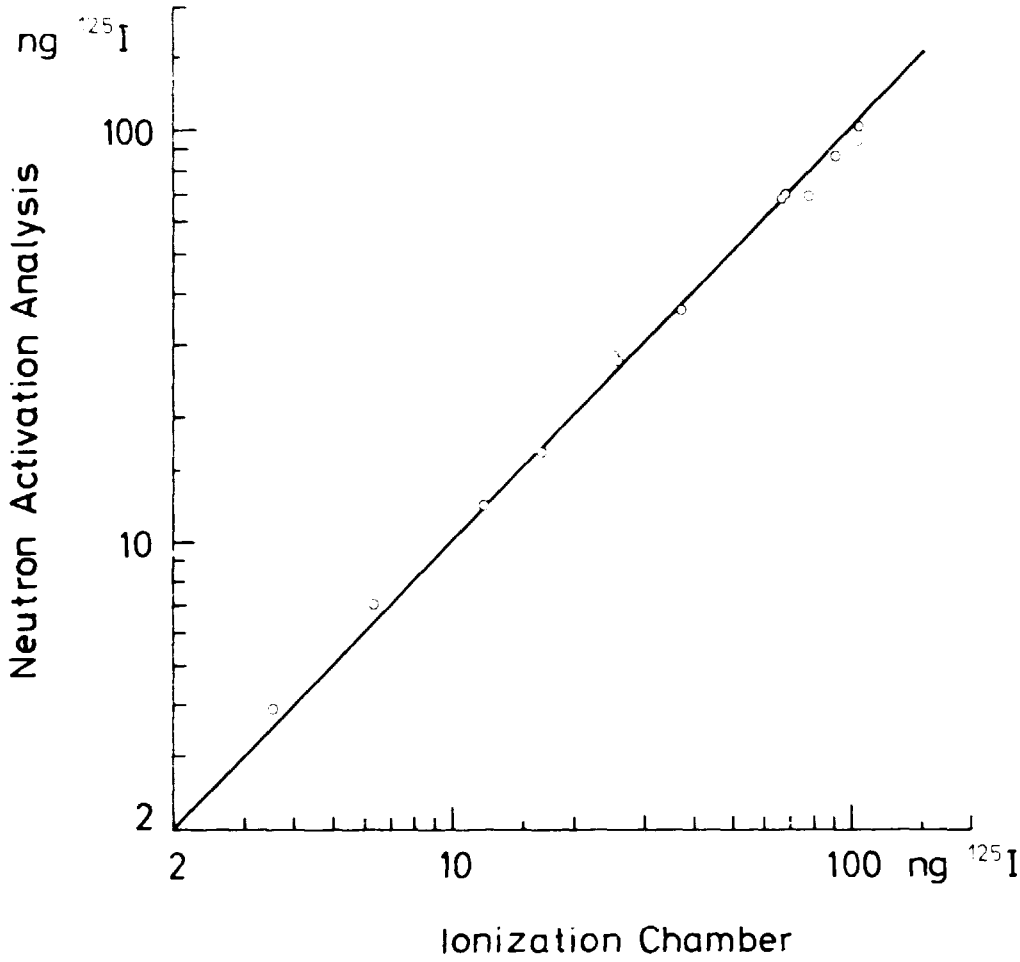

Fig. 33. Correlation between measured activity of ^{125}I and quantity determined by neutron activation analysis.

Among the primary radioisotopes in regular production at the Isotope Division, ^{36}Cl is the most long-lived and its specific activity is its most distinguished characteristic. This is measured by β-counting of ^{36}Cl followed by the determination of total content of chlorine by instrumental neutron activation analysis of the aqueous solution.

Neutron activation analysis based on the use of comparator standards automatically assumes identical isotopic composition of sample element and comparator. This assumption is far from correct in cases where a significant fraction of stable ^{35}Cl has been transformed into radioactive ^{36}Cl. However, the abundance of ^{37}Cl was assumed to be unchanged,[2] because its thermal neutron cross section is 2 orders of magnitude lower than that of ^{35}Cl, and a small loss would further be compensated by neutron absorption in ^{36}Cl. With ^{38}Cl as indicator,

2. The abundance of ^{37}Cl was later found to be normal within < 1 % relative.

correct results for total chlorine can therefore be determined with excellent accuracy by INAA, in spite of the abnormal isotopic composition.

Samples from 2 batches were taken in duplicate from 2 different dilutions, giving a total of 8 samples each containing about 2 μCi (70 kBq) ^{36}Cl. Results for the specific activity were subjected to a two-way Analysis of Variance with replicates, and no significant difference between the individual results could be found.

The overall mean was 7.31 mCi/g Cl (271 GBq/kg) with a standard error of the mean of 1 %.

A completely independent determination of the specific activity was carried out by determining the isotopic composition by mass spectrometry using a Varian MAT CH 5 instrument. With a liquid-nitrogen-cooled direct inlet system, a sample of 10 mm^3 of the stock solution containing 85 μCi ^{36}Cl per milliliter (~3 TBq/m^3) could be run several times at intervals of 15-30 minutes, each measurement being the result of 4-7 observations [Elfinn Larsen 1975].

Consecutive runs showed increasing ratios of mass divided by charge, m/e 37 ($^{37}Cl^+$, $H^{36}Cl$) to m/e 38 ($H^{37}Cl$), presumably caused by a slow exchange of natural HCl with sample HCl. Complete replacement was assumed when the difference between the maximum ratios for two consecutive samples became <0.5 %. This occurred only after a total of 5 samples and required a whole day's work; the contribution of m/e 38 to the total ion current, however, remained normal to within 1 % relative during the whole operation.

The final ratio of m/e 37 to m/e 38 was found to be 1.074 for the sample, which must be corrected for a ratio of 0.168 determined for natural HCl, to yield an isotopic ratio of ^{36}Cl to ^{37}Cl of

$$(1.074 - 0.168) = 0.906 \pm 0.005$$

The contribution to the uncertainty of the result from natural HCl is negligible.

This ratio is in principle exactly what was determined by neutron activation analysis of a sample with known ^{36}Cl activity, although the result was expressed as specific activity. With a half-life of 3.01 x 10^5 years [Holden 1973], one Curie is 0.842 N_A atoms of ^{36}Cl, and one mole of chlorine contains 0.242 N_A atoms of ^{37}Cl [Shields 1962].

The reported specific activity of 7.31 mCi/g Cl thus represents an isotopic ratio of ^{36}Cl to ^{37}Cl of

$$7.31 \cdot 10^{-3} \cdot 0.842 \cdot 35.45/0.242 = 0.901$$

With a standard error of 1 %, this value is in perfect agreement with the value obtained by mass spectrometry, and its accuracy is therefore consistent with the observed precision.

The *internal verification* of the accuracy of analytical results by the application of *duplicate methods* is basically part of the development stage of the analytical method - or perhaps rather the conclusion of the methodological development.

At this stage the analytical method is also in statistical control, which means that its precision can be monitored by the analysis of *duplicate samples*.

The appearance of new systematic as well as random errors gives rise to an increased variability of results, which will be detected by an analytical quality control system based on the Analysis of Precision [XI].

Verification of precision in a well-tested analytical method is therefore also a verification of the accuracy of results.

4.2. Detection of Systematic Errors by the Analysis of Precision

All random errors affecting the precision of analytical results can be included in the precision of the analytical method, either in the a priori precision or the counting statistics.

Some types of error cannot be estimated in this way; they are therefore referred to as non-random errors although the distinction is often made on a more pragmatic basis. Non-random errors are characterized by abnormal distributions, which may be used to distinguish between different types of such errors.

Gross errors affect individual samples or a group of samples with inaccuracies of unknown magnitude.

Systematic errors affect all samples of a certain type in a more or less consistent way. A special type of systematic error is the bias which affects all such samples in the same way and which is characteristic of some analytical methods or techniques employed.

Detection characteristics

When an analytical method is in statistical control and ready for routine application, a quality control system based on the Analysis of Precision will detect novel sources of variability whether they are random or not. A constant bias, however, will not be detected, and gross errors are detected only by a certain probability, unless a 100 % quality control is performed. Other systematic, variable errors are most likely to be detected sooner or later, and the nature of these errors often permits correction or sorting of the analytical results produced before the error is determined.

It may be expedient to estimate the maximum percentage, θ, of undetected gross errors that may be present in a set of N analytical

results, when quality control is carried out by the analysis of m duplicates.

The probability, P, for the absence of errors in a random sample of n items can be estimated from a Poisson distribution with

$$\lambda = n \times \theta \tag{88}$$

Values of n for the detection of gross error frequencies of $\theta = 5$ % and $\theta = 1$ % are independent of N, and Table 38 gives actual numbers for 3 different levels of significance.

For the approximately 2000 results of V in air filter samples reported previously [XI], altogether 319 duplicates were made. Table 38 shows that the percentage of undetected gross errors is definitely less than 5 % and probably less than 1 %.

The considerable number of duplicates needed to verify the absence of gross errors illustrates the necessity to prevent the occurrence of this type of error by mechanical or administrative precautions. Only by such a priori measures can θ be reduced to insignificance.

Experimental performance

During several years of routine determination of V and other elements in air filter samples [XI], both gross errors and systematic errors were detected and identified by the Analysis of Precision. Duplicate control data is presented graphically by plotting values of T as a function of the number of duplicates at suitable intervals. Action limits are indicated at 1 % and 99 % levels of significance.

Errors affecting relatively *few samples* were detected from the duplicate controls, as shown in Figs. 34 and 35.

The error in Fig. 34 appeared at the early stage of routine operation and could be attributed to a particular batch in which all samples were uniformly high.

The corresponding comparator standard was found to be about 15 % lower than required, and this incident led to the introduction of continuous quality control of recorded counts per microgram of vanadium comparator.

The error in Fig. 35 (a) was traced to a set of samples with no apparent common denominator. A duplicate re-run of the set gave excellent agreement with expected variability, as shown in Fig. 35 (b).

Assuming accidental interchange of some samples in the period of about 2 weeks between the original run and the corresponding duplicate run, the total quantity of vanadium in the set should be in statistical agreement with the duplicates.

This was found to be the case for the three measurements:

Table 38

Number of duplicates required to detect gross errors

Probability of detection 1 - P	Percentage gross errors ≤ 5 %	≤ 1 %
95 %	60	300
99 %	92	460
99.9 %	140	700

Original run	73.4 ± 3.6 ng
Duplicate run	80.7 ± 3.6 ng
Re-run	75.7 ± 3.7 ng

The complete agreement between the observed and the estimated standard deviation of these results shows that the analytical method was not out of statistical control

While efforts have been made to eliminate the risk of accidental interchange of samples, it must be realized that inadvertent exchange of two samples is not likely to be detected by the present quality control system with only about 12 % of duplicates. On the other hand, a shift affecting all samples in a batch will definitely be revealed by the presence of at least one duplicate sample in each batch.

Errors affecting essentially *all samples* of a certain type, but not necessarily all elements in the sample, were detected in several instances by the Analysis of Precision, as shown in Table 39.

In the *first case* the agreement of observed differences between duplicates with the variability expected from the calculated precision for Whatman No. 1 cellulose filters is very satisfactory for the elements V, Al, and Mn, but not for Cl.

All results are based on the single-comparator V-standard, which eliminates possible errors from a chloride comparator standard.

A classical Analysis of Variance confirmed a significant loss of chlorine from the fibrous cellulose filters during the 1-2 weeks between the first and second analysis.

In the *second case* an unexpected source of variation appeared in the same cellulose filters for the Al results only, while statistical control was maintained over the V results. In this case the Analysis of Variance did not establish any significant difference between the first and second determinations, so that no loss of material could be envisaged.

Contamination with Al is of course a potential possibility, but transfer of the irradiated Whatman No. 1 filter to an un-irradiated polyethylene

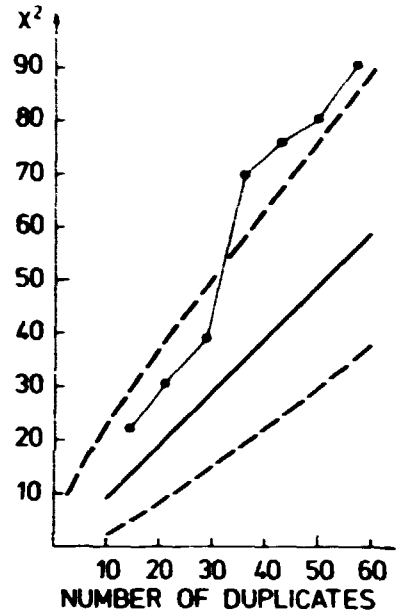

Fig. 34. Quality control plot of the statistic T showing the effect of an inaccurate comparator standard.

envelope before counting had been sufficient to overcome this problem until the beginning of 1976. A new pneumatic tube system based on pressurised air was installed to connect the irradiation terminal in the Radioisotope Laboratory with the DR 3 reactor, instead of the vacuum-operated system connecting to the DR 2 reactor. The change to the new system was made by the end of October 1975, and no additional sources of variation were observed as a consequence of this transition, as described in Section 3.3.

However, from January 1976 utilization was started of the same ca. 300 m long pneumatic tube for the transport of various materials irradiated for production purposes in other facilities of the DR 3 reactor. These materials are all encapsulated in aluminium cans, from which airborne aluminum-oxide contamination might conceivably be introduced into the system. Some of it could be activated and blown out at the irradiation terminal at the same time as an activated sample. The aluminium cans themselves are automatically diverted to a specially designed, shielded terminal in another part of the Radioisotope Laboratory. Under these assumed circumstances the ^{28}Al contamination adheres to the exterior surface of the polyethylene envelope, from which it may easily be displaced or lost in the course of normal handling of the envelope between the two successive countings of the same sample. This was in fact found to be the case, so that the apparent half-life of ^{28}Al determined from a sample proved significantly shorter than the actual half-life of 2.3 minutes.

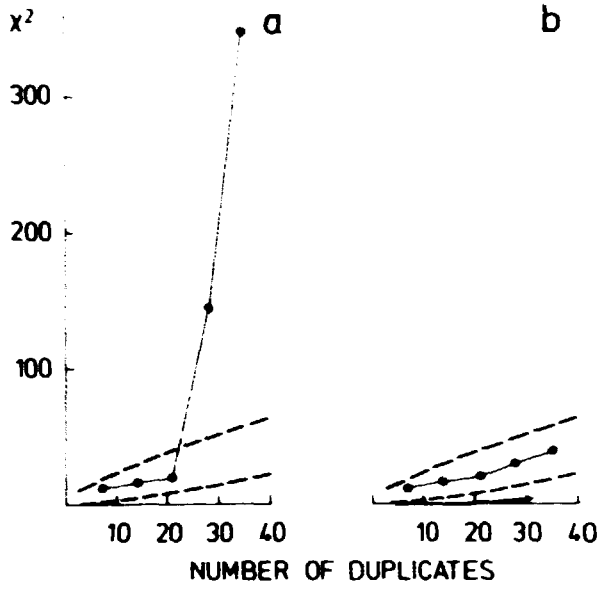

Fig. 35. Quality control plots of the statistic T showing the effect of (a) accidental interchange of samples together with (b) a re-run of the same sequence of samples.

In the *third case* in Table 39 the introduction of membrane filters instead of fibrous filters immediately gave rise to additional sources of variability between duplicates.

Both elements were lower in the second determination than in the first, indicating loss of particulate material from the filter surface; this loss, however, affected the two elements differently, as shown by the different Al to V ratios. This is in agreement with the tendency of Al to be found in the larger particles, while V concentrates in the small particulate.

In all 3 cases the systematic error was caused by the un-intentional transfer of material to or from the sample, and the remedy is *containment*.

The prevention of loss from the sample by containment necessarily increases the blank value, with a resulting loss of precision.

For the fibrous cellulose filters, the particulate material is firmly embedded in the filter material, and only the chlorine values will benefit from containment. Containment of the sample reduces the precision available for V determination, which is not acceptable in view of the modest volume of air samples. Containment was therefore rejected, admitting that results for Cl should be considered of questionable reliability.

For the membrane filters, the results are meaningless without containment during the entire sequence of operations, and therefore the procedure was modified for these samples; for actual samples the

Table 39

Control of sample stability by the Analysis of Precision [XI]

Matrix	Element	d.f.	T	$P(\chi^2 \geq T)$
Whatman No. 1	V	14	14.8	0.40
	Al	14	12.4	0.57
	Mn	14	11.4	0.65
	Cl	14	27.7	0.015
Whatman No. 1	V	12	9.2	0.69
	Al	12	26.8	0.008
Cellulose acetate	V	6	41	$<10^{-5}$
	Al	6	173	$<<10^{-5}$
	Al:V	6	96	$<<10^{-5}$

increased blank values and inferior precision is not completely offset by the increased volume of air sampled.

The prevention of contamination with airborne ^{28}Al dust particles is achieved by containment of the irradiation terminal as well as by performing all operations in a perspex box outside the active area of the hood.

Electronic errors certainly qualify for inclusion in the preceding groups of non-random errors. Malfunction, maladjustment, or faulty operation are gross errors which are usually discovered and remedied without great efforts, and thus deserve no further discussion.

Limitations in the design and operation of the available equipment may produce systematic errors under conditions such as very high count-rates, see Section 2.6., or particularly low signal levels. These types of errors are, however, predictable and should be considered in connection with the investigation of the performance characteristics of the analytical method.

In the present context electronic errors refer to the systematic errors present in equipment that is well within its design specification, that is used properly and appears to be in perfect operating condition.

One such example is shown for V in Fig. 36, in which a gradual increase in variability was observed after the 2 years of smooth operation reported in [XI]. Slow deterioration of electronic equipment is a source of error not easily detected by any other method, but an exact diagnosis is often difficult.

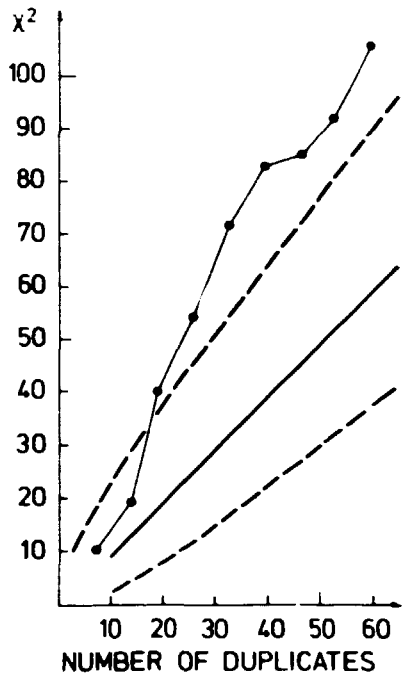

Fig. 36. Quality control plot of the statistic T showing the effect of inadequate stability of instrumentation.

The shape of the curve here differs from those characteristic of gross errors, shown in Figs. 34 and 35, and a novel source of chronic variability is therefore sought.

The present case was only diagnosed after several weeks of searching as an occasional slip in the memory of the computer that controls the multichannel-analyser. This only had a detrimental effect when it took place between the two countings of the same sample.

Another example is illustrated in Fig. 37 (a) showing the quality control plot of all 4 elements for 7 duplicate samples in the order of increasing γ-ray energy. Figure 37 (b) gives the same plot of a re-run showing that the error is intermittent instead of chronic, as in the preceding example.

In this case the error was identified as an occasional drop-out of 8 channels in the punched paper-tape print-out. This affects the calculation of the different parts of the spectrum in different manners, and in the case in question the effect on the calculation of V concentration was very small.

It should be noted that the total number of channels printed out was correct, and therefore the paper-tape had passed the usual check-system without being apprehended.

Without a continuous quality control system, electronic errors of this type might have remained undetected for a long period of time, and a

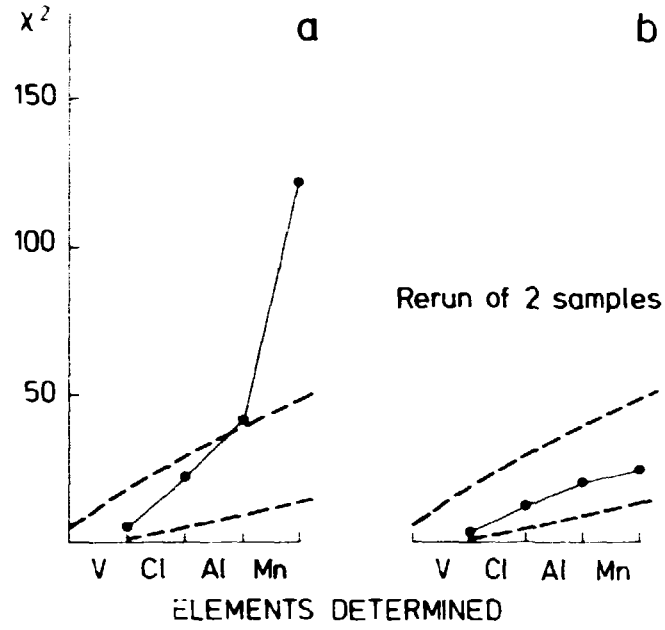

Fig. 37. Quality control plot for a set of 7 duplicates showing the effect of occasionally missing channel numbers (a) before and (b) after two samples were repeated.

large number of results of questionable value would have been produced.

These errors are typical systematic errors, which can usually be eliminated without great difficulty once they have been identified. However, the actual identification of a source of variability is not made by the quality control system, but depends entirely on the competence and insight of the analyst.

The detection of systematic errors by the Analysis of Precision is only possible when the errors lead to increased variability of results; a constant bias or a blank value cannot be detected by this method.

Electronic errors of this category are quite possible, and the quality control system should therefore, wherever possible, be based on results of identical samples analyzed on *duplicate equipment*. If only a single system is available, a change of conversion gain or similar alternative mode of operation should be introduced for the analysis of a duplicate sample. Under these circumstances a constant, electronic bias will not escape detection by the Analysis of Precision.

The control or detection of other types of blank values will be discussed in the next chapter.

4.3. Blanks

Perhaps the most significant characteristic of activation analysis is that it is free of blank problems; it must be kept in mind, however, that this applies only to the irradiated sample. Neglect of blank problems from the time of sampling to the end of irradiation may lead to very serious errors in the analytical results.

For the purpose of the subsequent discussions, the blank of a particular sample must be assigned a value, which is the expectation value of the result found by analysis of a sample that is identical with the particular sample in all respects except the absence of determinand.

A blank value is not a numerical performance characteristic [Wilson 1974] of the analytical method, but depends on the circumstances and the samples to which the method is applied. The usefulness of a blank value is therefore strongly dependent upon the degree of control that can be exercised over these conditions.

From an operational point of view, 3 types of blank distinguish themselves:

(a) the known blank for which exact *correction* is *possible* with complete confidence and often without significant loss of precision,

(b) the estimated blank depends on supplementary information on the particular sample analyzed, and correction is therefore more uncertain; a *maximum error* can usually be given,

(c) the unknown blank, for which *no correction* can be applied even when potential sources of blank errors are known; the only solution to this blank problem is to reduce the individual blank contributions to insignificance.

Known blank

In the first case the magnitude of the blank correction is not important, and in some cases a high, but constant blank value may be preferable to a lower, but less constant blank. In the other two cases, a reduction of the blank value automatically improves the accuracy of the analytical results; wherever possible these blank errors should be reduced to a level below the detection limit of the analytical method.

The *detection limit* is the concentration at which the difference between a sample and a blank is no longer significant; it is therefore determined by the standard deviation of a blank. This itself is referred to by Wilson [1973] as the Power of detection; however, in general, blank values in analytical chemistry have a very skew distribution, so that the standard deviation of the blank has no strict meaning.

In activation analysis the blank value does not distinguish itself from any other determination, and its distribution may be based on the usual

assumption of Poisson statistics. This degenerates in all practical cases into a normal distribution, and its standard deviation multiplied by a factor of $\sqrt{2}$ was used in [I] to express the detectivity of the instrumental determination of V in an oxine-pyridine matrix.

Several other related concepts are discussed by Currie [1968], who also defined the a priori detection limit, which is the concept used in the present work. Under practical conditions of counting the risk of making errors of both the first and the second kinds is less than 5 % when a factor of 3.29 is applied to the standard deviation of a blank sample.

This standard deviation will be determined from the actual signal recorded, which may be counts or ionization current; it must be converted to concentration units by division by the sensitivity of the method, which may be calculated from the irradiation conditions [V]. It should be noted that sensitivity and detection limit are inversely correlated within a relatively wide range of irradiation conditions.

This does not mean, however, that the accuracy of the results is improved by increasing sensitivity, which does not usually affect the blank value as such. For cases 2 and 3, where the blank correction is uncertain, Meinke [1973] recommends stating the *limiting error* as ± blank value.

A straightforward example of known blanks is the filter materials used in the air filter samples analyzed in [XI]. Here, the accumulation of blank values from successive batches not only serves to detect possible contamination or other blank errors, but also to verify the assumed normal distribution of these blank values.

Treating N blank values Y_{i_0} as sample replicates with calculated standard deviation $\hat{\sigma}_0$, one can test for the absence of significant deviations from the weighted mean $\hat{\mu}_0$ by the statistic T:

$$T = \sum_{i_0}^{N} \frac{(y_{i_0} - \hat{\mu}_0)^2}{\hat{\sigma}_{i_0}^2} \qquad (39)$$

which is approximated by a χ^2-distribution [VIII] with N-1 degrees of freedom.

Agreement between the observed and the calculated variability of blank values was very satisfactory for all three types of material, as shown in Table 40.

This means that the weighted mean of all proper blank values gives the best sample correction; moreover, its reduced uncertainty does not affect the precision of sample results.

In addition, the demonstrated adequacy of the calculated standard deviation of the blank permits the reliable prediction of the limit of detection for the three filter materials.

Table 40

Blank values and detection limits
for vanadium in air filter samples [Xl]

Type of filter Condition	Cellulose Whatman No. 1 transferred	Membrane filters dissolved in 1 ml pyridine	Membrane filters in sealed polyethylene bag
Blank value	0.13 ± 0.07 ng	3.7 ± 1.0 ng	27.6 ± 0.7 ng
Degrees of freedom	249	34	36
T	261.7	30.9	44.5
$P(X^2 \geq T)$	0.28	0.62	0.16
Limit of detection*	4 ng	19 ng	14 ng
in air	~ 2 ng/m³	~ 5 ng/m³	~ 4 ng/m³

*Assuming $P_\alpha = P_\beta = 0.05$

For the determination of concentrations of atmospheric vanadium in remote, non-urban regions in Greenland or the Faroe Islands, lower detection limits are required. This was achieved by increasing the volume of air drawn through a membrane filter, but also by eliminating the containment of the air filter samples needed to avoid loss of particulate matter [Xl]. In this way the limit of detection in air was reduced by 2 orders of magnitude; but in no cases could V be detected in actual samples from these areas. This is, however, not inconsistent with the results found by Zoller [1973], who reports V-levels in antarctic air that are lower by an additional two orders of magnitude.

Similar considerations apply to other types of instrumental neutron activation analysis, where the irradiation container represents a known blank. Transfer of the irradiated sample to an inactive counting vial gives the lowest blank value and the best detection limit [I], but it is not always feasible. In the case of Li [Heydorn et al. 1977], the short half-life of ^{8}Li does not leave time for transfer, and in other cases there is a risk of losing volatile elements such as Cl or I [Ii].

Our usual half-dram vials from the Olympic Plastic Corporation, used for both irradiation and counting, are made of very pure low-density polyethylene with impurity levels below 1 mg/kg. For many matrix materials, the detection limits for the majority of elements are not affected by the contribution from the polyvial, and it is therefore very suitable for instrumental neutron activation analysis at the mg/kg level - below which the credibility of results deteriorates rapidly anyway

Table 41

Selected impurity levels in half-dram polyvials* and polyethylene film

Element	Damsgaard 1975 µg	Yule 1966 µg	Harrison 1975 mg/kg
Na	0.6	0.30	3 4
Al	0.2	0.25	1.0
Cl	0.7	< 0.02	5.3
K	0.6	< 0.1	
V	0.001	< 0.02	0.2
Mn	0.02	0.008	~ 0.2
Cu	0.10	< 0.008	
As	0.002	< 0.01	
Se	<0.003	< 0.1	< 0.003

* Weight 1.1 g

[Meinke 1973]. Experimentally determined quantities of selected elements are summarized in Table 41, and although not quite as good as the material available to Yule [1966], it is far superior to the polyethylene films investigated by Harrison [1975].

In the re-irradiation yield determination of As by the ionization chamber measurement described in [V], a blank value of 0.02 mg As was determined experimentally, and its variation was found to be insignificant compared with the background fluctuation. This blank value represents not only the polyvial, but also the approximately 1 cm^3 of ammonium-sulfide solution used to dissolve the sulphide precipitate of As. It is the total activity that is measured by the ionization chamber, and only a small fraction is caused by the presence of As; but as long as the blank value is constant, its origin is not important.

Variations in otherwise known blank values may occur, when the addition of reagents is not controlled adequately, or their contribution is much greater than anticipated. An example of this is the addition of sodium-citrate solution to blood samples to prevent clotting; here the unexpected increase of As concentration by almost 2 orders of magnitude [IV] required the addition to be controlled more accurately than was actually possible. The uncertainty of the corrected values for As in plasma was therefore greatly increased, when the 20 % variation in the blank value was taken into account.

Table 42

Vanadium impurity in various reagents [I]

Reagent	V µg/kg	ml of reagent ≡ 1 ng of V
Potassium hydrogen phthalate, approx. 10 %	< 0.3	> 3
Chloroform, A.R.	< 0.3	> 2
Nitric acid, approx. 70 %	< 0.3	> 2
Pyridine, A.R.	< 0.5	> 1.5
Distilled, yellow fuming nitric acid	< 0.8	> 1
Ammonium hydroxide, approx. 58 %	< 2	> 0.5
Sulphuric acid, 96 %	< 2.4	> 0.2
Nitric acid, approx. 90 %	< 4	> 0.15
Oxine, 8-hydroxy quinoline 1 % in chloroform	~ 5	~ 20

Similar considerations necessitated the determination of V in the individual reagents used for the pre-irradiation separation procedure reported in [I]. Only upper limits ($P \geq 0.95$) could be determined, and these are given in Table 42 together with the calculated least amount of reagent needed to increase the blank value of V by 1 ng. This immediately draws our attention to those reagents that require the most accurate control of addition, but in all cases routine analytical technique appears entirely adequate to keep variations in blank value well below 1 ng of V.

A closer study of the actual procedure, however, reveals some of the fundamental problems of blank corrections. The initial addition of sulphuric acid can easily be reproduced to 0.1 cm^3 or better; the successive addition of nitric acid portions can probably be duplicated in the corresponding blank sample with satisfactory precision. Unless the sample to be analyzed is pure water, however, the quantity of ammonia used to neutralize the acid sample solution before extraction will differ from that of the blank. Consequently, the resulting blank can no longer be used with complete confidence, and exact correction is impossible.

Estimated blank

The measured blank is therefore replaced by the estimated blank, which is based on a detailed knowledge of the origin of all contributions to the final blank value.

There are, however, two problems involved in this estimation of blank value. One is the accurate determination of the concentration in each reagent, rather than the upper limits; this must usually be carried out by the actual method of analysis under investigation with all the problems involved. The second problem is the fundamental question of how to correct blank values for chemical yield, seeing that the recovery of an element certainly depends on the stage of the analytical method at which it is added. Finally, a positive verification of an estimated blank value must be made experimentally, in order to ascertain that all significant sources have been properly taken into consideration.

Identification of all such contributions was attempted in connection with efforts to duplicate the work of Levstek et al. [1972], who reported unexpected losses of V during dry ashing of plant materials such as Kale and SRM 1571 Orchard Leaves. In our improved pre-irradiation separation method for V in biological materials, we replaced the dry ashing by combustion in a completely sealed system in the form of a Schöniger flask [Rietz[3] 1972]

Here, the concentrations of V in the reagents determining the blank value were partly determined by purely instrumental neutron activation analysis, and partly by prior extraction with 8-hydroxy-chinaldine. No blank corrections were made, and the results are therefore positively biased.

No sulphuric acid is needed in the Schöniger combustion, but a minimum volume of 125 cm^3 was needed for efficient dissolution of the combustion products. The addition of nitric acid, as well as of chinaldine in chloroform, was therefore doubled in comparison with the original method. After special purification of 8-hydroxy-chinaldine to reduce its V content from 26 µg/kg to 5 µg/kg, results were found as shown in Table 43.

These experimental results demonstrate that the blank values are independent of the yield, which again proves that the contributions from the cellulose powder used as a blank material, as well as the filter paper strip used for ignition, together with the added ^{48}V tracer, are all without significance. No significant difference is found between the individual blanks, and their weighted mean is in excellent agreement with the blank value reported by Levstek [1972].

The results also show that a significant fraction of the observed blank value cannot be explained by the known additions of reagents, even though their concentration may be slightly overestimated. The only

3. Unpublished work.

Table 43

Vanadium blank values in Schöniger combustion

Observed blanks *		Estimated blank	
^{48}V yield	Vanadium ng	Contribution from	Vanadium ng
47 %	11.9 ± 2.2	Nitric acid	2.4 ± 0.9
84 %	11.0 ± 1.7	Ammonia	2.6 ± 0.4
56 %	11.2 ± 1.8	8-hydroxy-chinaldine	2.1 ± 1.0
96 %	10.4 ± 1.5		
46 %	9.8 ± 1.4	Estimated blank	7.1 ± 1.4
Mean	10.7 ± 0.8	Deficit	3.6 ± 1.6

* Rietz 1972

remaining source of V contribution seemed to be the pure oxygen used for combustion of the sample.

This was investigated by comparing the blank values of a small piece of filter paper ashed in pure oxygen with an identical piece ashed in atmospheric air:

It appears that a contribution of about 1 µg of V per m^3 of oxygen must be included in the estimation of blank value in order to achieve satisfactory agreement with experimentally determined blank values. From a practical point of view, these same experimentally determined blank values should be subtracted from sample data before any correction for chemical yield is made.

Interference from other elements may have to be taken into account in the estimation of blank values, but this is usually treated as a performance characteristic of the analytical method, as described in Section 2.5. A special case in the instrumental determination of V is the drastic increase in blank value resulting from the replacement of the 3" x 3" solid scintillation detector by the corresponding well-type detector; here the sum peak of ^{19}O at 1.55 MeV constitutes an important matrix interference, even after the usual pre-irradiation separation.

Samples not subjected to pre-irradiation separation are subject to nuclear interference from elements with atomic numbers one or two higher than the element to be determined. Transmutations by fast neutrons during sample irradiation produce the same indicator isotope, which gives rise to a blank value even after a complete radiochemical separation.

The magnitude of this interference is estimated from the (n,p) and (n,α) cross sections given by Calamand [1974], but in critical cases experimental determinations have to be made [IX]. High-purity interfering materials were irradiated together with the appropriate comparator standard within and without a 0.5 mm thick cadmium box in the pneumatic tube facility used for sample irradiation. Results for Mn, Se, and Cu [Damsgaard and Heydorn 1976] are presented in Table 44 together with cross sections based on a known thermal-to-fast-neutron ratio of 44 for the irradiation position used.

The successful estimation of blank values is based on the assumption that a particular sample can be assigned a blank with a particular expectation value. This is not always quite so. The loss of gaseous ^{41}Ar during measurement, for example, is a potential source of systematic difference between otherwise identical samples, but its effect on the blank values for instrumental determination of V in [I] was found to be insignificant. The reverse case of building up contamination from gaseous ^{203}Hg escaping from a succession of samples during measurement is another example that introduces a highly significant time dependence of the blank correction.

In such cases the uncertainty of the corrected results is increased, but a maximum systematic error can be given. In other cases the magnitude of the blank value escapes determination, and no correction can be made.

Unknown blank

In cases of unknown blank value, the only way to produce accurate results is to eliminate or reduce to insignificance the source of a blank contribution. Before this can be done, the presence of a potential blank problem must be realised, and in most cases its elimination is not particularly difficult. An obvious example is the selection of equipment for sampling of biological materials that does not contain the determinand [Larsen et al. 1972], e.g., the use of a platinum cannula for taking blood samples for the determination of Mn [IX]. This is an intrinsically safer method than the subsequent demonstration of insignificant contamination levels achieved using conventional equipment [Versieck 1972].

The presence of a blank problem, however, is not always obvious, as was clearly experienced in the determination of V in human serum [I]. Here, the use of porcelain crucibles for dry ashing produced consistently low pure water blank values, as well as analytical results for V in serum that were in good agreement with the only published data [Gofman 1961].

Table 44

Experimentally determined blank values and cross sections for nuclear interference [IX] based on thermal activation cross sections from Aliev [1970]

Determinand	Interfering reaction	Blank of 1 ng produced by	Equivalent* cross section	Literature value cross section	reference
Mn	$^{56}Fe(n,p)^{56}Mn$	660 ± 20 µg Fe	1.04 ± 0.03 mb	1.03 mb	Bresesti 1970
Cu	$^{64}Zn(n,p)^{64}Cu$	7.79 ± 0.07 µg Zn	37 ± 2 mb	36 mb	Kimura 1971
Se	$^{81}Br(n,p)^{81m}Se$	106 ± 3 µg Br	0.014 mb*	0.086 mb*	Pfrepper 1976

* 1 mb = 0.1 fm² * A discussion of these results is given by Heydorn [1976]

Results obtained by wet ashing yielded higher blank values, but lower serum values, which were later confirmed by dry ashing in quartz crucibles. This led to the conclusion that the glaze in the porcelain crucibles was the source of practically all the vanadium found in the corresponding samples. Serum ash slightly attacks the glaze of porcelain crucibles, which was found to contain appreciable quantities of V; the distilled water blank on the other hand did not attack the glaze. An exactly corresponding case was recently described by Wuyts [1976] in a critical investigation of results for Cu in brain tissue reported by Greiner [1975]. Also here it was shown that the dry ash attacked the glaze in the porcelain crucibles, so that the sample was contaminated with Cu, etc. In such cases there is no way of correcting for a blank, because the blank is only produced by the sample itself. The problem with vanadium was eliminated by the use of crucibles made of synthetic quartz with a V content that was more than two orders of magnitude lower.

Only after the end of irradiation is the blank problem eliminated, and during irradiation a container blank may well be introduced for all elements present in amounts comparable to the quantity of the corresponding determinand in the sample being irradiated. For the elements involved in this study, Table 41 gives the levels found in the half-dram polyvials.

For the determination of As, Se, and Mn in biological materials, the blank problem was investigated by the analysis of redistilled water irradiated in the same half-dram polyethylene vials [VII]. No As or Se could be detected in the water, but up to 10 % of the Mn could be released during irradiation. The limited contact between a tissue sample and the container wall makes a tissue blank lower than that of water, and a blank value of <3 ng was assumed. For the method described in [VII] this means that all three elements have blank values well below the limit of detection for actual tissue samples, and no blank correction is therefore required.

For samples with ultratrace levels of determinand, an irradiation blank of several ng is not acceptable, and where possible the exterior surface of the sample is removed after irradiation by etching or mechanical treatment. This is usually impossible with biological samples.

The actual contribution to the blank value must therefore be reduced by selection of:

(a) cleaning methods that remove surface contamination without attacking the surface itself,
(b) irradiation containers with particularly low levels of critical impurities.

For the determination of Mn in serum, we found that the half-dram polyethylene vials used for irradiation of tissue samples released more manganese during irradiation to a redistilled water blank than was expected to be present in the actual serum sample [IX]. This agrees with the results of Brune [1966], whereas Mitchell [1973] reports no leaching of Mn from polyethylene surfaces. Cleaning with nitric acid, as recommended by Robertson [1972], instead of with redistilled water reduced the total Mn by about 25 % in good agreement with the investigations of Karin [1975]. At the same time the release of manganese from these vials dropped by more than a factor of 2, but the variation in blank values was not acceptable. A 3 % hydrogen-peroxide solution proved just as effective as nitric acid, and less variation in blank values was observed.

A significant positive correlation was found between the total amount of manganese in the polyvial and the quantity released to the redistilled water blank, and a search was made for polyethylene vials with lower manganese content.

One such container made available to us[4] contained about half as much manganese, of which only about 1 % was released during irradiation. No correlation between total manganese and blank values was observed, and a correction of values for manganese in serum by 0.15 mg/m^3 (ng/ml) would presumably yield acceptable results, although the observed variation in blank values would reduce their precision. Similar results were recently found by Maziere [1977] with very pure, synthetic quartz ampoules.

Finally, a polyethylene ampoule of undetermined manganese content was investigated, and a significant release of sodium was observed during irradiation. However, the release of manganese was near the limit of detection of the method for the analysis of serum. At the same time the release of arsenic and selenium was also below their limits of detection, as shown in Table 45, and this container was therefore selected for the irradiation of serum samples.

In contrast to the other polyethylene containers, the total manganese content of the polyethylene ampoule selected could not be determined by instrumental neutron activation analysis, because of its high sodium content. The irradiated ampoule was therefore brought into solution by alternate charring with hot sulphuric acid and oxidation with 50 % hydrogen peroxide. The manganese contents, determined after radiochemical separation, showed considerable variation between individual ampoules, but their average was not lower than for containers with considerably higher blank values.

The results of these investigations are summarized in Table 46, from which it is quite obvious that the total manganese content of irradiation

4. Courtesy of Dr. Nic Spronk, Vrije Universiteit, Amsterdam.

Table 45

Limits of detection and blank values
for the determination of As, Mn, and Se in serum [IX]

Element	Detection limit µg/kg	Bias	Blank value µg/kg
As	0.25*	-1 %	< 0.2
Mn	0.02	-1 %	0.02 ± 0.01
Se	10	+2 %	< 0.2

*Assuming no bromine interference

containers is no useful guide to the selection of containers with low blank values.

The use of a different irradiation container for serum and for the comparator standards gives rise to a systematic error because of the vertical flux gradient in the reactor. This error has been estimated to yield a 1 % negative bias for arsenic and manganese and a 2 % positive bias for selenium. For the actual concentrations of these elements in serum, these errors are below the limits of detection given in Table 45, and no correction has been made in the results.

With the blank values for arsenic and selenium below the detection limits, only the origin of the manganese blank needs to be investigated in order to decide whether a blank correction should be made.

Duplicate samples of freshly made redistilled water were taken from the same bottle at the same time and transferred to polyethylene ampoules in a clean room. One sample, serving as a reference, was irradiated and analyzed for manganese in the usual manner. The other was frozen and surrounded by solid carbondioxide during an entire one-hour irradiation. After irradiation the frozen sample was removed from the container, and the surface layers were allowed to melt away. Only a solid core of ice [Brune and Landström 1966], which had not been in contact with the container wall, was used for analysis.

The difference between the two results was 0.0035 ng/ml, 3.5 µg/m^3, which represents the contribution to the blank value from the irradiation container. Although probably significant, its contribution is definitely below our limit of detection for manganese in serum.

The greater part of the manganese blank value must represent the actual presence of manganese in the redistilled water samples, the origin of which is not known. It would be misleading to apply corrections on this basis, and consequently analytical results for all three elements in human serum are presented without blank correction.

Table 46

Manganese blank values for different irradiation containers [IX]

Irradiation container		Total Mn	Blank value		
Type	Supplier	ng	Volume cm^3	Number of analyses	Concentration of Mn mg/m^3
Polyvial	Olympic Plastics Company	24 ± 7	1.1	15	1.1 ± 0.7
H_2O_2 cleaned		22 ± 8		10	0.5 ± 0.1
HNO_3 cleaned		18 ± 5		12	0.4 ± 0.4
Polyvial	Dr. Nic Spronk	14 ± 3	1.1	10	0.15 ± 0.05
Polyethylene ampoule	Atomic Industrial	19 ± 11	4.5	10	0.02 ± 0.01

Table 47

Copper blank values for different irradiation containers [Heydorn et al. 1976]

Irradiation container		Total Cu	Blank value		
Type	Supplier	ng	Volume cm^3	Number of analyses	Concentration of Cu mg/m^3
Polyvial	Olympic Plastics Company	91 ± 41	1.1	10	3.4 ± 0.4
H_2O_2 cleaned		105 ± 49		10	10 ± 4
HNO_3 cleaned		101 ± 15		10	33 ± 9
Polyethylene ampoule	Atomic Industrial		4.5	6	7.2 ± 0.9

The later addition of Cu to the determinands in the analysis of biological materials, including serum, does not give rise to comparable blank problems, because of the much higher concentrations of this element in practically all samples, including human blood. Only in some analyses of amniotic fluid were the concentrations similar to the impurity levels of the irradiation containers. Here again, Brune [1966] reported the release of Cu from a polyethylene surface, whereas Mitchell [1973] failed to detect any leaching. An investigation similar to the previous one for Mn was therefore carried out for Cu [Damsgaard and Heydorn 1976], and the results are presented in Table 47.

In contrast to the previous study, a simple rinsing of a polyvial with redistilled water is superior to the classical nitric acid procedure [Robertson 1972], as well as to the other alternatives.

Again the copper content of the redistilled water was determined by the irradiation of a frozen sample and analysis of the core only. In this way the copper content was found to be 3 ng/ml (mg/m^3), which indicates that no Cu is released from the polyvial during irradiation; no blank correction should therefore be made of the analytical results, unless the sample has been adulterated by redistilled water!

Insignificant blank

When all potential blanks have been either corrected for or eliminated, it still remains to demonstrate that all *residual blanks* are actually reduced to insignificance. This can be done by the analysis of sub-samples with contents of determinand near the limit of detection, and comparison with results for the larger sample.

This was the method used in the determination of Li in SRM 1571 Orchard Leaves by instrumental neutron activation analysis [Heydorn et al. 1977]. Here 5 different samples with weights between 0.2 and 0.5 g were analyzed 4 times each; linear regression of the sample means on the sample weights is shown in Fig. 38, with a correlation coefficient of 0.985 and no significant blank correction.

The slope of 0.77 mg/kg was in perfect agreement with the weighted mean of all results - but not with the information value given by the National Bureau of Standards.

This simple type of correlation analysis not only serves to verify the absence of significant blank errors, but may also be used as a method of quality control whenever the same material is analyzed a number of times by the same method.

In neutron activation analysis with radiochemical separation, however, sample weights should be corrected for chemical yield, and correlated with actually recovered quantities of determinand. In this case both variables are random, and often with similar relative standard

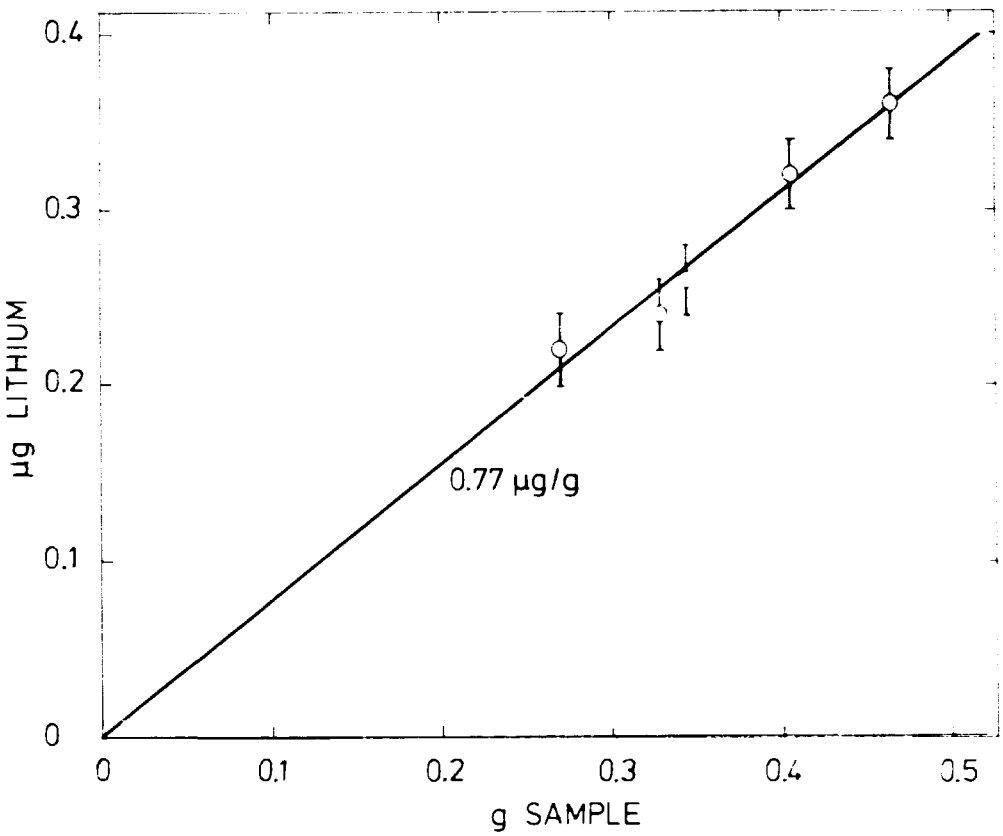

Fig. 38. Correlation between observed quantity of Li and weight of Orchard Leaves SRM 1571 shows the absence of significant blank correction.

deviations from irradiation and re-irradiation. The absence of significant blank errors cannot be demonstrated by regression analysis under these circumstances.

The functional relation between sample weight and determinand results in a structural relation between the stochastically independent variables m_i and q_i, which manifests itself by a high degree of correlation [Kendall 1973]. If a significant correlation coefficient is found for samples of different weights, the absence of blank errors may be verified by the statistic

$$B = \sum_{1}^{N} \frac{(q_i - \hat{\mu} m_i)^2}{\hat{\sigma}_{qi}^2 + \hat{\mu}^2 \hat{\sigma}_{mi}^2} \qquad (90)$$

which is approximately χ^2 distributed, when $\hat{\mu}$ is the weighted mean of N independent results q_i/m_i. If the analytical method is in statistical control, as demonstrated by the T statistic according to equation (89), significant deviations from the expected value of (90) signal the presence of a blank problem.

The only material for which a reasonable number of determinations has been made is SRM 1571 Orchard Leaves, which was the subject of a special investigation for its concentration of arsenic [Damsgaard and Heydorn 1973]. A test for the absence of blank value was made by means of the statistic in equation (90) using the results of the radiochemical separation, shown in Table 48.

A similar type of verification of the absence of blank values is the analysis of a number of sub-samples to compare with the analysis of a sample of normal size. Such a study was made in the investigation of the concentration of V in small polyethylene bags used for the containment of membrane filters; it was found that the weighted mean of results close to the limit of detection varied in complete accord with the size of the polyethylene bag. This proved that the concentration of 0.10 mg/kg V from the polyethylene was the only significant blank contribution.

Another comparison has been made between the concentration of As in tissue determined by Larsen [1972] on samples weighing about 1 g, and the overall mean of samples of human skin weighing 2 orders of magnitude less. The agreement between these results is shown in Section 5.1 and again lends support to the assumption of no blank value.

It is usually possible in neutron activation analysis to reduce blank values to the limit of detection, as shown in Table 45. This also solves the problem of deciding to what extent correction has to be made.

The *information content* of an analytical result is then satisfactorily determined by its precision [Malissa 1975], which again is contained in the analytical method [VIII]. Instructions for the estimation of the standard deviation σ of the analytical results constitute a performance characteristic, which may be used for the calculation of information content I, according to Eckschlager [1969].

$$I = \log_2 \frac{100\sqrt{N}}{\sigma\sqrt{2\pi e}} \quad \text{bits} \tag{91}$$

where N is the number of measurements.

Attempts to combine precision and accuracy to make a *total error* are tempting when systematic errors like blank values cannot be completely eliminated. Eckschlager [1972] endorses with minor modifications the expression proposed by McFarren [1970]

$$100\,\frac{|\eta| + 2\sigma}{\mu} \tag{92}$$

where η is the bias or blank value and μ the mean value. This expression was also used by Lauwerys [1975] in an evaluation of the acceptability

Table 48

Test for the absence of blank values
in the determination of As in SRM 1571 Orchard Leaves

Yield-corrected sample weight g	Quantity of arsenic µg
0.297 ± 0.010	2.90 ± 0.10
0.204 ± 0.007	1.95 ± 0.07
0.210 ± 0.007	1.97 ± 0.07
0.190 ± 0.007	1.72 ± 0.06
0.192 ± 0.007	1.79 ± 0.06
0.212 ± 0.007	2.04 ± 0.07
0.156 ± 0.005	1.66 ± 0.06
0.174 ± 0.006	1.87 ± 0.07
Correlation coefficient	r = 0.964
Significance at 6 d.f.	P < 0.1 %
Mean value	9.70 ± 0.17 mg/kg
Test statistic	B = 10.71
Probability for 7 d.f.	P > 10 %

of Pb and Cd measurements by non-activation methods, whereas Rosenberg [1976] used a les well-defined combination of errors in INAA. Further refinements of the concept of total error are presented by Midgley [1977].

It seems that such combined errors do not convey much useful information, particularly when one approaches the detection limit of the analytical method, where blank variability is a major problem. On the contrary, in agreement with Campion [1973], random uncertainties and systematic uncertainties should be reported separately.

This is particularly important when analytical results are compared with certified data for Standard Reference Materials, where precision and accuracy may be tested simultaneously.

4.4. Reference Materials

While the presence of many unknown systematic errors may be detected by the Analysis of Precision, and the absence of many known systematic errors may be ascertained by Correlation Analysis, there

remain some types of systematic error that cannot be found by any of these methods: unknown, systematic errors which do not contribute to the variability of results. Examples are:

Systematic errors of calculation or fundamental assumptions, calibration errors and other types of constant, relative biases, including erroneous comparator standards.

Such errors are detected when the same samples are analyzed by other laboratories or other analytical techniques. This might be done by a simple exchange of standards, which means materials of known composition. However, the use of a material having a composition close to that of the actual samples being analyzed is preferable, because it is more likely to be affected by the same types of error; and only in this case is it possible to perform the analysis as a blind experiment.

The most important property of such a material is its degree of homogeneity, and this may be tested by the Analysis of Precision; this is, however, a major task, which is usually left to institutions specializing in preparing such *Reference Materials*. This adds another crucial property to the required characteristics, namely stability with time so that lack of agreement need not be attributed to different times of analysis of the same material. According to the level of ambition of the institutions producing Reference Materials, we may distinguish between several types of Material.

The simplest type serves to extend intra-laboratory consistency to inter-laboratory comparisons of results obtained by the same method. This is important in clinical chemistry, and Reference Materials in the form of dried sera for such *Intercalibration* exercises have been commercially available for a number of years. Discrepancies between laboratories may be eliminated by standardizing the analytical techniques used, until comparable precision and absence of significant bias has been reached. Unless a *Referee Method* [Cali 1972] is used, however, there is no reason to expect that the results reported are unbiased

This was the situation for many elements with respect to trace analysis in biological materials until about 10 years ago when only one method was available for a determination with reasonable precision; in most cases this method was neutron activation analysis with radiochemical separation (RNAA).

The advent of other methods with comparable precision at the trace level, particularly Atomic Absorption Spectrometry (AAS), changed this situation significantly, and Reference Materials are now usually analyzed by several different analytical methods. Results from such *Intercomparisons* are now much more useful for investigations of the accuracy of analytical results, and agreement between independent analytical methods with entirely different systematic errors is usually

accepted as a confirmation of the absence of significant bias in the result. However, if significant differences are found between different methods, there is no way of telling which method has the smallest bias, and no conclusion can be made before further investigations. Thus the weakness of such intercomparisons is that no certain answer is given to the question of analytical accuracy, except in cases where consensus has already been reached between several methods.

Results of intercomparisons sometimes form the basis for a *certification* of a Reference Material by the originating institution. The pooling of data from different laboratories to obtain a consensus value has been made on the assumption that all participating laboratories are equally competent to perform a particular analysis, and that their systematic differences from the true value follow a normal distribution [Sutarno 1975]. This is perhaps a satisfactory approximation [Mandel 1977] to the intercalibration process, where the same analytical method with local deviations is used by everybody - but only if all participating laboratories report the same number of results!

Different levels of experience have surprisingly little influence on the precision of the results [Lauwerys 1975], but the use of several entirely different analytical methods to improve accuracy in the intercomparison causes severe deviations from normality of the inter-laboratory variation. Weighting of the laboratory mean values has been used in this situation, but the simple weighting by the number of replicates leads to logically inconsistent estimates of best mean and standard error [Mandel 1970]. Other weighting factors may under special circumstances be calculated by iteration, but the conditions required are seldom achieved in practice.

Another problem is the rejection of outliers in the data material, which means results that have an inordinate effect on both mean and standard deviation. The most widely applied methods are Dixon's test and Chauvenet's criterion, both of which were used by Fukai [1976] in his treatment of results for 14 trace elements from 60 different laboratories. Chauvenet's criterion rejected more results than Dixon's test and gave more consistent results for most elements.

However, the best mean and standard deviation from an intercomparison exercise cannot be based on purely statistical considerations; the majority of cases requires an intimate knowledge of the problems associated with analysis.

Such knowledge is only available in the relatively few institutions actually carrying out all determinations by several different methods leading to the certification of a Reference Material. Only the National Bureau of Standards has actually produced such Certified Reference Material (CRM) in the biological field with a considerable number of trace elements included in the certification. According to Cali [1976],

measurement by at least two independent and reliable methods without significant systematic error is required for certification. This approach was assumed by the National Bureau of Standards to guarantee the accuracy of a certified value [Meinke 1973] so that the *true value* was found within the stated overall uncertainty. Recently, however, it was conceded that NBS does not claim to be infallible [Cali 1976].

Nevertheless, the measurement of the bias of an analytical method [Wilson 1974] is without doubt best carried out by comparison with certified concentrations in a suitable Standard Reference Material (SRM) from the National Bureau of Standards.

Biological reference materials

In the present investigations the analysis of international biological Reference Materials served two purposes:
(a) as a homogeneous material it served in the Analysis of Precision to verify the absence of unknown sources of variation,
(b) being available to all research workers, the publication of results serves to pinpoint possible systematic deviations.

In most cases, results were published before consensus values or certified concentrations were available, and therefore they were not used at the development stage to ascertain the absence of significant bias, but rather as a final demonstration of the quality of the analytical method.

Participation in the analytical intercomparisons organised by the *International Atomic Energy Agency* is a useful exercise for all participating laboratories, and the results reflect the level of competence achieved in the various analytical techniques for the determination of trace elements.

Here, the best value for the concentration of an element is not the simple mean and its associated standard error, calculated from the results submitted by all participating laboratories. This is clearly brought out by the report on the determinations of trace elements in Animal Blood [Tugsavul 1970], which presents a mean value of 0.19 mg/kg of manganese with a standard deviation between laboratories of 0.11 mg/kg.

From exactly the same data, a mean value of 151 ± 4 µg/kg was extracted in [IX] by pooling results from only 3 laboratories. Although not significantly different from the overall mean, the standard error of the mean is hereby reduced by an order of magnitude, and therefore it becomes useful for checking the accuracy of new analytical procedures [IX].

For copper, a value of 1.76 ± 0.02 mg/kg in Animal Blood was submitted in 1968 to the IAEA and contributed as Lab. No. 8 to an

overall mean value of 2.15 ppm, with a standard deviation between laboratories of 0.66 ppm [Tugsavul 1970]. The only individual results published were also based on neutron activation and the use of solvent extraction with re-irradiation yield determination [Steinnes 1971]; the mean of 5 determinations was 1.76 ± 0.07 mg/kg Cu.

This experience confirms that it is much easier to reach agreement with other laboratories in an intercalibration using the same analytical method than in an intercomparison using different methods.

This, of course, also applies to the first real biological Reference Material, Kale, introduced by *H.J.M. Bowen* [1965]. The very large number of results, accumulated over the years for practically all elements by a host of different methods, puts this material in a class by itself. Although no certification is made, the quality of this Reference Material is certainly comparable to a Standard Reference Material [Smith 1967], and the values recommended by Bowen [1974] are in many cases not grand means. With satisfactory data available for the certification of 35 elements [Bowen 1974], Kale retains its position as the most important international, biological reference material for trace elements, IBS.

Our own results for kale are presented in Table 49 together with selected values from recent literature.

Not until 1971 did the *National Bureau of Standards* distribute a botanical Standard Reference Material similar to IBS, namely SRM 1571 Orchard Leaves, but already in 1972 this was supplemented by SRM 1577 Bovine Liver. These two materials together are certified for 19 different elements, and coupled with IBS they constitute the basis for ascertaining the accuracy of an analytical method for the determination of trace elements in biological material.

Our results for these two CRM (SRM) are presented in Table 50 together with selected values from recent literature.

Arsenic in SRM 1571

In most cases our results agree very well with the subsequent certified or recommended values. However, one conspicuous difference was observed for As in SRM 1571 Orchard Leaves, which was therefore made the subject of a special investigation [Damsgaard and Heydorn 1973].

SRM 1571 Orchard Leaves, released on January 28, 1971, was the first biological Standard Reference Material from the National Bureau of Standards. A provisional certificate dated October 1, 1971, became available some time after publication of our results [VII], and the certified value for As of 14 ± 2 mg/kg dried material was much higher than our value of 8.7 mg/kg.

Table 49

Trace element results for the International Biological Standard, Bowen's Kale, in mg/kg dry weight

Element	Recommended value::	Risø results		Selected literature values		
		Reference	Mean value	Method	Result	Reference
As	0.141	[IV]	0.118 ± 0.004	RNAA	0.118 ± 0.002	Byrne 1974
Cu	4.99	*	4.57 ± 0.06	AAS	4.8 ± 0.2	Schramel 1973
Mn	14.73	[VII]	14.6 ± 0.3	AAS	14.8 ± 0.4	Schramel 1973
Se	0.121	[VII]	0.132 ± 0.008	RNAA	0.132 ± 0.006	Hoede 1975

:: Bowen 1974, considering results up to and including 1972

* Heydorn et al. 1976

Table 50

Trace element results for Standard Reference Materials in mg/kg dry weight

SRM 1571 Orchard Leaves

Element	NBS values::	Risø results		Selected literature values			
		Reference	Mean value	Method	Result	Reference	
As	11 ± 2	*	9.86 ± 0.11	INAA	10.1 ± 0.2	Rancitelli	1976
Cu	12 ± 1	**	11.70 ± 0.11	RNAA	11.4 ± 0.2	Ravnik	1974
Mn	91 ± 4	[VII]	87.1 ± 1.6	RNAA	87.8 ± 2.4	Ravnik	1974
Se	0.08 ± 0.01	[VII]	0.088 ± 0.007	Fluori-metry	0.077 ± 0.005	Chan	1976

SRM 1577 Bovine Liver

As	(0.055)	[X]	0.0566 ± 0.0012	RNAA	0.0540±0.0011	Byrne	1974
Cu	193 ± 10	**	186 ± ?	RNAA	183 ± 8	Gladney	1977
Mn	10.3 ± 1.0	**	9.3 ± 0.2	RNAA	10.3 ± 0.8	Gaudry	1976
Se	1.1 ± 0.1	**	1.09 ± 0.03	RNAA	1.07 ± 0.01	Steinnes	1975

:: Revised by LaFleur 1974

* Damsgaard and Heydorn 1973

** Heydorn et al. 1976

This discrepancy led to an exhaustive study of all imaginable systematic errors in the analytical method, as well as in the conditioning of the sample [Damsgaard and Heydorn 1973].

Experimental investigations were made of the following possible errors at the various stages of analysis:

(i) No indication of inhomogeneity was found at the 250 mg level, nor was there any difference between bottles.

No loss of As was observed during the two years 1971 - 1973, and no As was found adhering to the inner surface of the bottle, nor was As detected in the air from an unopened bottle.

(ii) The loss of As during drying at 90°C was checked by analyzing dried as well as undried material. Taking into account a weight loss by drying of 5.86 %, the results obtained were identical; consequently there could be no significant loss of As during drying.

Even repeated drying did not result in the detection of any loss of As.

(iii) The stability of the comparator standard was checked by chemical analysis of the stock solution of 10 mg/ml (kg/m^3), and a change of less than 2 % was found during 7 years of storage.

Comparator solutions of 10 µg/ml (g/m^3), prepared at regular intervals by dilution with redistilled water, are always compared with their predecessor and usually agree within 1 %.

(iv) Loss of As by radiation decomposition or of ^{76}As by nuclear recoil during irradiation was checked by counting a sample both before the irradiation container was opened and after transfer of the sample to an identical container. No loss was detected.

(v) Losses during wet ashing were determined by decomposition of the irradiated sample in a closed system to trap the possible volatile As compounds and collect a distillate during ashing. The distillate and the trapped fumes were counted for ^{76}As, and only insignificant amounts were found.

Carrier exchange was investigated by the addition of carrier-free ^{74}As as a tracer in two steps:

1. a test for exchange between the As from irradiated orchard leaves and the ^{74}As tracer,
2. a test for exchange between irradiated As-carrier and the ^{74}As tracer.

In both cases the ratio between the ^{76}As and the ^{74}As peak was measured before decomposition and in the separated As sample, and no difference was found.

(vi) Counting errors caused by the limited resolution of a NaI(Tl) scintillation detector were eliminated by using a high-resolution Ge(Li) semiconductor detector instead. In the γ-spectrum of Orchard Leaves in Fig. 39 about 40 hours after the irradiation, all peaks were identified and none of the isotopes interfered with the As peak at 559 keV. No

assignment error from inaccurate calibration could be envisaged, because the region around the ^{76}As peak forms a very characteristic triad, as shown in Fig. 40, with the As peak surrounded by a ^{82}Br peak and a ^{122}Sb peak.

Loss of counts from the 559 keV photo-peak by pile-up or by erroneous dead-time compensation was reduced to insignificance by reducing the total count-rate to less than 500 counts/s; in addition, at this low count-rate no change in resolution is found. During the measurement of actual samples for instrumental determination of As, the samples were turned 90° every 20 minutes to compensate for a possible uneven distribution inside the counting vial.

In the radiochemically separated sample the presence of ^{74}As in the carrier permits the simultaneous measurement of indicator activity and yield; hereby the errors discussed in the preceding paragraph are eliminated and also other errors such as differences in self-absorption between samples and comparator, owing to the small difference in γ-energy between the two isotopes of As, 596 keV and 559 keV, respectively.

(vii) Losses of As during radiochemical separation are accounted for by the determination of yield, and too high yields produce the same low final results as the loss of As before carrier equilibration.

Investigations were made of the reproducibility of carrier addition, and of the possible presence of As in any of the reagents added. No significant contribution to the overall uncertainty could be found, but the re-irradiation yield determination with measurement in an ionization chamber is susceptible to interference [V] also from elements other than As when they occur in the ammonium-sulphide solution.

In order to eliminate even small positive biases in the yield correction, ^{74}As was used as a yield indicator in 4 investigations.

(viii) Calculations based on photo-peak integration [VI] are sensitive to incomplete separation of neighbouring peaks. Possible errors from the 554 keV or 564 keV photo-peaks were therefore reduced by graphical stripping using a 559 keV reference peak as a model.

This process was found to have no significant influence on the ^{76}As net photo-peak area.

The final outcome of the investigations did not point to any error or combination of errors that could explain the difference between our results for As in SRM 1571 Orchard Leaves and the result certified by the National Bureau of Standards.

The final results of the investigation, including both instrumental and radiochemical neutron activation analysis by 2 different analysts, are shown in Table 51. No significant difference was found between analysts or between techniques by the Analysis of Precision, so that no additional unknown sources of variation were discovered.

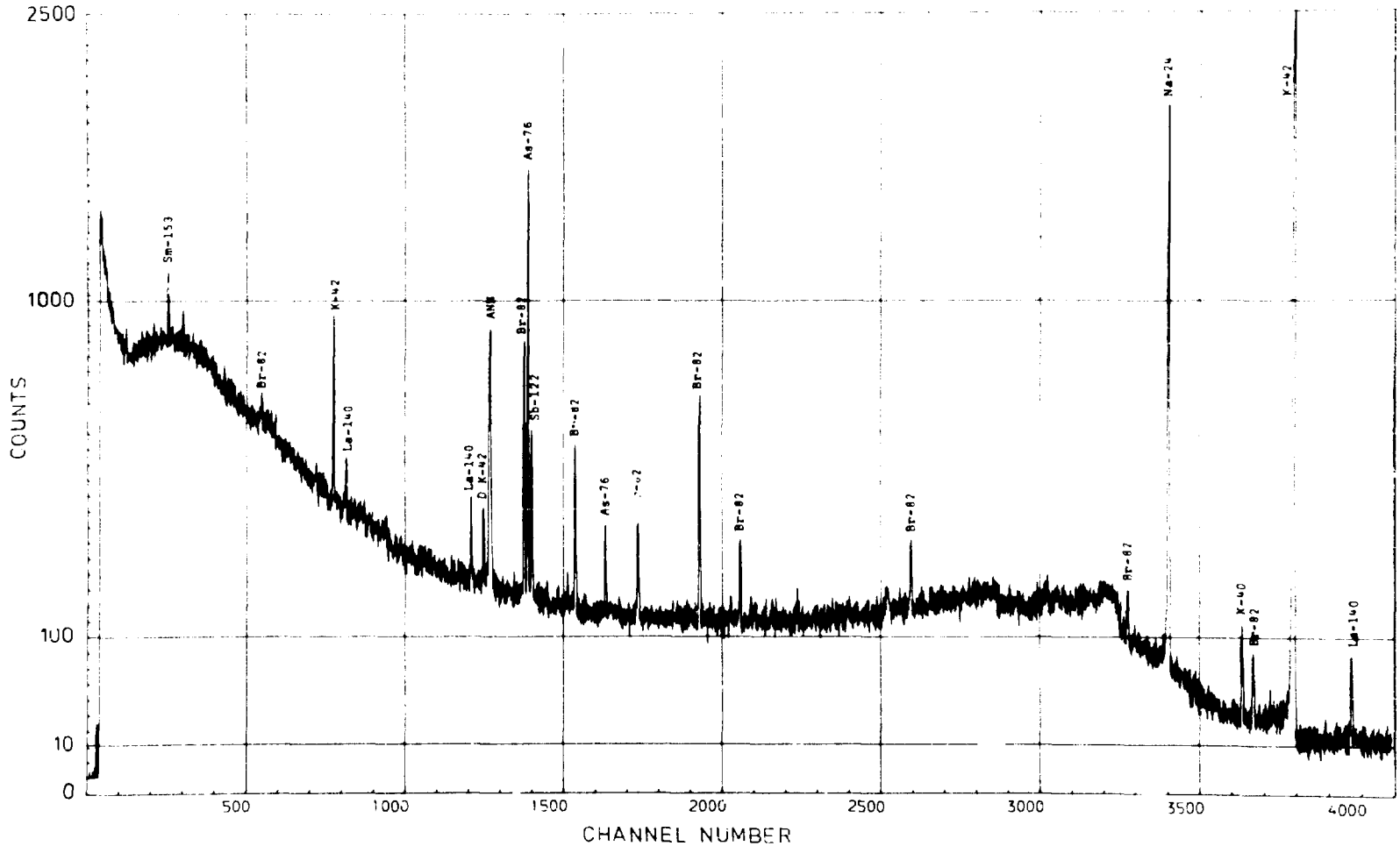

Fig. 39. γ-spectrum of SRM 1571 Orchard Leaves on square root graph paper [Verall 1969]. Counted for 80 min at approx. 40 h after irradiation at a count-rate of ~200 counts/s.

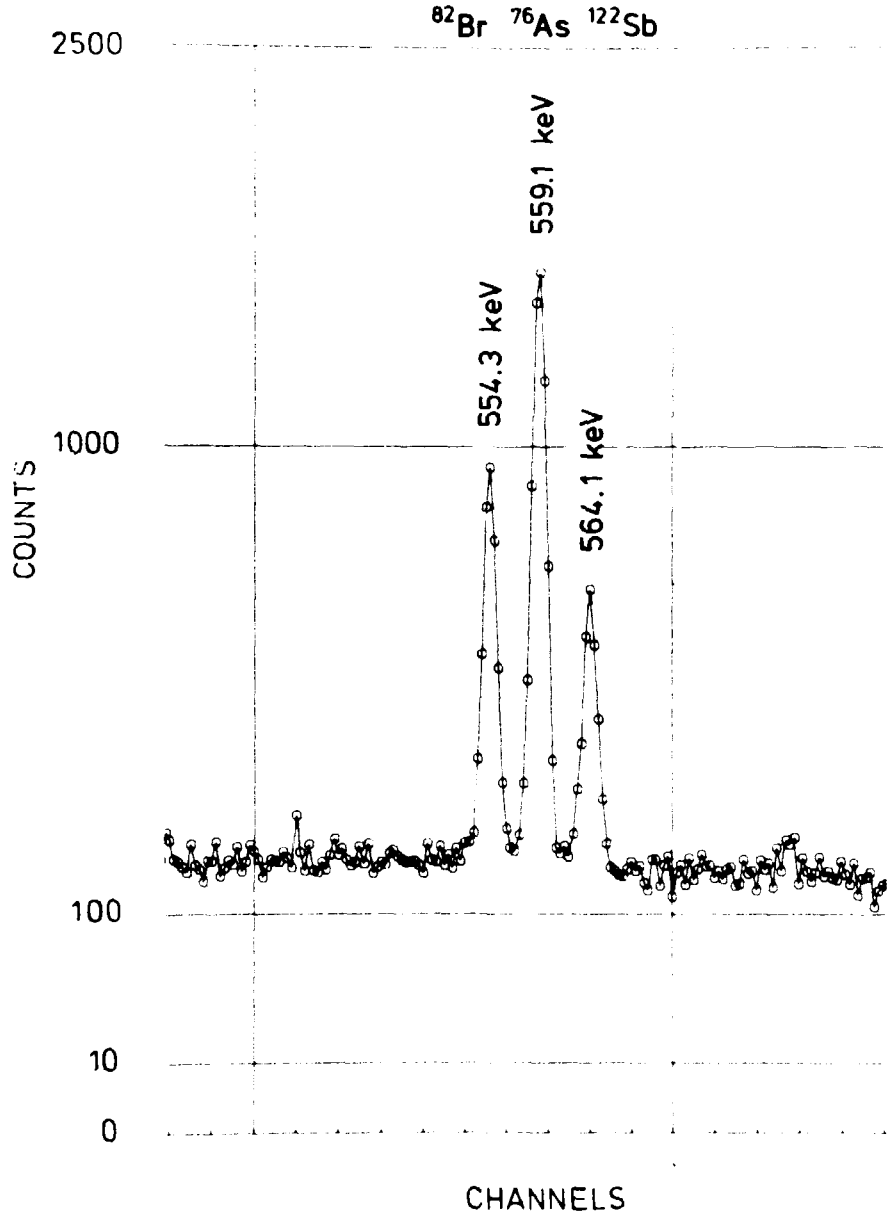

Fig. 40. Region of interest for the instrumental determination of As in SRM 1571 Orchard Leaves by activation spectrometry. [Damsgaard and Heydorn 1973].

These results are higher than those reported originally [VII], although no definitive error was detected. The presence of sodium contamination in a particular batch of ammonium-sulphide solution has since been revealed; this gives rise to too high yields, when the ammonium-sulphide is irradiated for yield determination and measured in an ionization chamber. The low results at 8.7 mg/kg may be attributed to this factor.

Table 51

Concentration of arsenic in SRM 1571 Orchard Leaves
[Damsgaard and Heydorn 1973]

NAA Method	Mean value mg/kg	Number of results	Number of analysts
Radiochemical	9.7 ± 0.2	4	2
Instrumental	9.93 ± 0.13	12	2
Weighted mean	9.86 ± 0.11	16	2

The mere agreement between instrumental and radiochemical neutron activation analysis may not be sufficient to ascertain the absence of systematic errors. This appears not only from the fact that the certification of 14 ± 2 mg/kg by the National Bureau of Standards is based on agreement between these same - allegedly independent - methods, but also from the same agreement between instrumental and radiochemical neutron activation analysis reported by Nadkarni [1969] for As in Kale - more than a factor of 10 higher than the recommended value given in Table 49.

Subsequent to brief personal contact in Octooer and December 1972 with Dr. Philip D. LaFleur of the National Bureau of Standards, who was in charge of the technical measurements leading to the certification of SRM 1571, we decided to put our case before the scientific community as a contribution to the meeting on Recent Developments in Neutron Activation Analysis in July 1973 [Damsgaard and Heydorn 1973]. Here, we recommended a value of 9.7 mg/kg instead of the certified value of 14 mg/kg

Since no representative of the National Bureau of Standards was present at the meeting in Cambridge, UK, a written version of our contribution was forwarded to Dr. LaFleur on August 15 together with some supplementary information.

On August 30 when Dr. LaFleur presented a paper at the IAEA Symposium on Nuclear Techniques in Comparative Studies of Food and Environmental Contamination [LaFleur 1974] in Otnäs, Finland, he altered the certified value for As in SRM 1571 to 11 ± 2 mg/kg - but offered no comments on this change.

Ensuing correspondance with the NBS confirmed that the originally certified value was in fact erroneous, and it was stated that a new, revised certificate would be prepared about January 1, 1974. The new value was based on the mean value for a number of samples of

10.8 ± 0.8 mg/kg, and this is in excellent agreement with the grand mean 10.8 mg/kg of the 20 mean values known to us by December 1973. Their distribution is, however, significantly skew and much better described by two different, normal distributions, one around 14 mg/kg with a standard deviation of 1 mg/kg, and the other around 9.7 mg/kg with a standard deviation of 0.6 mg/kg and a skewness of zero.

This is illustrated in Fig. 41, which was transmitted to the NBS in 1973 together with a suggestion to replace the certified mean value by 10 mg/kg instead of 11 mg/kg.

By the end of 1976 no revised certificate had been issued by the National Bureau of Standards, and the 1975-76 Catalog of NBS Standard Reference Materials continues to quote 14 mg/kg of As for SRM 1571, and no revision of the certificate is listed even in the 1977 supplement.[5] A considerable number of analysts are thus unaware of the alteration in certification, and the obsolete value of 14 mg/kg continues to be quoted in the literature during 1975, 1976, 1977 and 1978, see Table 52.

Consensus values

Certification of trace element concentrations by even the most competent analytical authority is thus no absolute guarantee of accuracy, and the analysis of Certified Reference Materials is therefore only one of several methods for the detection of systematic errors. An additional problem is the interaction between the certified value and the reported results, pointed out by Byrne [1976], who found unrealistically high accuracy and precision of results achieved for some Standard Reference Materials.

A similar effect was observed in the literature values for As in SRM 1571 Orchard Leaves during the years 1974 - 1976; all reports of mean values significantly higher than 10 mg/kg quoted the originally certified concentration of 14 mg/kg instead of the later value of 11 mg/kg.

The validity of a CRM, certified reference material, is therefore best before certification. The way to control the accuracy of analytical results is therefore analysis of all Reference Materials as soon as they become available; no special preference need be given to materials destined for certification compared with reference materials analyzed by many different laboratories and several different methods for intercomparison.

5. Only in 1978 did the NBS announce [Alvarez et al. 1978] a revised certificate for SRM 1571. The new certificate, dated August 15, 1976, gives much more detailed information on the certification process than the previous certificate of October 1, 1971; but only one certified value is changed; arsenic. The revised value is backed up by the agreement between results of 4 different analytical methods used by 9 different NBS staff members and 2 external, co-operating analysts. The final certification value of 10 ± 2 µg/g is also in full agreement with our recommendation - based on Fig. 41 - forwarded to the NBS in 1973.

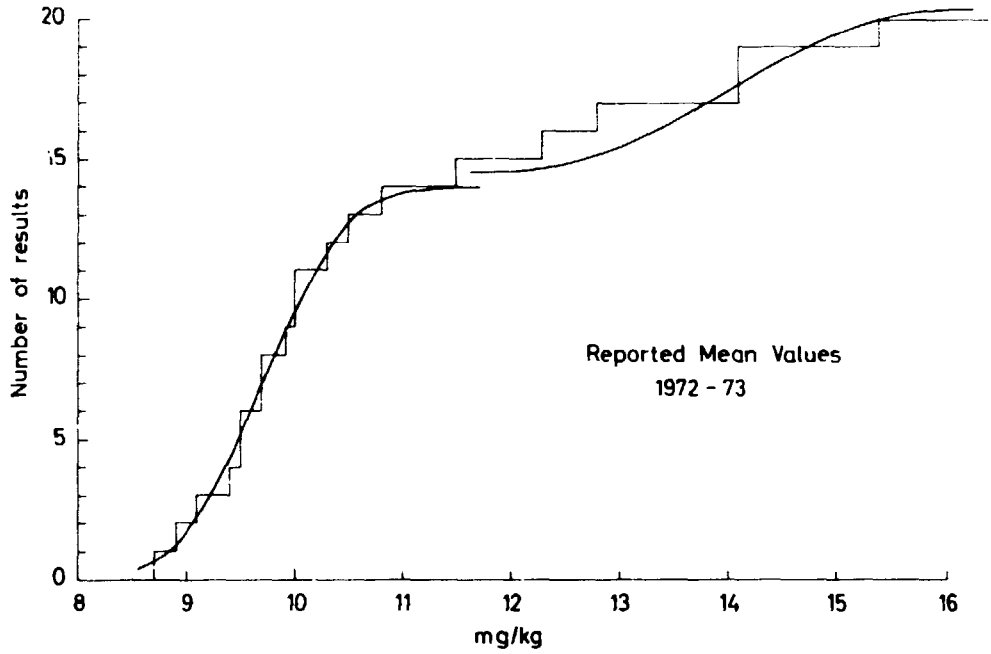

Fig. 41. Less than ogives for normal distributions of reported concentrations of As in SRM 1571 Orchard Leaves during 1972-73.

Such intercomparisons continue to be held by the International Atomic Energy Agency, and recently Fukai [1976] presented results from 60 laboratories on 14 trace elements in Mediterranean Oyster Homogenate. Here, the excellent agreement between results of AAS and NAA for elements such as Mn and Fe is based on results from about 10 laboratories or more for each method. No amount of official certification could improve on this, but at the same time significant systematic differences are found for some other elements, including Hg.

Our results for Mediterranean Oyster Homogenate were published in [Heydorn et al. 1976] and are presented in Table 53. No significant difference was found by the T-test between our results and the consensus values, i.e. the average values obtained after rejection of outlying values by Chauvenet's criterion. However, deeper insight was gained by comparison with the separate averages from neutron activation analysis and the method used most frequently for the remaining results - in most cases atomic absorption spectrometry (AAS). Very satisfactory agreement with both methods for Mn confirmed the certifiability of this element, and the excellent agreement with the more precise AAS values for Cu justifies its certifiability. For As and Se, the agreement with the consensus value is basically only an agreement with other results found by means of neutron activation analysis; but these are not substantiated by agreement with other methods and therefore an actual certification of accuracy could not be

Table 52

Arsenic in SRM 1571 Orchard Leaves 1974-1978

Reference	Found mg/kg	Assumed	Analytical method
Erdtmann 1974	11.8 , 13 3	14 ± 2	RNAA
Guzzi* 1974	12.15 ± 0.43	14 ± 2	RNAA
Bolton 1975	13.0 ± 0.1	14 ± 2	INAA
Kucera 1976	12 ± 1	14 ± 2	INAA
Roscoe 1976	14.6 ± 0.4	14 ± 2	INAA
Zeisler 1976	16 , 18	14 ± 2	HiXE
Bando 1977	15 ± 0.1	14 ± 2	RNAA
Furr 1978	14 ± 1	14 ± 2	INAA

*Also published in 1976

based on them. It should be noted, however, that the Se results from Risø are based on ^{81m}Se as indicator, whereas all other results are almost certainly based on the use of ^{75}Se; the agreement between results for Se is therefore more significant than for As.

The large number of data available for trace element concentrations in the IBS Kale were studied by Bowen [1975] in order to determine possible biases of different analytical methods. However, considerable improvements of the precision and accuracy of many analytical techniques, in particular atomic absorption, have been made over the 10 years during which the analytical data for Kale were being produced. This makes both the treatment and the interpretation of these data much more difficult than in the preceding case of an IAEA Intercomparisor, where all results are contemporary.

Such investigations have not been published for Standard Reference Materials and hence the veracity of these materials relies heavily on the reputation of the certifying institution.

Improved certifications

The satisfactory agreement of the present results with recommended values (Table 49), certified values (Table 50) or consensus values (Table 53) indicates that our systematic errors are insignificant in comparison with the uncertainty of these values, but not necessarily compared with the precision of our results.

This problem was discussed by Sutarno [1975], who defined a *certification factor* as an expression of the uncertainty of the consensus value relative to the precision of analytical results. With the

Table 53

Trace element results for IAEA Reference Material MA-M-1 in mg/kg dry weight

Element	Heydorn et al. 1976		NAA Fukai 1976		AAS	
	Number	Mean value	Number*	Mean value	Number	Mean value
As	3	12.3 ± 0.3	10	11.5 ± 0.7	2	9.7 ± 0.1
Cu	4	331 ± 6	9	290 ± 20	23	334 ± 5
Mn	3	68.2 ± 1.5	8	71 ± 3	15	70 ± 2
Se	4	2.26 ± 0.08	12	2.36 ± 0.09	1	1.90*

* not including Risø results

* by fluorimetry

recommended certification factor less than 4, the standard error of the consensus value is smaller than the standard deviation of a single analytical result.

With the 3.5 % relative standard deviation characteristic of our single determinations only Mn in SRM 1571 and Cu in SRM 1577 yield acceptable certification factors; in the remaining cases a reduction of the uncertainty is required.

Such improvement of the accuracy of the certification may be achieved on the basis of literature values appearing from time to time. This has been done for As in SRM 1571, and in Table 54 are listed values reported in the literature after the original certification was altered.

The most precise value is reported by Rancitelli [1976], who used INAA with counting on an anticoincidence shielded Ge(Li) spectrometer. All other values in Table 54 are in agreement with this result within their quoted standard deviations, as shown by the T-test, $\chi^2_{(13)} = 14.08$. It may therefore be assumed that the value of 10.1 ± 0.2 mg/kg is an unbiased estimate of the true concentration of As in SRM 1571, and it represents a significant improvement on the revised, certified value. It is also in excellent agreement with our results presented in [Damsgaard and Heydorn 1973].

All known published values from the same period not consistent with this value rely on a certified value of 14 ± 2 mg/kg and are included in Table 52.

Similar considerations have led to the selection of more recent, more precise values than the certificate values for the other elements of interest in the two Standard Reference Materials 1571 and 1577. These results are included in Table 50, and it can be seen that, with a single exception, the most precise values are based on neutron activation analysis with radiochemical separation.

Improved values for Bowen's Kale were selected from the recent literature as the most precise results obtained by a method of established accuracy from a laboratory with great experience.

For the following elements, the methods of choice were:

Arsenic: no real alternative to neutron activation analysis with radiochemical separation was found.

Copper: results of the intercomparison presented in Table 53
Manganese: for both these elements establish the accuracy of (flameless) atomic absorption spectrometry.

Selenium: the only alternative to neutron activation analysis with radiochemical separation is fluorimetry, the accuracy of which may be strongly dependent on the experience of the laboratory.

Table 54

Arsenic in SRM 1571 Orchard Leaves 1974-1976 in mg/kg

References		Value	Analytical method
Becker	1974	10.7 ± 1.0	INAA
Byrne	1974	9.85 ± 0.54	RNAA
Chattopadhyay	1974	10.2 ± 1.0	IPAA
Guinn	1974	11.4 ± 0.7	RNAA
Hislop	1974	10.5 ± 1.0	IPAA
Nadkarni	1974	11.6 ± 1.3	INAA
Orvini	1974	10.6 ± 0.8	RNAA
Reuter	1975	12.0 ± 1.3	XRF
Ricci	1975	9.7 ± 0.4	INAA
Fiorino	1976	11 ± 1	AAS
Kato	1976	10.5 ± 0.5	IPAA
Meloni	1976	10.8 ± 0.9	RNAA
Rancitelli	1976	10.1 ± 0.2	INAA
Vijan	1976	9.4 ± 0.4	AAS

The selected results are included in Table 49 and are generally in better agreement with our results than with the recommended values, based on the literature up to 1972.

Inspection of Tables 49, 50 and 53 confirms that even with the greatly improved values for concentrations in the 4 Reference Materials, no significant bias was found for any of the 4 elements.

Of course, strictly speaking this observation only applies to the matrices represented and to the concentration ranges covered. The composition of these Reference Materials is probably fairly representative of dry tissue, but the concentrations of trace elements are much higher than in the wet tissue which mainly concerns us here. Additional biological reference materials are being prepared by the IAEA, and additional botanical materials by the NBS, some of them with lower trace element concentrations than those presently available.

If we include the results from IAEA Animal Blood for Mn and Cu, the accuracy of the methods and the procedures employed is ascertained over a concentration range covering more than 2 decades for As, Cu, and Mn, and slightly more than 1 decade for Se.

This means that the absence of significant proportional, systematic errors has been verified for matrices with a natural distribution of trace elements.

5. Practical Applications

The ultimate purpose of activation analysis - as well as of any other type of chemical analysis - is to provide meaningful analytical results for real, practical samples with unknown content of determinand. These results should be highly accurate and have an adequate precision to permit conclusions to be drawn with a prescribed level of confidence.

This object was achieved already long ago with respect to the determination of major elements with concentrations exceeding 0.01 %, and for many elements and matrices reliable results are also available at trace levels.

The true challenge, as expressed by Meinke [1973], lies in the achievement of accurate analytical results at the ppb levels of concentration. Here the use of neutron activation analysis appears particularly appropriate for trace elements in biological materials [Tobias 1949], and the present studies would seem to be of fundamental as well as of practical importance.

Activation spectrometry, or INAA, is useful for many applications at the trace levels, but has a credibility limit when 10 ppb (10 µg/kg) is approached as pointed out by Meinke [1973]. This coincides with the present definition of the ultratrace level of concentration, although other definitions have been proposed [Zief 1976].

Thus at ultratrace levels only RNAA is expected to be capable of yielding accurate results, and other methods will have to be judged by comparison with this method.

Types of problems

The importance of accuracy is perhaps most readily appreciated in the analysis of *medical samples*, because it is often necessary to make comparisons between samples analyzed by different laboratories, or at different times or by different methods. Only when accuracy can be trusted is it possible to base conclusions on reference values from the literature. Unfortunately there is a scarcity of accurate values for trace element concentrations in the human body, and it is one of the purposes of the present study to contribute to filling this need.

While no effort is spared to ascertain the accuracy of these results, there is no need to improve precision beyond a certain point where analytical variability becomes insignificant compared to other sources of variation.

With the analytical method in statistical control, other sources of variation are readily identified. Lack of homogeneity can be unmistakably proven and may be a limiting factor in the analysis of some biological materials. Individual biological variations may be quite large without necessarily indicating disease, but it is important to accurately determine the distribution of normal values in order to establish significant deviations. The type of distribution also has a bearing on the essentiality of the particular element [Liebscher 1968].

More special features of neutron activation analysis may be used to solve *other problems*, where other methods fail because of chemical problems.This applies particularly when the *total* quantity of an element is sought, regardless of its chemical - or even isotopic - composition. When multi- or oligo-phase systems are analyzed, the result of chemical or radiochemical analysis depends on the degree of mineralisation achieved for the different phases by the chosen method of decomposition. For INAA, however, this micro-heterogeneity has no influence on the results for the total element present. Some standard reference materials belong to this category - just like the real materials they simulate. These problems are therefore of fundamental importance in the comparison of analytical data from different laboratories.

The challenge in *Ultratrace Analysis* by RNAA is characterized by the difficulty of obtaining statistical control of the analysis. When sample heterogeneity can be disregarded, contamination problems are undoubtedly the main offenders [Speecke 1976].

Without statistical control, single results are not useful because their precision is unknown. Every determination has therefore to be made in duplicate or triplicate, and more samples are then needed to obtain the same level of confidence.

With some modification the Analysis of Precision is still helpful in detecting and eliminating systematic errors, and it is clearly found that the use of RNAA is not per se a guarantee of accuracy. Many conclusions in the literature are based on totally erroneous results, and the efforts involved in verifying and confirming results at the ultratrace level are not usually appreciated.

In addition to the well-known absence of reagent blanks, the most significant contribution of RNAA to ultratrace analysis is perhaps that the a priori precision is independent of concentration level, and this permits the accurate estimation of the precision of a single result. The stepwise identification of all observed sources of variation contributes to the reliability of the final results, even when they disagree with those of established authorities.

5.1. Medical Samples

The analysis of biological material was the most important goal of the present studies, and particularly the determination of elements such as As in human tissue, for which no other satisfactory method is available.

Such samples must be very well characterized from a medical point of view to be of any value; they must be taken by a physician and only in full accordance with the ethics of his profession. Their availability is therefore quite limited, particularly with respect to material from normal, healthy persons.

It was therefore found appropriate to better utilize the samples by determining several trace elements in the same sample, and methods were therefore developed for simultaneous determination of Se, Mn, and Cu as well. The determination in a small sample of elements ranging from sub-nanogram to milligram quantities by the same method and with known precision is characteristic of neutron activation analysis.

This made possible the utilization of trace element ratios, which are sensitive to contamination or losses, but not to moisture content, cf. Table 6.

Biological variation

With the exception of reference materials, all biological samples reflect a biological variation that is the overall result of many sources of variability, most of which cannot be controlled experimentally. It is therefore not expedient to push the precision of the analytical method beyond a certain point, or vice versa: it is not worth making biologically insignificant effects statistically significant.

This may be expressed as an *Index of Determination*, calculated from the average precision of a single analytical result $\bar{\sigma}_y^2$, and the assumed population variance σ_x^2

$$ID = 1 - \frac{\bar{\sigma}^2_{results}}{\sigma^2_{samples}} = \frac{\sigma_x^2}{\sigma_x^2 + \bar{\sigma}_y^2} \qquad (93)$$

This index approaches zero when the analytical precision is not sufficiently good to detect any additional variability in the sample material, and approximates 1 or 100 % when the analytical variation becomes completely negligible compared with other sources of variability. It is an expression of the sufficiency of the analytical method for the detection of variations of an anticipated magnitude.

The detectability of σ_x^2 is expressed by

$$F_U \times \bar{\sigma}^2 = s^2_{samples} = F_L \times (\sigma_x^2 + \bar{\sigma}_y^2) \qquad (94)$$

where F_U and F_L are upper and lower limits of the χ^2/ν - distribution cf. Currie [1972]. For any given ID, this equation determines the degrees of freedom required for the appropriate values of errors of the first (α) and second (β) kinds.

If the analytical method is in statistical control, and we wish to detect a population standard deviation with statistical significance ($P_\alpha < 0.01$), more often than not ($P_\beta < 0.5$), Table 55 gives the number of results required for varying values of the Index of Determination.

It can be seen that there is no need to increase the Index of Determination beyond 0.8 - 0.9, and with a population standard deviation of 10 % this is equivalent to average precisions of 3.5 - 5 %.

This again means that the methods developed in the present study have a very satisfactory *a priori* precision for application to biological samples; the only requirements are that the levels are well above the detection limits and that the analytical method is in satisfactory statistical control.

For biological samples, it is particularly important to ascertain that all sources of variation are in statistical control including the process of sampling. For selenium and copper, this does not cause any problems, but for the lowest levels of As very considerable effort is required to reduce contamination to insignificance. For Mn in human serum, satisfactory statistical control could not be achieved [IX].

The analysis of precision of duplicate results of arsenic determinations in human serum revealed the presence of two sources of variability: sampling conditions and bromine interference [X]. Over the years 1970 - 1972 these factors were gradually brought under control, and the resulting improvements are itemized in Table 56. Final verification of the precision of the complete analytical method for As including sampling was demonstrated in 1973 by the Analysis of Precision of the results of a set of serum samples taken in duplicate under almost perfect conditions. The 14 results, covering the range of a decade, are presented in Table 59 and confirm that all significant sources of variation are in statistical control.

A summary of the results for As, Mn, and Se in normal human serum is presented in Table 57 together with the estimated Index of Determination for these elements. In spite of sampling problems, the ID is very satisfactory not only for As, but also for Mn, whereas the levels of Se at less than 10× the detection limit yield a less satisfactory value.

It must be kept in mind that sampling conditions are very difficult to control, particularly for biological materials. The analysis of duplicate

Table 55

Number of samples required to detect a non-zero population variance with 99 % significance in the majority of cases as a function of the Index of Determination

Index of Determination	Number of samples
0.95	2
0.90	3
0.85	3
0.80	4
0.75	5
0.70	7
0.65	9
0.60	11
0.55	15
0.50	20

samples with subsequent Analysis of Precision of a reasonable number of results constitutes therefore a mandatory part of the analytical quality control. This has been discussed in some detail in Section 3.3., and in the following discussion of the analytical results we assume that the precision of these results is known.

Equipped with this important information, we may set out to apprehend and identify *real sources of variability*. This expression refers to effects that actually affect the true concentration of the determinand in the original location and condition of the material to be analyzed. As an example we may take the influence of storage; variability of results caused by the influence of the duration of storage between sampling and analysis must be included in the precision of the analytical method. However, variability of results caused by the period of time between the time of death and the time of autopsy is a real source of variability, because storage takes place before sampling.

Factors affecting the concentration of microconstituents in biological tissue may be divided into 2 categories, as shown in Table 58. Fixed factors are chosen according to the purpose of the investigation, and they usually assume discrete values only. Variable factors in a way represent the environment in which the investigation is carried out; in some investigations the environment should be kept constant throughout, but other studies aim at determining the influence of the environment, particularly on normal samples. The genetic effect as a

Table 56

Arsenic in serum from normal Danish adults [X]

Sampling conditions	Correction for bromine	Number of cases	a posteriori error $\bar{s}$ in mg/m³	Mean value mg/m³	Population $\hat{\sigma}_x$ in mg/m³
1970	none	16	2.4	2.4 ± 0.6	1.9
1971	none	11	0.85	2.1 ± 0.3	0.85
	usual	12	0.32	1.4 ± 0.3	0.55
1972	usual	10	0.12	0.94 ± 0.14	0.44
	alternative	11	0.11	1.07 ± 0.14	0.44

Table 57

Summary of determinations of arsenic, manganese and selenium in normal, human serum [IX]

Element	Distribution of mg/m^3		Index of Determination
	Mean value	Standard deviation	
As	1.07 ± 0.14	41 %	0.94
Mn	0.54 ± 0.05	30 %	0.90
Se	89 ± 4	12 %	0.67

variable factor is related to the presence - or absence - of the menstrual cycle.

The influence of many variable factors on the results of routine clinical analysis is well established [Young 1976], but for most trace elements very little is known about the significance of these sources of variation.

In the present study of As, Se, Mn, and Cu, several different types of tissue have been analyzed in order to obtain information both on the average level in the body and on the distribution. We compared results for normal patients with those for patients suffering from specific diseases, and work was done on autopsy samples, as well as biopsies and blood.

Arsenic was determined, usually together with one or more of the essential elements, in all samples. In the following the influence of a number of variable factors on the concentration of As is discussed in relation to its probable lack of essentiality.

Short-term effects

Short-term factors include the intake of food or medication and their effect as a function of time after ingestion, time after sleep, or time of the day.

Obviously, the study of these factors requires frequent sampling of normal persons, and blood is therefore the only type of tissue that has been investigated. In one set of experiments we studied the stability of arsenic and selenium concentrations in human serum under the influence of normal, human activity.

Samples were taken in 1973 from 2 normal persons every 4 hours during a 24-hour period, using the sampling technique reported in [IX]. Their intake of food and drink was carefully recorded, but no restrictions were imposed on their activities.

Table 58

Factors influencing the concentration of trace elements in tissue

Fixed factors	Variable factors
Elements	Genetic
Tissue	Long-term physiological
Disease	Short-term physiological

Duplicate samples were taken in all cases but analyzed only for one person. Results for As and Se are presented in Table 59 for the period from December 19 at 8 am to December 20 at about the same time. Results are given with a standard deviation representing the overall effect of an a priori error of 5 % and counting statistics.

An analysis of precision based on 28 duplicate results for As and Se for KMT indicates no significant, unexpected sources of variability with T=21.3 at 14 degrees of freedom. Weighted means and their reduced standard deviations may therefore be used instead of duplicates without loss of information.

In spite of the increased precision of selenium concentrations in KMT, no significant difference between results from different times of the day could be detected by the chi-squared test [VIII] with T=12.5 at 12 degrees of freedom.

All selenium results for each person may therefore be pooled, and a highly significant difference is found between the two persons with respective weighted mean values of 83 ± 2 mg/m^3 and 99 ± 3 mg/m^3. Both agree well, however, with the population distribution reported in [IX] with a mean value 89 mg/m^3 and a 12 % relative standard deviation.

The results for As are very different, as can be seen from the data plotted in Fig. 42 for the two subjects. In both cases the first sample after supper is higher than any other. Careful study of the records showed that the only item consumed by both subjects at about supper time - and not by either of them at any other time - was an open sandwich with fillet of plaice.

If we assume that the sudden increase in arsenic concentration coincides with their intake of supper, a common half-life for the disappearance of arsenic from the blood-stream is estimated at about 5 hours. The corresponding decay curves are shown in the figure to illustrate the high degree of similarity between the two sets of observations.

Table 59

Concentrations of arsenic and selenium in normal, human serum of the 1973 series [Heydorn et al. 1979]

Identification		KMT		HL	KMT		HL
Time Date and hour		Arsenic mg/m³	Weighted mean	Arsenic mg/m³	Selenium mg/m³	Weighted mean	Selenium mg/m³
December 19		1.36 ± 0.23 1.53 ± 0.21	1.45	1.85 ± 0.17	91 ± 12 77 ± 6	80 ± 5	108 ± 7
	noon	0.72 ± 0.18 1.04 ± 0.20	0.86	1.66 ± 0.22	97 ± 14 72 ± 5	75 ± 5	106 ± 7
		0.91 ± 0.23 1.06 ± 0.18	1.00	1.35 ± 0.18	107 ± 9 77 ± 6	86 ± 5	103 ± 8
	8 p.m.	1.78 ± 0.24 1.16 ± 0.26	1.49	1.68 ± 0.21	81 ± 8 81 ± 7	81 ± 5	97 ± 8
December 20		6.74 ± 0.49 6.91 ± 0.39	6.84	7.71 ± 0.44	74 ± 14 84 ± 7	82 ± 6	92 ± 7
		4.45 ± 0.27 4.32 ± 0.25	4.38	6.01 ± 0.37	90 ± 7 79 ± 7	84 ± 5	100 ± 8
	8 a.m.	2.63 ± 0.17 3.11 ± 0.25	2.78	3.84 ± 0.23	88 ± 7 94 ± 8	91 ± 5	88 ± 7
Degrees of freedom		7			7	6	6
Test of precision		T = 7.8			T = 13.6	T = 6.1	T = 6.5

Table 60

Results for arsenic in normal, human serum of the 1975 series [Heydorn et al. 1979]

Identification		JO ♂		GK ♀
Time references Date, hour, activity		Arsenic mg/m³	Weighted mean	Arsenic mg/m³
31		5.04 ± 0.30 5.32 ± 0.32	5.17	0.51 ± 0.10
				0.43 ± 0.10
November 1	Sleep	4.84 ± 0.28 5.37 ± 0.31	5.08	0.22 ± 0.11
				0.16 ± 0.11
		4.33 ± 0.24 4.59 ± 0.27	4.45	0.27 ± 0.09
				0.37 ± 0.11
	Supper GK Supper JO	3.77 ± 0.21 4.06 ± 0.23	3.90	7.67 ± 0.43
				18.38 ± 0.97
November 2	Sleep	13.63 ± 0.71 12.34 ± 0.64	12.92	13.79 ± 0.72
	Breakfast			10.18 ± 0.55
		8.48 ± 0.45 7.44 ± 0.40	7.90	7.02 ± 0.37
Degrees of freedom		6		
Test of precision		T = 8.2		

Association is no proof, and the increase in arsenic concentration could conceivably be attributed to other factors varying with the time of the day, similar to the diurnal variation of serum iron confirmed by Wiltink [1973]. The experiment was therefore repeated in 1975 under much more rigid conditions.

The duration of the investigation was extended to 42 hours, again with simultaneous sampling of a male and a female subject every 4 hours on the hour. The 24-hour period preceding the test period was a normal working day with the sole restriction that no fish be eaten.

In the first 24 hours of the test period no food was consumed, and only tea was given as a beverage. Supper was then served for GK, and 2 hours later for JO; breakfast was eaten the next morning and the last samples were taken before lunch. In both cases the supper included sandwiches with fillet of plaice.

All samples were taken in duplicate, and 6 were analyzed in duplicate in order to ascertain that the analytical method was in statistical control. Results with estimated standard deviations based on a 5 % a priori precision are presented in Table 60 for arsenic and in Table 61 for selenium.

The analysis of precision showed excellent agreement between duplicate results for As and Se in JO with T=11.58 at 11 degrees of freedom, and weighted means and standard deviations were calculated for the 6 sampling times with 8 hours interval. For GK, the same number of samples were analyzed as single determinations every 4 hours.

During the 24-hour fast period no significant difference between the selenium results was found by the T-test, whereas the arsenic levels in JO displayed a slow, but significant drop, as shown in Fig. 43.

The first samples after supper show a steep increase in the concentration of As for both persons, but no corresponding change for selenium, except perhaps for the last measurement before sleep.

After a maximum at 5-6 hours after supper, the As concentration drops exponentially with a half-life of approximately 500 minutes for both persons, as shown in Fig. 44.

This investigation indicates the absence of significant short-term sources of variation for selenium in normal, human serum; this is entirely consistent with its status as an essential trace element. At the same time, highly significant differences between individuals were observed, but they were in good agreement with the rather narrow distribution of values previously reported [IX].

The arsenic concentrations absolutely lack the stability of the selenium levels; not only do the starting levels of As differ by an order of magnitude between the two persons in the 1975 series, but even during the rather short period of fasting no stable concentration was

Table 61

Results for selenium in normal, human serum of the 1975 series [Heydorn et al. 1979]

Identification		JO ♂		GK ♀
Time references Date, hour, activity		Selenium mg/m³	Weighted mean	Selenium mg/m³
31		96 ± 8 100 ± 12	97 ± 7	65 ± 5
November 1				79 ± 6
	Sleep	91 ± 8	91 ± 8	67 ± 6
				67 ± 5
		94 ± 6 93 ± 6	94 ± 4	80 ± 6
				76 ± 5
	Supper GK Supper JO	85 ± 6 88 ± 7	86 ± 4	66 ± 4
November 2				90 ± 6
	Sleep	89 ± 7 79 ± 6	83 ± 5	72 ± 7
	Breakfast			64 ± 5
		90 ± 6 78 ± 6	84 ± 4	75 ± 6
Degrees of freedom		5	5	
Test of precision		T = 3.4	T = 5.9	

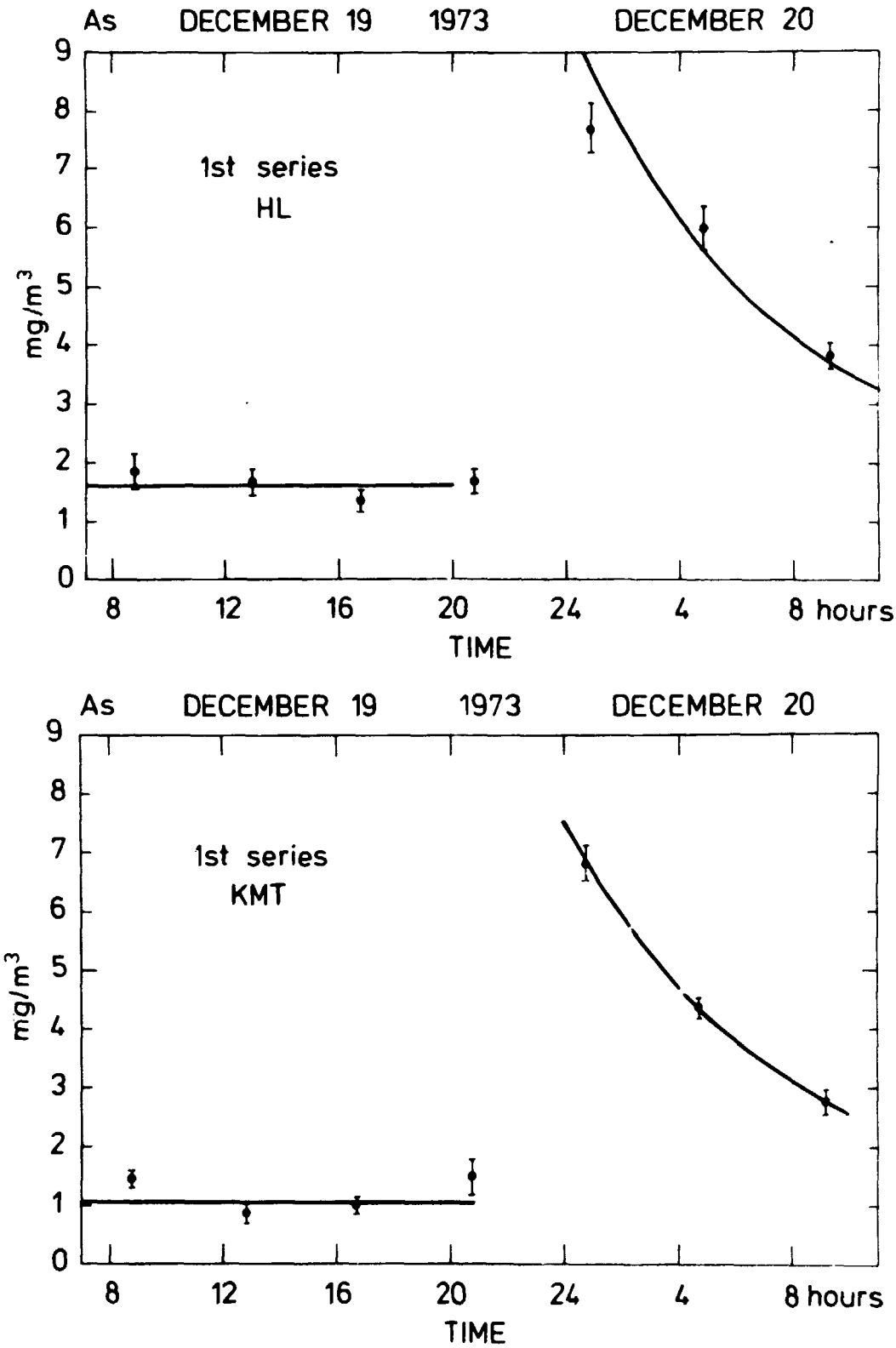

Fig. 42. Concentrations of arsenic in human serum from two normal persons during a 24-hour period of normal activity. [Heydorn et al. 1979].

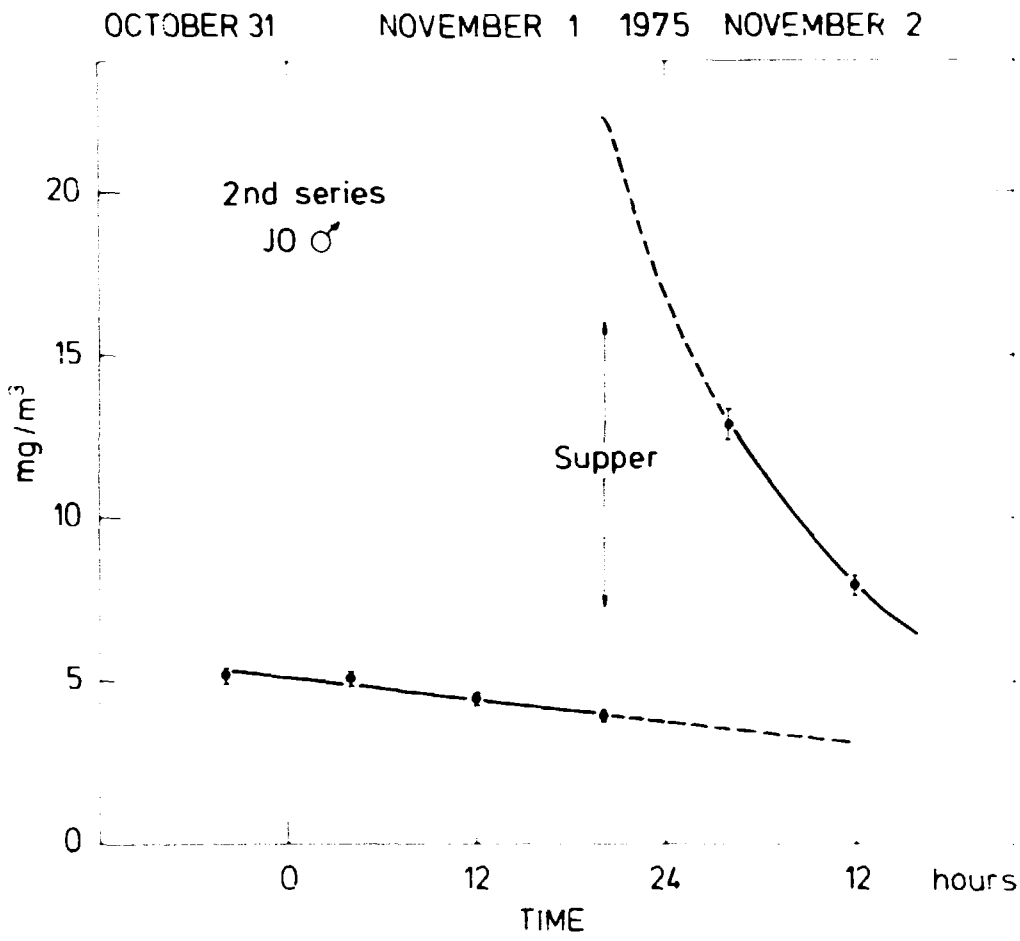

Fig. 43. Concentration of arsenic in serum from a male student during 24 hours of fasting followed by supper at 8 pm. [Heydorn et al. 1979].

maintained. This behaviour supports the assumption that As is not an essential element. At the same time no circadian variation is indicated, and the concentration of As in human serum is therefore a function of the intake of As-containing food. The only type of food common to all 4 cases of a sudden increase in the concentration of As in serum was the fillet of plaice. This was analyzed, and an As content of 2.4 ± 0.1 mg/kg was found, in excellent agreement with Hamilton [1973] - but in violation of the maximum permissible concentration of 1 ppm As in food introduced into the UK, in 1903, and reiterated in the Arsenic in Food Regulations, 1959 [amended 1973]; cf. Hinton [1960].

With a fish fillet weight of 30-40 g, the amount of As ingested is sufficient to account for the observed As-concentration in an average, total serum volume of about 3 liters. Such intake of food is a typical short-term factor, the effect of which is usually eliminated by sampling pre-prandially instead of post-prandially.

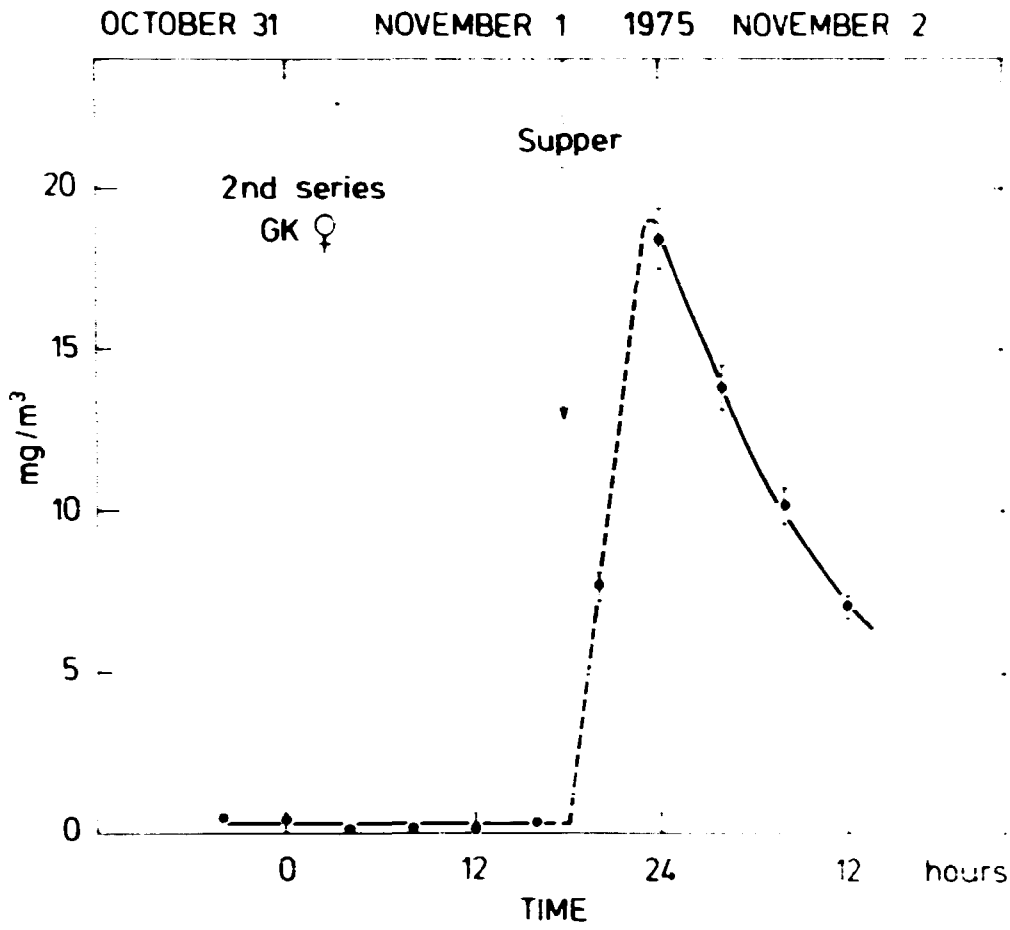

Fig. 44. Concentration of arsenic in serum from a female student during 24 hours fasting followed by supper at 6 pm. [Heydorn et al. 1979].

However, the time constant for elimination of increased arsenic levels in serum is not short compared to the intervals between eating; the average level of As in the diet therefore becomes an important long-term physiological factor.

Long-term arsenic

The composition of the diet in Denmark is not very different from that of most other European countries, whereas the diet in the developing countries and in countries with entirely differing cultural backgrounds may be much more dependent on the local environment. The combined effect of these many complex factors is often collectively termed the geographical variation.

These factors were studied in an investigation of arsenic concentrations in human plasma and cells from normal Taiwanese subjects [IV].

The levels observed were much higher than in Denmark, so the intake of As must be higher.

The composition of the *diet* in Taiwan was studied by Yang and Blackwell [1961], who found strong reliance on locally produced food, particularly sweet potatoes, which together with rice and beans made up 90 % of the calorie intake. In spite of this remarkable difference from the Danish diet, where 50 % of the calorie intake comes from animal products, the average level of As is only expected to double when the contribution from the various components is calculated from the values reported by Schroeder [1966], cf. also Schrauzer [1977].

A factor of two is not sufficient to account for the observed difference between Denmark and Taiwan, and it was therefore concluded that the general level of As in the *environment*, including the local food, is significantly higher in Taiwan. This is not inconsistent with geochemical considerations [Vinogradov 1959].

The distribution of results was assumed to be either Gaussian or logarithmic normal [Flynn 1974], the choice being made by means of a chi-squared test.

The sampling was carried out without regard to the time of the latest meal, so that the observed levels of As in plasma or serum are random samples of the exponentially decreasing concentration of the ingested As, shown in Figs. 42 to 44.

Under the circumstances it is quite reasonable to assume that there is very little day-to-day variation in the menu, so that the daily intake of As is relatively constant. The distribution of results is therefore approximately a logarithmic normal distribution with a standard deviation depending on the average time from eating to sampling.

The observed distribution of results for As in normal Taiwanese blood plasma is shown in Fig. 45, and the results for 2 different locations are given in Table 62. No significant difference is found between samples from Taipei and those from the area of endemic Blackfoot disease ($P >> 0.05$), and the results can be pooled to represent normal Taiwanese subjects. The contribution from precision to sample variance is comparatively small, and the pooled results are well approximated by a log-normal distribution, as illustrated in Fig. 45.

The adequacy of the log-normal distribution for the results from Taiwan is supported by the close agreement between the whole-blood mean values, calculated from corresponding red cells and plasma determinations, and those calculated from the means of the total number of determinations in the two groups. When both the shape of the distribution and the level of arsenic in normal plasma is known, the influence of *chronic disease* may be investigated.

Blackfoot disease is endemic to the area under investigation in Taiwan and has been associated with the presence of As in drinking

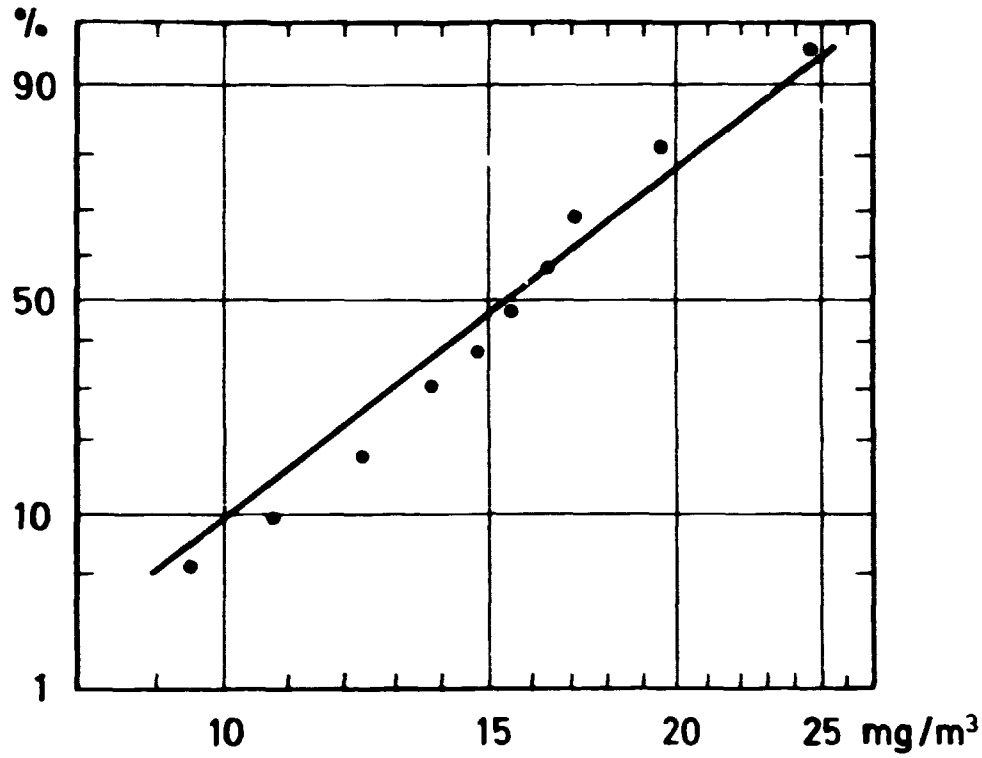

Fig. 45. Cumulative distribution of arsenic determinations in normal Taiwanese blood plasma.

water from artesian wells [How 1963]. Concentrations of As in plasma from Blackfoot patients were significantly higher than normal, and results are presented in Table 63.

However, concentrations of As in plasma from family members without Blackfoot disease were indistinguishable from the patient group. The results were therefore pooled to represent Blackfoot families, and their distribution is in very good agreement with the logarithmic normal distribution, as shown in Fig. 46.

The disease is consequently not responsible for the increased levels of As in serum. A particularly high level of As in food or in drinking water, consumed by the whole family, is undoubtedly the culprit. Among the Blackfoot families a higher than average fraction consumed *drinking water* from artesian wells with arsenic levels up to about 1 ppm, which is more than sufficient to explain the increased levels in plasma.

Comparable levels of As in drinking water are found in other localities, including California and Chile, but Goldsmith [1972] found no evidence of association with Blackfoot disease or any other specific illness.

Table 62

Arsenic in plasma from normal Taiwanese individuals [IV]

Habitat	Mean value mg/m³	Number of samples	Standard deviation s_{log}
Taipei	15.0	6	1.37
Blackfoot area	15.7	11	1.42
Taiwan	15.4	17	1.39

Chronic exposure to arsenic and other trace elements, partly or entirely excreted via the kidney, may also be a result of renal malfunction. Elevated concentrations of arsenic in blood from uraemic patients were reported by Brune et al. [1966] and were suggested by Christoffersen et al. [1969] as a factor in the evolution of uraemic polyneuropathy.

Investigations of the long-term physiological effects of *uraemia* on the concentrations of arsenic, manganese, and selenium in organs were carried out in co-operation with Larsen et al. [1972]. Autopsy samples of peripheral nerves, organs, and muscle tissue from normal and uraemic patients were analyzed [VII]. Only As was found to be significantly higher than normal in the uraemic patients, and highly significant differences between the patients were found. Neither in normal nor in uraemic patients was a preference for any particular organ apparent for As; the organ-to-organ variation was much smaller than the variation between patients. Results for normal persons are presented in Table 66 together with results for foetuses, which are discussed later.

One final possibility of chronic exposure to arsenic is the intake of arsenic-containing tonics or drugs, such as Fowler's solution[1] with 1 % arsenic trioxide, which had a restricted use in the treatment of *psoriasis* until very recently.

In spite of the fact that arsenic is not a cumulative poison [Peoples 1969], it is generally accepted [IARC 1973] that it may cause cancer of the skin in humans with a latency of from 18 to 20 years.

Ingestion of radioactive arsenicals has shown that, although most of the dissolved arsenic is rapidly excreted via the urine, some arsenic is deposited in the hair and nails, and also to some extent in the skin, in the form of tricysteinylarsine [Challenger 1976].

Concentrations of arsenic in skin biopsies from patients known to have been treated with arsenical drugs were therefore determined by

1. Pharmacopoeia Danica 1948

Table 63

Arsenic in plasma from Blackfoot families [IV]

Population group	Mean value mg/m³	Number of samples	Standard deviation σ_{log}
Blackfoot patients	32.3	33	1.75
Family members	45.2	14	2.01
Blackfoot families	38.1	47	1.90

neutron activation analysis and compared with concentrations in normal individuals not exposed to arsenicals [Heydorn and Wanscher 1978]. Sample weights averaged 20 mg with a thickness of 1-3 mm skin depth.

Results are presented in Table 64 for 16 patients with known intakes of arsenic, as well as for 16 controls. The estimated standard deviations do not include any *a priori* precision, which is insignificant in all cases.

Duplicates from psoriatic patients represent the same skin areas left and right, so that one is clearly psoriatic skin, the other apparently normal. As shown in the lowest part of Table 64, the Analysis of Precision indicates complete agreement between duplicates, and consequently no difference in arsenic content of psoriatic and normal skin samples.

The weighted means of the duplicates were subjected to the same test, which shows that the difference between individual psoriasis patients is not significant. These results are now pooled with the 8 single results from other patients treated with arsenicals, but still the difference between patients is not significant ($P > 0.1$).

Individuality of the control samples has already been suppressed by pooling before analysis, and the 5 groups do not exhibit significantly different average values. However, when combined with the results of the above 16 patients, the Analysis of Precision reveals a highly significant difference between the samples and controls, as shown in Table 65.

The significance of this finding does not lie in its novelty, but rather in the fact that the confirmation of an *a priori* assumption that arsenic ingestion leads to increased arsenic concentration in the skin, lends support to the accuracy of the analytical data in spite of their poor precision.

This conclusion could not have been made on the basis of conventional statistical treatment, as described, e.g., by Gottschalk [1976], of the data without regard to their estimated precision. The

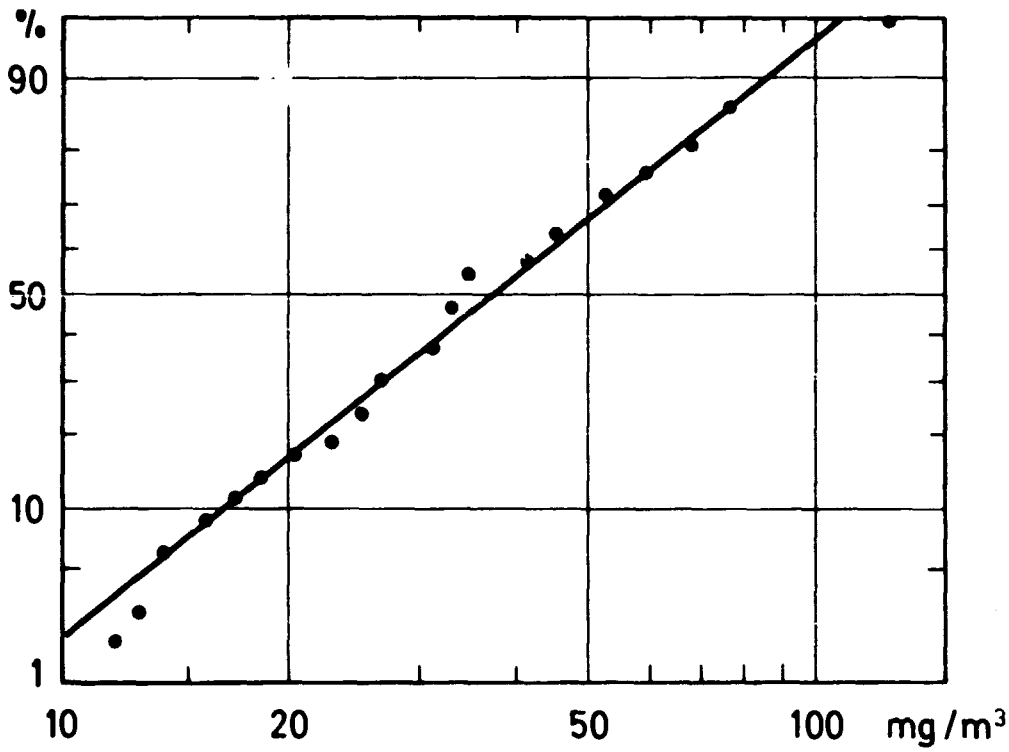

Fig. 46. Cumulative distribution of arsenic determinations in blood plasma from Blackfoot families.

analysis of variance is also shown in Table 65 and it confirms that no significant, individual differences are found in the group of 16 patients either with or without the control group. The difference between the mean values of the arsenic patient group and those of the control group may be tested, assuming unequal variances by means of a t-statistic, and it was found just barely significant at the 5 % level.

The superiority of the Analysis of Precision is in this case associated with the more efficient utilization of the results from the controls, which were pooled before the analysis to yield a larger sample with correspondingly better precision. This quality is not properly taken advantage of in the Analysis of Variance, and the small number of samples prevents definite conclusions from being drawn from the t-test with unequal variances.

Only when the estimated standard deviations are taken into account are the results significantly different from random numbers, and the Index of Determination significantly greater than zero. The accuracy of the results is substantiated by the very good agreement of the overall mean of 4.9 µg/kg and standard deviation 45 % with the value of 4.5 µg/kg of normal muscle tissue found on the 50 times larger samples included in Table 66.

Table 64

Concentrations of arsenic in skin biopsies of normal and psoriatic skin from psoriasis patients and a control group [Heydorn 1978]

PATIENTS TREATED WITH ARSENICAL DRUGS Psoriatic and/or un-psoriatic skin			NORMAL CONTROL Pooled skin samples	
Duplicates µg/kg	Weighted mean	Single µg/kg	Number	µg/kg
1.5 ± 2.7 8.1 ± 3.3	4.1 ± 2.1	9.9 ± 2.4	2	5.7 ± 1.0
5.1 ± 2.2 4.8 ± 2.3	5.0 ± 1.6	6.0 ± 3.0	4	4.0 ± 0.7
5.2 ± 1.9 10.6 ± 3.1	6.6 ± 1.6	3.1 ± 2.4		
6.5 ± 1.9 3.4 ± 2.4	5.3 ± 1.5	3.1 ± 1.9	5	2.8 ± 0.5
2.8 ± 1.8 0.6 ± 2.2	1.9 ± 1.4	9.5 ± 2.1		
5.6 ± 2.3 5.2 ± 1.8	5.3 ± 1.4	2.7 ± 3.6	3	2.1 ± 1.1
4.9 ± 1.5 3.0 ± 2.0	4.2 ± 1.2	7.8 ± 2.8		
8.5 ± 2.8 10.9 ± 4.0	9.3 ± 2.3	3.9 ± 3.1	2	2.4 ± 1.2
8 d.f. T = 7.1	7 d.f. T = 10.1	15 d.f. T = 22.1		4 d.f. T = 9.2

Essential elements

One additional long-term physiological factor to be considered is age, but no significant correlation with age was found in our work. This means that the normal values reported for As, Se and Mn in tissue can be used without correction for age differences. This, however, applies only to adults, whereas the considerable physiological changes taking place during childhood, and particularly during the foetal stage, certainly affect the distribution of some trace elements, particularly Cu.

Measurements of As, Se, and Mn in foetal tissue were reported in [Heydorn et al. 1976] and are presented in Table 66 together with the

Table 65

Comparison of data processing of results with and without estimated precision [Heydorn 1978]

Characteristic group Type	Cases	Number of results	Variability within groups			Variability between groups			Mean value wet weight
Analysis of Precision									
			d.f.	T	P	d.f.	T	P	weighted
Patients treated with Arsenicals	16	24	15	22.1	>0.05	20	41.3	<0.005	5.1 µg/kg **(*)
Normal, controls	16	5	4	9.2	>0.05				3.3 µg/kg
Analysis of Variance									
			d.f.	F	P	d.f.	F	P	simple
Patients treated with Arsenicals	16	24	15,8	1.3	~0.4	1,19	3.2	>0.05	4.9 µg/kg
Normal, controls	16	5	20,8	1.2	~0.4				

Table 66

Concentrations of arsenic, manganese and selenium in normal, human tissue and in foetal tissue

Tissue	Arsenic in fresh tissue						Manganese in fresh tissue				Selenium in fresh tissue			
	Normal (1)			Foetal (2)			Normal (1)		Foetal (2)		Normal (1)		Foetal (2)	
	Mean ng/g	Cases	σ_x %	Mean ng/g	Cases	σ_x %	Mean ng/g	Cases σ_x %	Mean ng/g	Cases σ_x %	Mean ng/g	Cases σ_x %	Mean ng/g	Cases σ_x %
Liver	11	7	60	4	6	87	1100	7 17	600	6 28	390	7 32	240	6 38
Pancreas	5	7	73	4	6	95	1150	7 22	300	6 38	220	7 27	130	6 46
Kidney	7	7	53	2	6	35	520	7 24	200	6 15	580	7 22	160	6 18
Lung	10	7	78	2	6	44	130	7 -	90	6 17	160	7 18	100	5 11
Spleen	3	7	63	3	6	67	91	7 15	130	6 19	240	7 14	210	6 38
Muscle	4	6	42	2	5	57	64	6 23	80	5 71	170	6 22	65	4 32

(1) Larsen et al. 1972

(2) Heydorn et al. 1976

previous results for normal adults. The distribution between the different organs is far less pronounced than in the adults, and the overall levels of all 3 trace elements are significantly lower in the foetal samples; but at the same time the average coefficient of variation between individuals is unchanged at 50-60 % for arsenic and 20-25 % for Mn and Se. This agrees with the assumption that overall trace element variations in the foetuses simply reflect variations in their environment, i.e. individual maternal variation. The distribution of individual tissue concentrations is expected to be Gaussian for essential elements such as Se and Mn, but logarithmic normal for non-essential elements such as As [Liebscher 1968].

Only *genetic factors* give rise to truly personal differences between foetuses of the same gestational age, and these are manifested only in the distribution of trace elements within the foetus, not in their absolute concentrations.

This was demonstrated in a study of the concentrations of Cu in a foetus suspected of having *Menkes' disease*, reported by Heydorn et al. [1975]. Menkes' disease is an X-linked genetic defect, leading to death in early childhood from an apparent copper deficiency.

Results for organs representing about 60 % of the total weight of the foetus showed a considerably reduced content of Cu in the liver; however, increased concentrations in the other organs brought the total foetal copper back to normal.

Controls of 4 normal foetuses had 80 ± 5 % of total copper in the liver, whereas the Menkes' foetus had only 25 % as illustrated in Fig. 47. One of the controls was female, but at this stage of development there was no significant difference between the sexes.

Although sex is the most conspicuous genetic factor, it does not seem to cause significant differences in the trace element pattern of As, Se, and Mn. During pregnancy the concentration of Cu in serum increases, but if a woman is pregnant she does not qualify as one of our select, normal persons, and similar changes for As, Se, and Mn would not be detected in our present studies.

Diagnostic verification of genetic defects has been made in several cases for Menkes' disease by the determination of Cu in the placenta, and also for Wilson's disease by the determination of Cu in a liver biopsy. In both cases the concentrations were an order of magnitude higher than normal.

The large number of factors affecting trace element concentrations in human tissue, blood, etc., cannot be evaluated by any one laboratory, but require the combination of results from many laboratories all over the world. Such combination depends far more on the absolute accuracy of the data than on their relative precision. Unfortunately, most values reported for trace element concentrations do not give

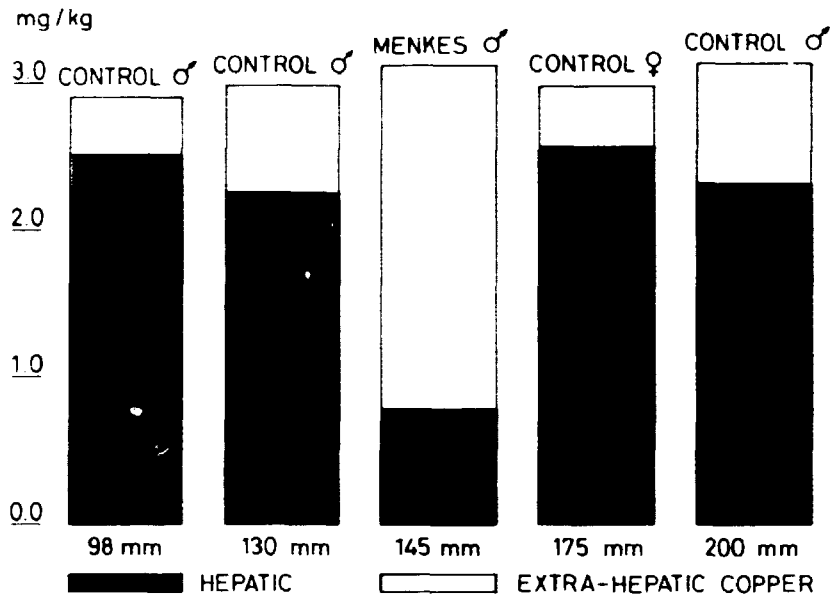

Fig. 47. Total copper found in tissue and its distribution between liver and the other organs analyzed. Each foetus is characterized by its crown-rump length. [Heydorn et al. 1975].

information that enables other laboratories to judge their accuracy. Only by comparing apparently identical types of investigation, such as the trace element levels found in human serum, can such information be provided.

This method is reasonably good for elements that are homeostatically well controlled within a rather narrow range of concentrations, such as selenium and several other essential elements. For non-essential elements such as arsenic where no such mechanism operates, even order-of-magnitude differences do not rule out the perfect agreement of absolute accuracy.

Results for selenium in serum or plasma found in recent literature are summarized in Table 67. Individual results reported by Lukens [1965] conform to a Gaussian distribution with a relative standard deviation of only 16 %, in good agreement with results for other essential elements, including Cu [Kasperek 1972], as well as many other constituents of human blood [Flynn 1974].

Coefficients of variation were subjected to Bartlett's test, which showed no significant disagreement between the 12 values reported, and the pooled variance gives an overall relative standard deviation of 19 %; this again confirms the remarkable stability of selenium concentrations in serum.

While no significant difference between serum and plasma is seen, considerable differences between the mean values reported by different

Table 67

Concentrations of selenium in serum or plasma from normal adults

Sample type	Mean mg/m³	Standard deviation	Investigation Reference	Year	Number of cases
Serum	100	-	Gofman	1964	39
-	102	16 %	Lukens	1965	93
Plasma	144	20 %	Dickson	1967	253
-	111	21 %	Levine	1970	4
Serum	63	-	Nadkarni	1971	12
-	59	27 %	Behne	1972	4
-	98	18 %	Kasperek	1972	184
Plasma	56	-	Maxia	1972	46
Serum	131	15 %	Hahn	1972	8
Plasma	102	18 %	Rhead	1972	7
Serum	129	26 %	Morss	1972	11
-	89	-	Maziere	1973	-
-	89	12 %	Damsgaard	1973	11
Plasma	102	18 %	Schrauzer	1973	17
Serum	103	-	Behne	1975	-
-	118	22 %	McConell	1975	18
-	130	15 %	Versieck	1977	36

investigators are observed, indicating the presence of significant geographical variations.

Our values for Se in Danes [IX] are not significantly lower than the overall geographical mean value of 100 ng/ml (mg/m³). This may not be unimportant, because the intake of selenium is inversely correlated with cancer mortality in man [Schrauzer 1976].

For arsenic, the Danish situation is much less clear, because the effect of small quantities of As in relation to Se is not yet satisfactorily elucidated.

In conclusion we may say that abnormal values for concentrations of essential elements such as selenium and copper are almost certainly a symptom of abnormal physiological conditions. Abnormal concentrations of non-essential elements only indicate the presence of unusual environmental conditions.

5.2. Other Problems

The use of neutron activation analysis with radiochemical separation for the determination of trace elements in biological materials is second to no other analytical method. However, many other analytical problems lend themselves to solution by NAA, and some of them exploit features of the method that are not always realised.

Instrumental neutron activation spectrometry is well known for the multi-elemental analysis of environmental samples, etc., by the use of high-resolution Ge(Li) counters. However, the estimated precision of results is not often used to investigate sample homogeneity by means of the Analysis of Precision [XI].

This separation of analytical precision and *sample homogeneity* is very important in all types of investigations of trace-element concentrations. Even in the best Certified Reference Materials the distribution of uncertified trace elements is not established, and in ordinary, unknown samples the homogeneity is too rarely questioned.

Another special feature of NAA that is often overlooked is that it does not determine elements, but nuclides. Simultaneous determination of two or more nuclides of the same element in one sample has been used to determine stable *isotope ratios* of elements, but in the present study the determination of highly abnormal isotopic compositions of radionuclides with high specific activity was carried out in the case of carrier-free ^{125}I [III] from different suppliers.

Finally, for some elements with short-lived indicators, the use of neutron activation analysis is chosen for its inherent *speed*. This is particularly true for Li with an indicator half-life of 0.8 seconds, but also to some extent for V, in combination with the scarcity of alternative methods with sufficient sensitivity.

Radioiodine isotope ratios

The *specific activity* of a primary radioisotope preparation is the activity of the radionuclide in question per unit weight of element, regardless of chemical state and isotopic composition.

^{125}I for protein iodination is supplied in an aqueous solution containing NaOH or Na_2SO_4 and stated to be in the form of iodide. This may be true of freshly prepared solutions, but in the course of a few weeks it may gradually be transformed into various iodine-containing compounds of unknown composition.[2] These compounds may be volatile and do not exchange with added iodide even after the addition of Na_2SO_3 or $Na_2S_2O_3$ [Moldenhawer 1966]. The questionable chemical state of the iodine rules out the direct application of chemical

2. Ulf Jacobsen, private communication.

methods, while extensive chemical treatment to restore a well-defined chemical state, such as digestion and distillation [Bale 1962], is impracticable for small samples and introduces a blank problem.

^{125}I is produced by reactor neutron irradiation of xenon, which means that the isotopic impurities are limited to ^{126}I and ^{127}I. ^{126}I is present at an activity level of 1 % or less, and with a half-life of 12.8 days, the corresponding amount of iodine is negligible compared with that of 60-day ^{125}I. The total amount of iodine in a ^{125}I solution is thus represented by the nuclides ^{125}I and ^{127}I, which can be determined by measuring the ^{126}I and ^{128}I formed by thermal neutron activation of a sample. It was therefore decided to use instrumental neutron activation analysis for the determination of total iodine, regardless of its chemical state.

Results of the determination of ^{125}I are presented in Table 68 along with the standard deviations based on counting statistics. The photopeak areas from the 386 keV γ-rays of ^{126}I, determined before and after irradiation, were converted to γ-quanta per second by means of an absolutely standardized ^{113}Sn solution emitting 393 keV γ-rays. All disintegration rates where corrected to the end of the irradiation, assuming a ^{126}I half-life of 12.8 days.

The loss of activity by purging with CO_2 is limited to a few per cent with the exception of sample ICN, which lost almost 20 %. This particular sample was supplied in Na_2SO_4 solution (20 mol/m^3) while all the others were in NaOH solution.

The ^{125}I determinations from Table 68 were corrected to the time of receipt, assuming a half-life of 60 days. A conversion factor of 644 PBq/kg gives the activity in MBq, and both figures are given in Table 69 along with the sample volumes.

Determination of ^{127}I by means of total peak area according to Yule [1966] gave the figures for ^{127}I given in Table 69 along with their standard deviations based on counting statistics.

Total iodine in a sample is now the sum of the masses of ^{125}I and ^{127}I; ^{126}I has a mass of only 0.33 fg/Bq and can be completely ignored. Finally, the specific activity of the product is presented in the last column of Table 69 as the ratio between the ^{125}I activity and total iodine.

The specific activities of the seven different commercial ^{125}I preparations were found to range from 280 to 600 PBq/kg, corresponding to 44-92 % of that of the truly carrier-free product. The specific activities of samples RCC and UC agreed well with the 11.0-11.4 mCi/μg (~415 PBq/kg) and 16.6 mCi/μg (615 PBq/kg) reported in the literature [Appleby 1967] for the corresponding products [Arino 1968].

Ratios of ^{126}I activity to ^{125}I activity ranged from less than 0.02 % to just above 1 % and in most cases reflected concurrent changes in

Table 68

Determinations of ^{125}I by neutron activation analysis [III]

Supply code	Decay days	Original 386 keV γ/s	CO_2 purging % loss	Irradiated 386 keV γ/s	Produced* 386 keV γ/s	^{125}I ng
RCC	101	284	1.5	1780	1505	105.0 ± 2.6
CEA	100	59	0.7	303	245	17.1 ± 0.4
NENC	92	33	6.0	450	421	29.4 ± 0.8
UC	114	1437	1.1	2832	1390	97.0 ± 2.4
TL	113	1265	4.7	2241	1025	71.5 ± 1.8
PD	99	7	6.2	113	106	7.4 ± 0.2
ICN	98	683	18.7	1520	1012	70.6 ± 1.8

All activities refer to the end of irradiation.

*Corrected for minor differences in irradiation conditions.

Table 69

Determinations of total iodine and specific activity of ^{125}I solutions [III]

Supply letter code	Sample volume mm³	^{125}I		^{127}I Stable iodine µg	Total iodine µg	Specific activity EBq/kg
		MBq	µg			
RCC	50	216	0.336	0.139 ± 0.033	0.475	0.45 ± 0.03
CEA	50	35	0.054	0.017 ± 0.012	0.071	0.49 ± 0.08
NENC	50	55	0.085	0.101 ± 0.017	0.186	0.30 ± 0.03
UC	50	233	0.362	0.031 ± 0.037	0.393	0.59 ± 0.06
TL	50	170	0.264	0.047 ± 0.019	0.311	0.55 ± 0.03
PD	100	15	0.023	0.023 ± 0.004	0.046	0.33 ± 0.03
ICN	50	141	0.219	0.282 ± 0.010	0.501	0.28 ± 0.01

All activities refer to the time of receipt of the samples.

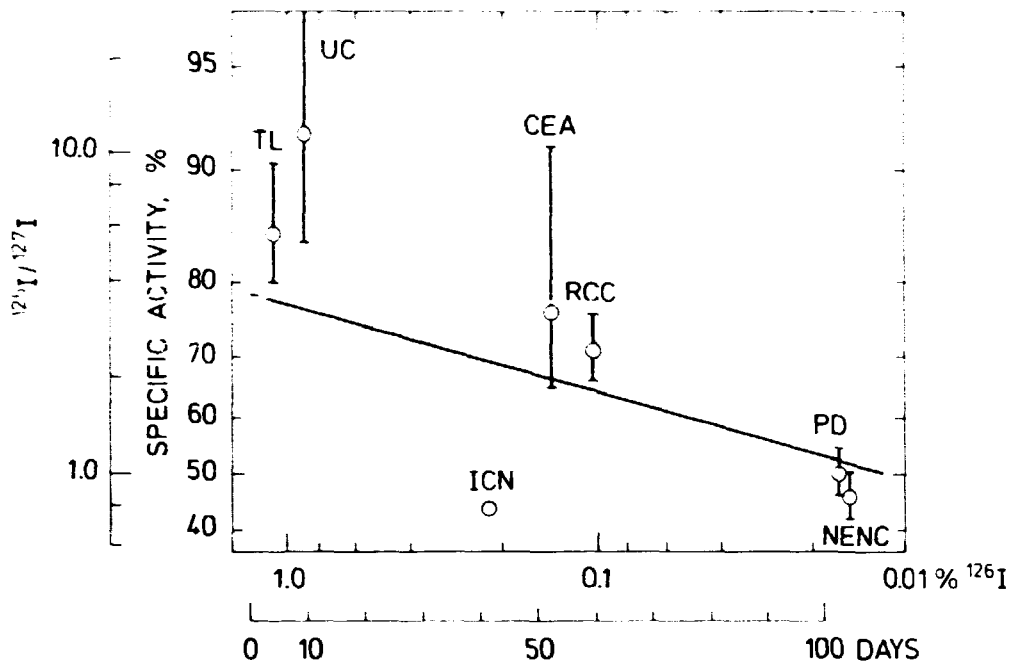

Fig. 48. Specific activities of ^{125}I samples vs. the $^{126}I/^{125}I$ ratios. Lines parallel to the sloping line indicate the change of specific activity and $^{126}I/^{125}I$ ratio with time for any given product.

specific activity. The correlation is presented graphically in Fig. 48 as a double logarithmic plot of the $^{125}I/^{127}I$ ratios *vs.* the $^{126}I/^{125}I$ ratios, supplemented by a linear scale of days corresponding to the 16.3 days half-life of the $^{126}I/^{125}I$ ratio.

Results for samples manufactured under identical conditions, but of different age, would fall on a straight line parallel to the line shown in the figure representing the 60-day half-life of the $^{125}I/^{127}I$ ratio.

Samples RCC, CEA, TL, and perhaps UC were probably produced under very similar conditions and differ only in age; the activity of the long-lived radionuclides ^{134}Cs and ^{137}Cs in these samples was less than 0.5 ppm (5×10^{-7}) of the ^{125}I activity.

Samples PD and NENC contained about 30 ppm (3×10^{-5}) of ^{134}Cs plus ^{137}Cs activity. They were probably older samples which may have decayed for rather a long time before chemical processing, so that stable iodine formed by the decay of 36-day ^{127}Xe reduced the specific activity.

Sample ICN had the same $^{134}Cs/^{137}Cs$ ratio of 1.0 as sample NENC, whereas UC, TL, and PD showed ratios of less than 0.25. The $^{134}Cs/^{125}I$ ratios of samples NENC and ICN were 15.0 and 6.8 ppm, respectively, corresponding to an age difference of about 70 days; this is in quite good agreement with the 63-day age difference shown in the figure by

the $^{126}I/^{125}I$ ratios. Therefore, samples NENC and ICN must have been irradiated under virtually identical conditions, and the low specific activity of sample ICN caused by contamination with stable iodine during chemical processing.

Determination of the specific activity of primary radioisotopes includes the regular quality control of ^{36}Cl preparations from Risø, as described in Section 4.1. The nuclide ^{36}Cl itself cannot be determined by neutron activation analysis, but has to be measured by β-counting only.

Instrumental neutron activation analysis for the determination of specific activities of radioisotope preparations is independent of the chemical state of the element to be determined. This is particularly important for carrier-free products supplied in extremely dilute aqueous solutions, in which the chemical state of the radioactive nuclides as well as of the accompanying stable element is often questionable.

The measurement of radionuclides by neutron activation analysis is still very unusual; in addition to ^{125}I [III] this method has only been used for ^{129}I [Schüttelkopf 1974] and ^{237}Np [Ruf 1976], but both these nuclides have half-lives exceeding 10^6 years.

Lithium in reference materials

The use of β-counting in instrumental neutron activation analysis is also rather unusual, and among the few practical applications up to now is the high-speed determination of Li [Heydorn et al. 1977] by Čerenkov counting of 0.84-second ^{8}Li. During the last month of operation of the DR 2 reactor more than 2000 rock samples were analyzed, thereby probably increasing the total number of Li-concentrations known in rocks by a factor of 2 or more.

Results included concentrations of Li in the 36 international geochemical reference materials shown in Table 70, and only 4 of these had recommended values for this element.

The biological reference materials Bowen's Kale and SRM 1571 Orchard Leaves have no recommended value for Li, nor has any value for Kale ever been reported [Bowen 1976]. Results by INAA are close to the limit of detection, and the lowest concentration was therefore checked by analyzing samples of different weights, as described in Section 4.3.

Results are presented in Table 71 together with an information value from the National Bureau of Standards. An Analysis of Precision does not indicate the presence of unknown sources of variation such as sample heterogeneity, but either the NBS value or the present value must have a large systematic error.

Table 70

Concentrations of Li in international geochemical reference samples [Heydorn et al. 1977]

Type of rock	Identification Source	Code No.	Li mg/kg This work	Flanagan 1973
Andesite	USGS	AGV-1	13.5	12
Anhydrite	ZGI	AN	4.3	-
Basalts	CRPG	BR	12.0	(9)
	GSJ	JB-1	10.7	10.2
	USGS	BCR-1	12.3	12.8
	ZGI	BM	72.5	70
Bauxite	ANRT	BX-N	45.8	-
Diabase	USGS	W-1	12.5	14.5
Diorite	ANRT	DR-N	42	-
Disthene	ANRT	DT-N	24.3	-
Dunites	USGS	DTS-1	2.0	(2)
	NIM	D	3.3	(5)
Felspar	ZGI	FK	6.8	-
Granites	CRPG	GA	7.6	(100)
	CRPG	GH	59	42
	NIM	G	12.7	(10)
	USGS	G1	21.6	22
	USGS	G2	34.9	34.8
	ZGI	GM	53	51
Granodiorite	GSJ	JG-1	70.3	100
Larvikite	ASKN	ASK-1	19.4	-
Lujavrite	NIM	L	40.7	(70)
Norite	NIM	N	5.8	(6)
Peridotite	USGS	PCC-1	0.9	2
Phlogopite	CRPG	Mica-Mg	110	-
Pyroxenite	NIM	P	3.8	(4)
Schist	ASKN	ASK-2	30.4	-
Serpentines	ANRT	UB-N	24.8	-
	ZGI	SW	1.7	-
Shale	ZGI	TS	38.6	-
Slate	ZGI	TB	107	114
Sulfide	CSRM	SU-1	12.4	-
	ASKN	ASK-3	1.5	-
Syenite	NIM	S	1.5	(3)
	CSRM	No. 2	114	-
	CSRM	No. 3	90	-

Vanadium in reference materials

In spite of the considerable interest in vanadium as an essential element [Schwarz 1971], very little information is available concerning its distribution in the biosphere. This is connected with the absence of suitable analytical methods for its determination at very low levels; even neutron activation analysis is only possible after suitable pre-concentration.

Pre-irradiation chemical separation defeats the most important characteristics of activation analysis and is therefore very unusual. For V in biological materials no other method with sufficient sensitivity seems available, but under these circumstances the analysis of biological reference materials for vanadium becomes of paramount importance.

Until April 1972 no results had been published for V in Kale [Bowen 1972] or in Orchard Leaves; at the IAEA Symposium on Nuclear Techniques in the Life Sciences in Bled, 2 laboratories published results of work carried out completely unaware of each other. Both laboratories used neutron activation analysis with pre-irradiation separation, and the results are shown in Table 72.

Levstek [1972] reports considerable losses of V during dry ashing for a variety of plant materials; combustion in a Schöniger flask was found to produce the higher results shown in Table 72.

For *Kale*, subsequent results found by other methods have not confirmed this finding, but they substantiate the result from Risø as shown in Table 73. The use of wet ashing in a post-irradiation radiochemical separation in neutron activation analysis, as well as in flameless atomic-absorption spectrometry, produce results that are in outstanding agreement with ours, and together the results fulfil the normal requirements for certification of the vanadium concentration in Kale.

No unexpected sources of variation were found by the Analysis of Precision of our own results, and no significant difference was found between the individual laboratories mentioned in Table 73. The disagreement with Levstek [1972] disappears when we compare with his results found by means of dry ashing only.

Instrumental neutron activation analysis was attempted by Nadkarni [1973] and by Pierce [1973], who found somewhat higher values with very modest precision; these and other results contribute to the mean value of 0.366 mg/kg reported by Bowen [1975]. These values as well as recent results by Marinov [1977] are all consistent with the much more precise value of 341 ± 25 µg/kg found by Damsgaard[3] in 1977 using special precautions to reduce all conceivable errors to insignificance.

3. Personal communication.

Table 71

Concentrations of Li in biological reference materials in mg/kg
[Heydorn et al. 1977]

Material	Bowen's Kale	SRM 1571 Orchard Leaves
Number of samples	4	5
Mean value	1.56 ± 0.03	0.77 ± 0.03
Analysis of Precision	T = 14.09	T = 18.02
Degrees of Freedom	12	14
$P(X^2 \geq T)$	0.30	0.21
Alternative value	none	14
Method	Personal communication	Nuclear track
Reference	Bowen 1976	Carpenter 1974

The situation for Kale is thus very satisfactory, and a recommended value of 0.33 mg/kg is not likely to have any significant systematic error; no heterogeneity has been detected by the Analysis of Precision.

For *Orchard Leaves*, the situation was much less satisfactory because our results were not in statistical control and hence the Analysis of Precision revealed significant influence from unknown sources of variablity [Damsgaard et al. 1972]. Lack of homogeneity with respect to this trace element was suggested, perhaps connected with the presence of the siliceous material left after digestion, which is mentioned in the Certificate [SRM 1571].

A number of results found by means of activation spectrometry or INAA have been published since 1972, and as shown in Table 74 their average value is in excellent agreement with the value reported by Levstek [1972] and shown in Table 72. At the same time Levstek [1972] reported a result after dry ashing of 340 ± 20 µg/kg, which is probably not significantly different from the Risø result in Table 72.

The inescapable conclusion seems to be that V from Orchard Leaves is in fact lost during dry ashing, i.e. before equilibrium with added ^{48}V tracer is established. We therefore decided to investigate the effect of alternative methods of sample decomposition on our results.

The experimental plan was carried out in steps (a) to (e) representing different modifications of the original method (a); key-word descriptions of the steps are given in Table 75.

Table 72

Concentrations of V in biological reference materials 1972-04-10

Laboratory	Institut Jozef Stefan	Research Establishment Risø
Bowen's Kale Orchard Leaves	414 ± 13 µg/kg 622 ± 23 µg/kg	332 ± 11 µg/kg 410 ± 40 µg/kg
Reference	Levstek et al. 1972	Damsgaard et al. 1972

(a) The variability of the original results reported in [Damsgaard et al. 1972] is strongly affected by the chemical yields, as determined by added ^{48}V tracer. Of the total variability of 10 %, only half is contributed from the vanadium actually extracted from the sample.

This suggests a variable component in Orchard Leaves with exchangeable, but only partially leachable, vanadium. It was concluded that Orchard Leaves is not homogeneous with respect to vanadium at the recommended sample size of 250 mg.

Alternatively, the dry ashing procedure with a slow rise in temperature up to 700°C is not reproducible but gives rise to different losses of ^{48}V and sample vanadium. Without exact knowledge of the chemical composition of the vanadium in Orchard Leaves, this possibility cannot be left out of consideration.

(b) The use of wet ashing by means of concentrated sulphuric and nitric acids, and in particular the subsequent neutralization with ammonia, introduces additional blank problems.

Significant blank values must be corrected for chemical yield before being applied to individual results, and this is only possible when the origin of the blank values, as well as the yields of different stages of the analytical procedure, are known.

In this case the total blank was assumed to consist of two components, a_0 and a_1, only one of which is corrected for overall yield, R_i

$$y_{io} = a_0 + a_1 \cdot R_i \qquad (95)$$

Values of a_0 and a_1 were determined experimentally by varying the quantities of decomposition mixture and extractant relative to each other

$a_0 = 2.1 \pm 1.0$ ng V, mainly from 8-hydroxy-chinaldine

$a_1 = 6.7 \pm 1.1$ ng V, mainly from NH_3 and ^{48}V tracer.

Table 73

Determinations of vanadium in Bowen's Kale 1972-73

Method of Decomposition	Method of Analysis	Mean value µg/kg	Number of samples	Reference
Dry ashing	INAA	332 ± 11	9	Damsgaard 1972
do	NAA	320 ± 30	8	Levstek 1972
$H_2SO_4 + H_2O_2$	RNAA	337 ± 18	5	Steinnes 1973
do	AAS	330 ± 4	10	Schramel 1973

Altogether 10 determinations of V in Orchard Leaves in 4 batches were individually corrected for blanks by means of equation (95). No additional source of variation could be detected by the Analysis of Precision, and the value given in Table 75 is the weighted mean and its estimated standard error.

(c) Schöniger combustion was carried out as described by Levstek [1972], except that the platinum sample-holder in the Schöniger flask was replaced by a quartz spiral. The same high blank values of 10 ± 2 ng V were obtained by this method, but attempts to identify the origin of this excess of vanadium were unsuccessful. A significant contribution from the oxygen gas used for combustion could not be excluded.

Blank corrections could therefore not be yield-corrected, and the Analysis of Precision of altogether 12 results showed the presence of unknown sources of variability. The value presented in Table 75 is therefore the simple mean with its a posteriori standard error, determined with 11 degrees of freedom.

The mean value and standard error of the 8 results with chemical yields >70 % is 410 ± 10 µg/kg.

(d) Post-irradiation radiochemical separation with carrier addition and yield determination avoids blank problems. However, very rapid decomposition and separation is necessary in order to preserve adequate sensitivity and precision; this in turn involves the risk of incomplete decomposition and carrier equilibration.

Rapid and complete decomposition is achieved by sulphuric acid in combination with 50 % hydrogen peroxide, but incomplete elimination of excess peroxide results in low yields, because the peroxyvanadan-ion is not extracted by 8-hydroxychinaldine. Fuming nitric acid may replace the hydrogen peroxide, resulting in slightly longer decomposition time.

Table 74

Determinations of vanadium in Orchard Leaves
by activation spectrometry

Number of samples	Mean value mg/kg	Reference
	<0.60	Morrison 1972
3	0.61 ± 0.04	Pierce 1973
5	0.57 ± 0.06	Hoffman 1974
3	0.58 ± 0.07	Ricci 1975
6	0.64 ± 0.13	Guinn 1976
	0.58	Bate 1976
	0.90 ± 0.02	Gallorini 1976

Results are presented in Table 76 together with other values based on chemical processing of the sample material.

(e) Instrumental neutron activation analysis as activation spectrometry is the only method that is independent of the chemical form of the vanadium in Orchard Leaves. Its precision is on the other hand poorer than that of any of the other methods used.

Interference from other elements is minimized by counting on a Ge(Li) detector with less than 2.0 keV resolution, and count-rates were kept at 10^3 counts/second or below to avoid the dead-time and pile-up problems mentioned in Section 2.6.

Photo-peak areas were determined by the method of Covell with peak boundary selection to yield maximum precision [VI]. The Analysis of Precision showed satisfactory agreement between the estimated and the actual variability of 11 results, and the weighted mean and standard error is given in Table 75. Calculations based on the methods of Sterlinsky, respectively our own method, are described in Section 2.8. and were in good agreement with the value reported here.

From the results of the preceding investigation several important *conclusions* can be drawn:

(i) The results of (a), (b), and (c) in Table 75 are in perfect agreement, which means that decomposition methods do not affect results; consequently, the dry ashing method does not cause any significant loss of V from Orchard Leaves.

(ii) The results of (c) are significantly lower than those reported by Levstek [1972] in Table 72 using the same method of decomposition.

Table 75

Experimental investigation of decomposition errors in the analysis of Orchard Leaves [Rietz 1972]

Method Label	Analytical Methodology		Critical balance		Mean value µg/kg	Number of samples
	Decomposition	Separation	Asset	Liability		
(a)	Dry ashing	Pre-irradiation	Low blank value	Possible loss by decomposition	410 ± 15	7
(b)	Wet ashing	Pre-irradiation	No loss expected by decomposition	High blank value	408 ± 16	10
(c)	Schöniger combustion	Pre-irradiation	No loss	High blank value	435 ± 20	12
(d)	Wet ashing	Radiochemical	No blank value	Possibly incomplete decomposition	377 ± 10	2
(e)	None	None	No loss	Poor precision	420 ± 100	11

Table 76

Alternative analytical results for V in Orchard Leaves 1972-73

Decomposition technique	Analytical method	Mean and µg/kg	number of samples	Reference
500°C ashing	NAA	340 ± 20	4	Levstek 1972
$H_2SO_4 + H_2O_2$	RNAA	384 ± 19	1	Damsgaard 1973*
$H_2SO_4 + HNO_3$	RNAA	374 ± 12	1	Rietz 1973*
$HClO_4 + HNO_3$	Catalysis	361 ± 9	6	Welch 1972*

*Personal communication

(iii) The results of (a), (b), and (c) are significantly lower than the results found by means of instrumental neutron activation analysis presented in Table 74.

(iv) The results of (d) are significantly lower than those of (a), (b), and (c) but they agree well with the only remaining published values presented in Table 76.

(v) The results of (e) are significantly less precise than the corresponding results found by means of activation spectrometry presented in Table 74; no significant differences could be found between mean values. This also applies to the far more precise result of 600 ± 30 µg/kg found by Damsgaard[4] in 1977.

All the results for vanadium in Orchard Leaves, except those of Levstek [1972] from Table 72, can be arranged in 3 categories according to the types of vanadium components included.

The *acid-soluble* vanadium comprises all the compounds that are brought into solution by the methods usually employed for the decomposition of plant material. The results in Table 76 represent 4 different decomposition methods, but the Analysis of Precision does not indicate any additional variability, as shown in Table 77.

The catalytic method used by Welch [1972] is completely different from RNAA, so that the weighted mean of all 12 samples qualifies for certification.

The *exchangeable* vanadium represents all compounds of V that may exchange completely with ^{48}V added as a tracer for yield determination. All the vanadium does not therefore have to be dissolved, but the exchange with solid material will depend upon the duration of contact with the tracer solution. In the post-irradiation radiochemical separations of V presented in Table 76 contact with the tracer solution is

4. Personal communication.

Table 77

Analysis of Precision and weighted mean values of results for vanadium in SRM 1571 Orchard Leaves

Form of Vanadium	Number of samples	Mean value µg/kg	Source Tables	Groups	d.f.	T	$P(\chi^2 \geq T)$
Acid-soluble	12	365 ± 6	76	Decomposition	3	3.32	0.34
Exchangeable	29	415 ± 10	75	methods	2	1.30	0.52
Total	28	580 ± 30	74	Laboratories	4	3.35	0.50

limited to a couple of minutes, whereas in the pre-irradiation separations of (a), (b), and (c) in Table 75 the time of contact is half an hour or more.

Pooled results of (a), (b), and (c) are therefore used in Table 77 to represent exhangeable V, although the Analysis of Precision indicates the presence of additional sources of variation. Lack of homogeneity with respect to the small amount of siliceous material was suggested in [Damsgaard et al. 1972], but differences in exchangeability caused by variations in surface area are another possibility.

Total vanadium comprises all vanadium compounds present in the sample, regardless of their origin or availability. The results found by means of activation spectrometry given in Table 74 are in good agreement, and the Analysis of Precision does not indicate unexpected sources of variation.

In Table 77 is shown the weighted mean of all reported values with the exception of that of Gallorini [1976].

It is surprising that only about 60 % of the total vanadium in Orchard Leaves can be brought into solution by normal decomposition methods. No such problem was found with Bowen's Kale, even after precise results by means of activation spectrometry became available. Neither were similar problems found in a Nordic intercalibration exercise dealing with trace elements in dried sewage sludge [Tjell 1974]. Here our result found by means of activation spectrometry of 39 $\pm$ 6 mg/kg was in perfect agreement with the value of 36 mg/kg found by atomic absorption spectrometry preceded by acid digestion.

The lack of homogeneity observed in the present investigation is of a different nature in comparison with the homogeneity tested by the usual procedures. Even though homogeneity is not guaranteed for uncertified elements, we have no reason to believe that V is not perfectly evenly distributed between samples of the usual >250 mg weight.

The lack of homogeneity between samples analyzed by different methods is attributed to insufficient micro-homogeneity, presumably caused by the presence of small quantities of siliceous material in the botanical matrix. This makes SRM 1571 unsuitable as a reference material for botanical investigations, because different analytical methods may give inconsistent results for Orchard Leaves, but at the same time give perfectly satisfactory results for other plant materials, like Kale.

5.3. Ultratrace Analysis

An upper level of 100 mg/kg (ppm) for trace concentrations is a generally accepted definition in trace analysis; thus, for example, Fe qualifies as the most abundant trace element in the human body [ICRP 1975]. Classical quantitative analysis therefore covers the 4 decades from 0.01 % - 100 %, and it has been proposed [X] to introduce the term *ultratrace analysis* for concentrations below 10 µg/kg (ppb), so that trace analysis covers the 4 decades from 0.01 ppm to 100 ppm.

Below 0.01 ppm the blank value becomes a very serious problem for all types of analysis [Meinke 1973], albeit less so for activation analysis. At the same time 10 µg/kg is the lowest reasonably attainable concentration level to be reached with generally available trace element techniques [Hofstader 1976]. Finally, with a single exception, no certified reference materials are available below 10 µg/kg.

In the present study the concentrations of the essential elements Mn, Cu, and Se in human tissues consistently belong to the field of trace analysis, whereas concentrations of As are more often than not at the ultratrace level. Nevertheless, as shown in Section 5.1, the Analysis of Precision of results for As has demonstrated that all sources of variation can be kept in statistical control, so that the problems characteristic of ultratrace analysis were not encountered.

Turning to *human serum*, two additional elements have been determined at the ultratrace level, namely V and Mn, for which statistical control could not be maintained.

Incomplete control of factors affecting precision and accuracy is thus characteristic of ultratrace analysis; this makes the selection of reliable values from the literature for typical, average concentrations of As, V, and Mn in human serum an impossible task, as shown in Table 78.

From the table it can be seen that Cu presents no problems, and it may be added that the order of magnitude variations of the Se concentration is not a sign of analytical problems, but only reflects the confusion of units preferred by different authors, and their lack of a sense of meaningful numbers in this field that covers a range of more than 4 decades in the same medium.[5]

Arsenic in serum

Arsenic in serum is characterized by its astonishing lack of stability, as shown in Section 5.1, where the concentration increases by more than 2 orders of magnitude within a matter of hours after a meal. In such cases the concentration of As in serum is higher than in many tissue samples, particularly nerve tissue [Larsen et al. 1972].

5. From 0.1 mg As m^3 in the investigation of Section 5.1, to 2.6 g Cu/m^3 of serum from the pregnant mother [Heydorn et al. 1975].

Table 78

Recommended values for trace elements in human serum in mg/m³

Element	Bowen 1966	Schroeder 1971	Varcoe 1974
As	40	190	190
Se	110	11	1100
Mn	2.9	8.3	8.3
Cu	1120	1160	1160
V	10	10	1000

It is therefore not surprising that arsenic values show considerable variations that originate from geographical, individual, and temporal sources [IV]. Comparatively few results are found in the literature, as shown in Table 79, which includes both serum, plasma, and whole blood.

In spite of the many sources of real variation, agreement between duplicates agrees with the estimated precision even at the lowest levels.

Vanadium in serum

Vanadium in serum displays a variation from observer to observer comparable to that of arsenic. Here the sources of variability are, however, not necessarily real, but have at least in one case [I] been identified as analytical errors.

Rather few results are found in the literature, as shown in Table 80, which includes whole blood. Our own results from 1966 [I] refer to serum samples supplied by the Walter Reed (Army) Hospital in Washington, and no control of the sampling procedure was possible.

The significantly lower results reported by Damsgaard et al. [1972] are therefore not necessarily an indication of geographical variation between Washington and Copenhagen, but could reflect differences in the quality of sampling.

Agreement with other serum values is non-existent, whereas results for whole blood are slightly more encouraging. Barg [1969] performed the analysis by a spectrographic method on ashed samples, whereas Kirzhner [1974] used exactly the same method as described in [I].

The precision of our results is not sufficient to determine variations between the individual samples, so that the Analysis of Precision will not detect any unknown sources of variation; all results were therefore pooled to give the best value of 4.6 ± 0.8 mg/m³ serum. Although the

Table 79

Concentrations of arsenic in normal human blood in mg/m^3

Serum or plasma	Whole blood	Samples	Reference
	66	146	Iwataki 1959
22		39	Gofman 1962
1.1		2	Bergström 1966
1.7		13	Giovanetti 1967
>3		1	Fritze 1968
15	22	17	Heydorn 1970
2.4	2.5	16	ibid.
	210	103	Hamilton 1972
0.009		8	Wester 1973
1.1		11	Damsgaard 1973

true concentration might be slightly lower, there is hardly any doubt that all the higher results reported are unreliable; as shown in Table 78, Schroeder [1971] himself reached the same conclusion.

Similar levels are found in human tissue [Damsgaard et al. 1972]; only in liver and sometimes in lung tissue were there levels significantly higher than the recommended serum value above.

Manganese in serum

Manganese levels in serum, on the other hand, are lower than in any other tissue analyzed [Larsen et al. 1972] by at least 2 orders of magnitude, and the determination of Mn has been reported in a large number of publications based on different analytical methods. A compilation of results for serum or plasma by neutron activation analysis up to 1971 is shown in Table 81 and by other methods in Table 82. Together, these tables represent the entire knowledge of concentrations of Mn in human serum available at the time we initiated our investigation of a possible manganese deficiency in uraemic patients.

With an overall median value of 13 mg/m^3, there is no significant difference between the results found by means of neutron activation analysis and those found by other methods when tested by the Wilcoxon rank test. At the same time the individual mean values differ so much that no useful information on the level of Mn in normal Danish serum can be extracted. In fact, no definite progress had taken place since 1940 when Kehoe et al. reported a value of about 70 mg/m^3 for Mn in human plasma.

Table 80

Concentrations of vanadium in normal human blood in mg/m^3

Serum or plasma	Whole blood	Samples	Reference
420			Schroeder 1963
<110		pooled	Gofman 1964
70			Lukens 1965
4.6		36	Heydorn 1966
	8	15	Barg 1969
800		4	de Jorge 1970
1.0		1	Damsgaard 1972
	4.6		Kirzhner 1974
20		300	Panteliadis 1975
30		3	Vis 1977
	22	5	Buono 1977

It was therefore necessary to analyze serum samples from Danish controls before an evaluation of possible deficiency could begin. A total of 12 samples were analyzed in duplicate and subjected to the Analysis of Precision, which showed highly significant additional variability between duplicates: an unaccounted standard deviation of 0.2 mg/m^3 (ng/ml) compared with an estimated standard deviation of 0.04 mg/m^3 (ng/ml). No significant variation between patients could be established.

We assumed that the standard deviation of 0.2 mg/m^3 was caused by variation in the contamination level of the individual samples, and the results were therefore treated by the Method of Least Duplicates described in Section 4.1. The mean value must be corrected for the estimated precision of the results

$$\hat{\mu} = \mu_{low} + \frac{\hat{\sigma}}{\sqrt{\pi}} \qquad (96)$$

and an estimated mean value of Mn in human serum of 0.52 ± 0.04 mg/m^3 was then reported in Cambridge by Damsgaard [1971]. Both this mean and this standard error, as well as the 28 % relative standard deviation of the distribution, could still have been influenced by contamination. However, this value was lower than any value previously published, so the level of contamination could not have been very high.

Table 81

Reported concentrations of manganese in human serum or plasma in mg/m³ determined by neutron activation analysis up to 1971

Mean value	Samples	Reference	
17	2	Bowen	1956
100	-	Jacobson	1961
9	1	Borg	1961
2.6	23	Papavasiliou	1961
170	7	Bethard	1962
45	10	Comar	1962
2.4	-	Bethard	1964
13	30	Kanabrocki	1964
22	98	Guinn	1965
4.3	12	Olehy	1966
0.6	14	Cotzias	1966
11	7	Haven	1966
1.4	128	Campero	1967
13	41	Kanabrocki	1967
1.4	55	Cotzias	1968

Additional work concentrated on reducing the risk of contamination during sampling, so that the true variability between individuals could be determined. At the same time the precision of the analytical method was thoroughly tested by analysis of Bowen's kale and dried animal blood from the IAEA [VIII] - in which the manganese concentrations differ by 2 orders of magnitude. The Analysis of Precision presented in Table 83 showed that the precision of the analytical method fully accounted for the variation observed between replicates of the two reference materials, but not between duplicates of serum samples.

The Analysis of Precision presented in Table 84 shows that the efforts to reduce contamination during sampling resulted in a highly significant reduction of variability. At the same time an Analysis of Variance shows that the variation between patients is now highly significant in comparison with the standard deviation of a single determination.

The reduction of the variability originating from sampling reduced not only the standard error of the mean, but also the mean itself, as shown in Table 85. Contamination from dust particles can reasonably be expected to have a Poisson distribution, which means that variance ratios equal the ratios of the means.

Table 82

Reported concentrations of manganese in human serum or plasma in mg/m^3 determined by chemical analysis up o 1971

Average	Samples	Reference		Method
10	80	Hegde	1961	Emission spectroscopy
70	9	Miller	1962	Spectro-photometry
0.6	12	Fernandez	1963	Catalysis
13	50	Butt	1964	Emission spectroscopy
7	150	Zhernakova	1967	Emission spectroscopy
17	62	Mertz	1968	Emission spectroscopy
24	40	Mahoney	1969	Atomic absorption
112	20	Malikova	1970	Emission spectroscopy
14	27	Arsagova	1971	Emission spectroscopy
20	105	Niedermeyer	1971	Emission spectroscopy

With a variance ratio of 75 and a difference of 0.08 ± 0.12 mg/m^3 between the means, the level of remaining contamination for the improved sampling conditions is estimated at well below 0.005 mg/m^3. Neither the mean value of 0.54 ± 0.05 mg/m^3 nor the standard deviation of the distribution of 30 %, reported in [IX], are significantly influenced by sample contamination; both agree completely with the values obtained using the method of least duplicates, as reported at Cambridge [1971].

In addition our final results are indistinguishable from the lowest value in either of Tables 81 and 82, indicating absence of a significant geographical variation. This allows us to pool our results with those of Fernandez [1963] and Cotzias [1966]; the grand mean of all 37 results becomes 0.59 mg/m^3 with a relative standard deviation of 27 %.

Almost simultaneously, work had been initiated along the same lines at the University of Ghent. This was published by Versieck, Speecke, Hoste and Barbier in 1973. Their results were in substantial agreement with ours and are included in the list of consistent values in Table 87.

Their individual results were included in the paper [Versieck 1973], and a detailed comparison with our own results in [IX] was therefore carried out and is shown in Table 86. It is interesting to note that the contributions from sampling conditions to the total variability are not significantly different, whereas the estimated analytical precision is significantly better in our work. The Index of Determination is therefore reduced slightly, but the difference is unimportant, as can be seen in Table 55.

Table 83

Analysis of Precision of manganese determinations [VIII]

Material	Kale	Blood	Serum
Manganese level	15 mg/kg	0.15 mg/kg	0.5 mg/m^3
M	1	1	11
N	4	6	22
Degrees of freedom*	5		11
T	2.73		285
$P(X^2 > T)$	0.75		$< 10^{-10}$

* Number of independent pairs = duplicates.

Versieck [1973] used disposable steel needles for collection of the blood samples instead of the re-usable platinum cannulae used by us. His higher mean value may be related to this [Versieck 1972], and in his subsequent investigations [Versieck 1974], included in Table 87, the steel needle was replaced by a Vygon plastic cannula. The total a posteriori precision, and thereby the variation from sampling conditions, remained unchanged [Versieck 1975], and the mean value of 0.57 ± 0.02 mg/m^3 was not significantly lower.

It appears that when the utmost care is taken to avoid all possible sources of contamination of serum samples with manganese, a variability with a standard deviation of ~0.04 mg/m^3 may be achieved, but not surpassed, with presently available techniques. Further reduction of variability is not likely to result from changes in the type and material of the cannula. No significant effect on the mean values has been established.

The remarkable agreement between the results of the University of Ghent and of Risø, in addition to the other important considerations previously discussed, leaves little doubt concerning the real concentrations of Mn in normal human serum. In Table 87 are included all known investigations that are compatible with ours, and with the exception of the first they are all based on neutron activation analysis with radiochemical separation.

The reported population variances were subjected to Bartlett's test, which showed no significant difference between the values, and the pooled estimate at 109 degrees of freedom gave a standard deviation of 0.15 mg/m^3.

The mean values were now tested by the statistic T for known precision, and with a value of T = 7.05 for 5 degrees of freedom their

Table 84

Comparison of original sampling conditions with improved standard hospital conditions [VIII]

Sampling conditions Standard error mg/m³	Original s_o = 0.34	Improved hospital $\hat{\sigma}_o$ = 0.34	 s_o = 0.04
Variation between	Samples	Duplicates	Samples
Number of materials	1	11	1
Number of results	11	22	11
Degrees of freedom	10	11	10
Test parameter	F* = 1.02	T = 0.29	F* = 10.43
Probability	0.50	$< 10^{-5}$	< 0.001

*Estimated population variance relative to the a posteriori precision of a single determination with 11 degrees of freedom

differences were considered insignificant. This indicates an absence of geographical variation, which is in good agreement with the impressive homeostatic control observed by Cotzias [1966].

At the time of writing no other group has reported results consistent with the grand mean value of 0.58 mg/m³ for 115 samples. Conclusions based on undoubtedly erroneous analytical results have though continued to appear after 1971, as shown in Table 88.

The distance and long-term stability of manganese concentrations in human serum, demonstrated in Table 87, was then supplemented by an investigation of the possible diurnal variation, such as reported by Scheving [1968] for rats, as well as of the temporal effect of food intake. This was carried out simultaneously with the investigation of As and Se concentrations described in Section 5.1., but for Mn the Analysis of Precision showed the presence of significant additional sources of variability from sample contamination. The results for Mn had therefore to be treated in a different way.

The analysis of blank samples of redistilled water treated like blood samples clearly showed that contamination could not be reduced to insignificance in these experiments; the additional variability of these results thus not only reduces the sensitivity towards small changes in the concentration of manganese, but also increases the average levels observed.

Both factors may be partly overcome by replicate analyses, so that a posteriori precision is improved by pooling of several results, and so that

Table 85

Mean and standard error for alternative sampling conditions [VIII]

Sampling conditions	Sample mean mg/m^3	Absolute standard error of	
		Mean	Sampling
Original	0.62	0.10	0.34
Improved	0.54	0.05	0.04

absolute levels are improved by the method of least duplicates previously described.

Altogether 37 results from 3 persons were tested for possible effects of food intake in two different ways: by comparison of mean values before and after a meal and by correlation with the concentrations of As shown in Section 5.1. to reflect food intake.

Both methods failed to detect any significant effect, and results were therefore combined without regard to meals according to the time of the day, as shown in Table 89. The presence of a 24-hour rhythm in normal, human serum manganese levels, similar to that reported by Scheving [1968] for rats, would result in a positive correlation between the average results for the 3 persons investigated. No such correlation was found, and it was concluded that the true manganese levels in serum did not change significantly with the time of the day.

At the same time it is clear that sampling errors are also independent of the time of the day, but that they change from one investigation to the other. Thus, the influence of 3 different levels of contamination of the results is also illustrated in Table 89. In all three investigations the sampling error is significantly larger than that reported in [IX], but it is apparent that improvement of precision results in lower values both for the simple mean and for the mean value of the least duplicates and for the single, lowest result.

The method of least duplicates gives a much more accurate mean value than the simple mean, but individual values are still not in statistical control when tested by the Analysis of Precision. If all additional variability is caused by contamination, the most accurate result is simply the lowest of each investigation. Both values are in satisfactory agreement with the results for normal serum appearing in Table 87.

Perhaps the most important *conclusion* to be drawn from Table 89 is that, unless results are in statistical control, the validity of observed

Table 86

Comparison between results from the University of Ghent and the Research Establishment Risø [IX] for manganese in human serum

Basis of comparison	Damsgaard 1973	Versieck 1973	Significance
Estimated analytical precision $\hat{\delta}$	0.03 mg/m³	¤0.05 mg/m³	***
Effect of sampling conditions s_o	0.04 -	0.05 -	n.s.
Total a posteriori precision	0.05 mg/m³	¤0.07 mg/m³	
Index of determination ID	0.90	0.85	n.s.
Cannula type	reusable platinum	disposable steel	
Mean value	0.54 ± 0.05 mg/m³	0.64 ± 0.04 mg/m³	n.s.
Population standard deviation σ_x	0.16 mg/m³	0.18 mg/m³	

¤Calculated from tables 4 and 5

Table 87

Selected, independent determinations of Mn concentrations in normal human serum or plasma

Mean value mg/m³	Population σ_{log}	Number of samples	Investigation Reference	Year	Country or State
0.63	20 %	12	Fernandez	1963	CA
0.59	31 %	14	Cotzias	1966	NY
0.52	28 %	12	Damsgaard	1971	DK
0.54	30 %	11	Damsgaard	1973	DK
0.64	28 %	20	Versieck	1973	B
0.57	23 %	46	Versieck	1974	B
0.58	26 %	115	Pooled	1963-1974	

values is not improved by increasing the number of replicates. Without the Analysis of Precision there is no way to tell that the simple mean is not the most unbiased estimate of the true mean value.

Table 88

Concentrations of Mn in human serum or plasma reported since 1972

Mean value mg/m³	Number of samples	Investigation Reference	Year	Analytical technique
4.6		Maziere	1972	Neutron activation
14	7	Bek	1972	Atomic absorption
1.8	28	Molokhia	1973	Neutron activation
2.5	42	Hagenfeldt	1973	Neutron activation
1.6	50	Grafflage	1974	Atomic absorption
23	6	Ross	1974	Atomic absorption
1.7	20	Hambidge	1974	Emission spectroscopy
35	20	Chandra	1974	Atomic absorption
9	9	Muzzarelli	1975	Atomic absorption
1.0	-	Seeling	1975	Atomic absorption
20	300	Panteliadis	1975	Emission spectroscopy
30	-	Pshetakovsky	1975	Emission spectroscopy
1.0	19	D'Amico	1976	Atomic absorption
63	71	Webb	1976	Emission spectroscopy
47	6	Vis	1977	PIXE

Table 89

Concentrations of Mn in human serum from three individuals at different times of the day

Time of the day hours		KMT mg/m³	KMT samples	GK mg/m³	GK samples	JO mg/m³	JO samples
20	8 p.m.	0.55	2	0.87	2	0.99	4
24	midnight	0.68	2	0.95	2	-	
04	4 a.m.	0.57	2	1.13	2	0.90	4
08	8 a.m.	0.69	4	0.58	2	-	
12	noon	0.63	2	0.82	2	0.85	4
16	4 p.m.	1.00	2	0.44	1	-	
A posteriori precision*		s = 0.11 mg/m³		s = 0.20 mg/m³		s = 0.34 mg/m³	
Simple mean		0.69	14	0.83	11	0.91	12
Least duplicate**		0.65	7	0.74	6	0.77	6
Lowest result		0.42		0.44		0.60	

* $s^2 = \Sigma d^2/2n$, where d is the difference between duplicates.

** corrected by $\hat{\sigma}/\sqrt{n}$, where $\hat{\sigma}$ is the estimated precision of a single result.

These considerations apply in particular to ultratrace analysis, where sampling errors or contamination cannot be brought into statistical control and are very difficult to reduce to insignificance. This situation is monitored by the Analysis of Precision which, together with the method of least duplicates, helps to avoid reporting the grossly inaccurate results that are so abundant in this field.

6. Conclusion

The precision and accuracy of an analytical result is just as important as the result itself, and the main thesis of the present studies is that the uncertainty of a single analytical determination can be estimated with confidence from sources of error characteristic of the analytical method.

For neutron activation analysis, potential sources of error have been taken into account and their contribution to the final precision and accuracy of individual results has been evaluated. A statistical T-test has been introduced to verify the agreement between expected and observed variability between replicate results.

Actual results with a standard deviation based on the overall effect of a priori precision and counting statistics produced a test statistic in very good agreement with the assumed chi-squared distribution, when all sources of variation had been identified and brought under statistical control.

When the complete analytical method is in statistical control, the Analysis of Precision may serve as a continuous quality control, and significant deviations of the T-statistic reveal the presence of unexpected random, as well as systematic errors.

For neutron activation analysis with carrier addition and radiochemical separation (RNAA) in particular, the a priori precision is independent of the concentration of the determinand. Detection of systematic errors from duplicate results is thus possible even at the ultratrace level of concentration (<10 µg/kg), when control of accuracy cannot be based on certified reference materials. Together with its well-known absence of reagent blanks, this property is a characteristic of RNAA, and it is far more important to trace analysis than the high specificity and sensitivity that are shared by an increasing number of other analytical techniques. Used as a referee method at the ultratrace level, RNAA helps to eliminate erroneous data and to discourage the use of such data for ill-founded conclusions.

This leads to the complementary question - is it possible to prove that an analytical result is true? This is not possible, but by judicious use of the Analysis of Precision we can test the absence of all conceivable errors.

And this is all we can ask for.

Appendix

Important symbols and variable names used throughout the text without explanation on each occasion.

Miscellaneous symbols and terms

Symbol	Name or description	
A	determinand, element to be determined	
α	error of the 1st kind	
β	error of the 2nd kind	
I	information content in bits	
M	matrix element	
μ	true mean value of normal distribution	
n	neutron	
p	proton	
P	probability	
σ_x^2	true variance of normal population	
X	an unspecified element	
$\bar{y}_{im}$	limiting mean of individual result	
$\bar{y}$	simple mean of a set of results	
$\dot{y}$	time derivative	dy/dt
Z	an interfering element	
ω	absolute, statistical weight	$1/\sigma^2$
$\pm$	precedes the expected, overall standard deviation $\hat{\sigma}$ of the preceding number	

Real quantities and derived parameters

Symbol	Definition, specification, etc., and SI base unit	
a	specific activity of an element	Bq/kg
a	quantity of element A	kg
A	total activity	Bq
A_r	relative atomic mass	kg/mol
b	quantity of element B	kg
c_x	concentration of X	mol/m^3
D	decontamination factor	1
E	γ-energy	keV
f	effective value	1
g	gain	keV/channel
η	limiting mean - true value	kg/m^3
i	ionization current	A
I	resonance integral	m^2
ID	Index of Determination	1
θ	plane angle	rad
k	factor close to unity	1
K	separation factor R·D·K=1	1
κ	correction factor	1
l	small length, thickness	m
λ	decay constant	s^{-1}
m	quantity of sample	kg
μ	absorption coefficient $\sigma_a N_A/A_r$	m^2/kg
$\hat{\mu}$	weighted mean value of results from a normal distribution	
N_A	Avogadro's constant	mol^{-1}
q	discrimination factor	1
q_{im}	analytical result	kg
Q_X	recovery of element X	1
r	correlation coefficient	1
R	chemical yield	1
R_{Cd}	cadmium ratio	1
ρ	mass density	kg/m^3
ρ_x	concentration of X	kg/m^3

s^2	$\Sigma_1^n (Y_i - \bar{Y})^2/(n-1)$	
SEM	Standard Error of Mean	$s/\sqrt{n}$
σ_a	absorption cross section	m^2
σ_m	standard deviation of the mean of n results	$\bar{\sigma}/\sqrt{n}$
σ_o	absolute standard deviation	
σ_r	relative standard deviation	
σ_{log}	logarithmic standard deviation	$\sim\sigma_r/\bar{y}$
$\bar{\sigma}$	average standard deviation $\bar{\sigma}^2 = 1/\bar{w}$	
$\hat{\sigma}_y^2$	a priori value of variance of result distribution	
t	time	s
t_i	irradiation time	s
t_c	decay time	s
t_d	difference time	s
$T_{1/2}$	half-life	s
τ	mean life $1/\lambda$	s
w	statistical weight $1/\hat{\sigma}^2$	
$\bar{w}$	average precision	
ø	neutron flux density	$mol/(m^2 \cdot s)$
Ø	fluence $\int_0^t ø(t)$	mol/m^2
Y_{im}	analytical result	kg/m^3
Y_m	true concentration	kg/m^3
Y_m^*	certified concentration	kg/m^3
ω	solid angle	sr

Integer variables and numbers associated with counting

Symbol	Definition, explanation and suitable unit	
a	photo-peak height	counts/channel
A	photo-peak area, Covell	counts
A_x	mass number of element X	nucleons
b	base height	counts/channel
β	base-to-peak ratio b/a	
h	integration half-width	channels
i	number of replicate analyses	
I	peak channel	channel
λt	mean and variance of Poisson distribution	
m	number of materials analyzed	
n	number of results in a set	
N	number of atoms, etc.	
ν	degrees of freedom	d.f.
P	Total Peak Area, TPA	counts
P_n	Poisson probability	$e^{-\lambda t}\cdot(\lambda t)^n/n!$
R	resolution w/I	
s	specific count-rate	counts/(kg s)
S_x	total counts from element X	counts/kg
σ_c	standard deviation based on counting statistics	
t	number of standard deviations	σ
T	test parameter for statistical control	χ^2
τ	number of channels in standard deviations	h/σ
V	variance estimate based on counting statistics	
w	Full Width at Half Maximum, FWHM	channels
χ^2	chi-square distribution of numbers	
Z	atomic number	unit charges

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The Radioisotope Laboratory with its characteristic combination of production and research has provided an environment of stimulating discussions with colleagues, while the efficiency of the entire staff has made it possible for me to complete these studies.

The widespread contacts of the laboratory with scientists using radionuclides in a variety of fields have been an inspiration and a guide for our own research and development of methods to ascertain the quality of analytical data based on neutron activation.

For more than 10 years many people, some no longer present, have contributed to the implementation of this work. Handwritten drafts have been transformed to legible manuscripts by the secretaries of the Radioisotope Laboratory, and subsequently language-polished by Mrs. Jennifer Paris. Rough sketches and ideas have been made into precise drawings by the Drawing Office, and obscure references brought to light by the Risø Library.

The completed manuscript was typed as a Risø Report by the competent staff of the Central Typing Office and later transformed into photo type at the Copenhagen University Computing Centre.

Sammendrag

Arbejdet søger at belyse præcisionen og nøjagtigheden af analytiske resultater opnået ved hjælp af aktivering med neutroner fra atomkærnereaktorer.

Ved et resultats præcision forstås spredningen af de talværdier, der fås ved gentagen analyse under identiske forhold. Ved nøjagtigheden forstås overensstemmelsen mellem middelværdien af et stort antal gentagelser af analysen og den sande værdi.

Medens præcisionen således kan forbedres ved forøgelse af antallet af målinger, kan nøjagtigheden kun forbedres ved eliminering af tilstedeværende fejl.

1. Problemstillingen formuleres her i form af en *These*, der postulerer den principielle mulighed for at forudberegne præcisionen af et enkelt analyseresultat. Det vil sige, at det ud fra et nøje kendskab til metodens variationskilder skulle være muligt at bestemme spredningen af et større antal resultater.

En eksperimentel undersøgelse af de praktiske vanskeligheder ved at realisere denne mulighed baseres her på en række aktiveringsanalytiske bestemmelser af grundstofferne As, Se, Mn, Cu, V og Li, fortrinsvis i biologisk materiale.

Disse resultater indgår for størstedelens vedkommende i en række tidligere offentliggjorte arbejder [I - XI], men behandles her tillige med en række nye data under en anden synsvinkel, der sigter på en afprøvning af den opstillede these inden for rammerne af de praktiske anvendelser.

Som en *Antithese* viser den virkelige spredning af et større antal gentagne bestemmelser sig oftest at være større end den beregnede. Men *Synthesen* fastslår, at en væsentlig uoverensstemmelse skyldes tilstedeværelsen af upåagtede variationskilder.

2. Undersøgelsen indledes med en detailleret gennemgang af alle de faktorer, der kan forventes at have indflydelse på den systematiske eller tilfældige variation af analyseresultaterne.

Udover de sædvanlige trin i en neutronaktiveringsanalyse: forbehandling, bestråling, separation, måling, korrektion for udbytte, og beregningsmetode, medtages her selve prøveudtagningen, der repræsenterer et vigtigt - men ofte overset - forbindelsesled mellem det, der ønskes analyseret, og det, der bliver analyseret.

Resultaternes nøjagtighed afhænger tillige af fejlkilder ved anvendelsen af forskellige metoder til normalisering af analyseværdier; disse fejl er særlig betydningsfulde ved sammenligning med resultater fra andre laboratorier, såvel som fra andre analysemetoder.

3. Ud fra de i det foregående gennemgåede bidrag til den analytiske variation kan nu beregnes en *a priori* præcision, der kan udtrykkes som en kombination af den resulterende absolutte og relative standard deviation af et analyseresultat. Denne parameter er en karakteristisk egenskab ved den anvendte analytiske metode og kan anvendes ved jævnføring med andre metoder.

Ved metoder som aktiveringsanalyse, der benytter sig af radioaktivitetsmåling kommer hertil et bidrag til resultatets variation fra den aktuelle tællestatistik. Det radioaktive henfald følger med stor nøjagtighed en Poisson-fordeling, der har samme middelværdi og spredning; bidrag fra tællingen kan derfor beregnes ud fra en enkeltmåling og kan kombineres med metodens præcision til det pågældende resultats samlede standard deviation.

Tilfredsstillende overensstemmelse mellem beregnet og observeret variation betegnes *statistisk kontrol*, og metoder til overvågning heraf er baseret på gentagelse af en vis del af analyserne, sædvanligvis i form af dobbeltbestemmelser. En parameter T er fundet velegnet til statistisk overvågning og kontrol, og signifikansniveauer kan baseres på en X^2-fordeling.

Konkrete tilfælde af manglende statistisk kontrol har henledt opmærksomheden på utilsigtede ændringer i arbejdsbetingelserne, og i visse tilfælde har nye variationskilder måttet accepteres, medens andre har kunnet reduceres til det betydningsløse.

Alle de i undersøgelsen anvendte aktiveringsanalytiske metoder, instrumentelle såvel som metoder med kemisk separation, har kunnet bringes i statistisk kontrol.

4. Forskellige metoder til kontrol af nøjagtigheden af analytiske resultater er alle afhængige af et kendskab til resultatets præcision. Verifikation af nøjagtigheden for virkelige prøver er betinget af adgang til alternative analysemetoder med sammenlignelig følsomhed og præcision. I nærværende arbejde er givet eksempler på en sådan kontrol, men som generel kontrolmetode er denne vej næppe farbar.

Anvendelse af analysemetoder i statistisk kontrol muliggør imidlertid opdagelse af ukontrollerede variationskilder, der oftest viser sig at medføre systematiske fejl. Eliminering eller korrektion af sådanne fejl forbedrer nøjagtigheden af de fundne resultater.

Tilstedeværelsen af en konstant blindværdi medfører normalt ikke manglende statistisk kontrol, og andre metoder må derfor tages i anvendelse for at opdage fejl af denne type. Herunder gennemgås kontrolmetoder baseret på referencematerialer med en kendt eller alment accepteret sammensætning; men manglende homogenitet kan undertiden påvises og give anledning til problemer.

5. Praktiske anvendelser af den foreslåede metodik er illustreret med eksempler inden for ultra-sporelement koncentrationer, hovedsagelig hentet fra medicinsk og biologisk forskning, hvor små prøver og lave koncentrationer af både essentielle og ikke-essentielle sporelementer har tiltrukket sig interesse.

Her er især neutronaktiverigsanalyse med radiokemisk separation af betydning, fordi metodens *a priori* præcision er uafhængig af det undersøgte koncentrationsniveau. Aktiveringsanalytiske metoder i statistisk kontrol muliggør påvisnig af andre vigtige variationskilder for disse ultra-spormængder, og eksempler på både tidsmæssige, geografiske, ernæringsmæssige og genetiske variationer bliver beskrevet.

Manglen af referencematerialer med ultralave koncentrationer opvejes i nogen grad af brugen af metoder i statistisk kontrol, og disse metoder er samtidig velegnede til bestemmelse af de nøjagtige indhold af sporelementer i referencematerialer, hvor der ikke i forvejen findes pålidelige undersøgelser. Ingen metode kan dog sikre helt mod systematiske fejl, og selv en autoriseret værdi - for arsen - i et Standard Reference Materiale fra National Bureau of Standards har kunnet vises af være fejlagtig.

Endelig muliggør en analyse af præcisionen af dobbeltprøver en indkredsning af årsagerne til manglende statistisk kontrol, således at en vurdering af indflydelsen på analyseresultatet kan vurderes, selv om effekten ikke kan elimineres. Et særligt eksempel herpå er Mn i humant serum, hvor prøveudtagningen ikke har kunnet bringes i statistisk kontrol, men hvor dens indflydelse på analyseresultatet har kunnet reduceres til betydningsløshed. En diskussion af det virkelige niveau af Mn i serum har pågået i en årrække, men er formentlig nær sin afslutning.

6. Det konkluderes, at etablering af statistisk kontrol er et væsentligt punkt i udvikling af metoder til bestemmelse af sporelementer i biologisk materiale. Brugt som analytisk kvalitetskontrol muliggør den opdagelse af ukendte fejlkilder, systematiske såvel som tilfældige, ved analyse af aktuelle prøver af varierende art.

Når alle fejlkilder er under kontrol, kan det analytiske resultat anses for at have ikke blot en velkendt præcision, men også en upåklagelig nøjagtighed.

References

I K. Heydorn and H.R. Lukens,
Pre-irradiation Separation for the Determination of Vanadium in Blood Serum by Reactor Neutron Activation Analysis.
Risø Report No. 138 (1966) 20 pp.

II K. Heydorn,
Improvement of Accuracy by Multiple Carrier Addition Followed by Re-irradiation Yield Determination in a Simple Method for the Determination of Arsenic in Biological Material.
In: Nuclear Activation Techniques in the Life Sciences
(IAEA, Vienna, 1967) 179-188.

III K. Heydorn,
Determination of the Specific Activity of Carrier-free I-125 Preparations by Neutron Activation Analysis.
J. Radioanal. Chem. **3** (1969) 225-232.

IV K. Heydorn,
Environmental Variation of Arsenic Levels in Human Blood Determined by Neutron Activation Analysis.
Clin. Chim. Acta **28** (1970) 349-357.

V K. Heydorn,
Use of the National Physical Laboratory Ionization Chamber Type 1383A in Neutron Activation Analysis.
J. Radioanal. Chem. **10** (1972) 245-256.

VI K. Heydorn and W. Lada,
Peak Boundary Selection in Photopeak Integration by the Method of Covell.
Anal. Chem. **44** (1972) 2313-2317.

VII K. Heydorn and E. Damsgaard,
Simultaneous Determination of Arsenic, Manganese, and Selenium in Biological Materials by Neutron-Activation Analysis.
Talanta **20** (1973) 1-11.

VIII K. Heydorn and K. Nørgaard,
Analysis of Precision of Activation-Analysis Methods.
Talanta **20** (1973) 835-842.

IX E. Damsgaard, K. Heydorn, N.A. Larsen, B. Nielsen,
Simultaneous Determination of Arsenic, Manganese and Selenium in Human Serum by Neutron Activation Analysis.
Risø Report No. 271 (1973) 35 pp.

X K. Heydorn,
Detection of Systematic Errors by the Analysis of Precision.
In: Accuracy in Trace Analysis, Proceedings of the 7th IMR Symposium,
National Bureau of Standards, Gaithersburg, Md., 7-11 October 1974
(NBS Special Publication 422, 1976) 127-139.

XI K. Heydorn,
Continuous Quality Control of Analytical Results from Neutron Activation Analysis of Air Filter Samples Based on the Analysis of Precision.
In: Development of Nuclear-Based Techniques for the Measurement, Detection and Control of Environmental Pollutants (IAEA, Vienna, 1976) 61-73.

Abdullaev, A.A. and A.P. Kim.
Selection of Optimum Activation and Waiting Times during Neutron Activation Determination of Rare Earth Elements. (In Russian).
Izv. Akad. Nauk Uzb. SSR Ser. Fiz.-Mat. Nauk **14** No. 4 (1970) 89-92.

Aliev, A.I., V.I. Drynkin, D.I. Leipunskaya, and V.A. Kasatkin.
Handbook of Nuclear Data for Neutron Activation Analysis. Translated by B. Benny. (Israel Program for Scientific Translations, Jerusalem, 1970) 168 pp.

Alvarez R., W.P. Reed and G.A. Uriano.
New Environmental SRM's.
Anal. Chem. **50** (1978) 324A

Anders, O.U.
Experiences with the Ge(Li)-Detector for High-Resolution Gamma Ray Spectrometry and a Practical Approach to the Pulse Pileup Problem.
Nucl. Instrum. Methods **68** (1969) 205-209.

Anders, O.U.
Representative Sampling and Proper Use of Reference Materials.
Anal. Chem. **49** (1977) 33A-36A

Anon.
Guide for Use of Terms in Reporting Data in Analytical Chemistry.
Anal. Chem. **47** (1975) 2527.

Appleby, A. and R.E. Spillett.
Cerium (IV)-Arsenite Reaction in Microdetermination of Iodine for Specific Activity Measurement.
RCC-M 210 (The Radiochemical Centre, Amersham, Bucks., 1967) 7 pp.

Arino, H. and H.H. Kramer.
Determination of Specific Activity of I-131 Solutions via an Iodide Electrode.
Nucl. Appl. **4** (1968) 356-360

Arsagova, N.S.
On the Nickel and Manganese Levels in Patients with Uterine Cancer.
Vopr. Onkol. **17** No.9 (1971) 53-56.

Baedecker, Philip A.
Digital Methods of Photopeak Integration in Activation Analysis.
Anal. Chem. **43** (1971) 405-410

Bale, W.F., R.W. Helmkamp, T.P. Davis, M.J. Izzo, R.L. Goodland and I.L. Spar.
High Specific Activity Labeling of Protein with I-131.
UR-604 (Univ. of Rochester, New York, 1962) 37 pp.

Bando, S. and T. Imahashi.
Determination of arsenic, mercury and selenium in biological standard reference materials by neutron activation analysis.
Bunseki Kagaku **26** (1977) 138-141

Barg, C.M. and G.M. Kogan.
The contents of several microelements in blood in case of hereditary degeneration of the macula.
Oftal'mol. Zh. **24** (1969) 10-14

Bartošek, J., F. Adams and J. Hoste.
A dead-time correction system for gamma-ray spectrometry of short-lived isotopes.
Nucl. Instrum. Methods **103** (1972) 45-47

Bate, L.C., S.E. Lindberg and A.W. Andren.
Elemental Analysis of Water and Air Solids by Neutron Activation Analysis.
J. Radioanal. Chem. **32** (1976) 125-135

Becker, D.A. and P.D. La Fleur.
Determination of Moisture Content in SRM Orchard Leaves.
In: Activation Analysis Section: Summary of Activities, July 1969 to June 1970 National Bureau Standards (U.S.), Technical Note 548 (1970) 50-53.

Becker, D.A. and P.D. La Fleur.
Characterization of a Nuclear Reactor for Neutron Activation Analysis.
J. Radioanal. Chem. **19** (1974) 149-157

Becker, D.A.
Accuracy and Precision in Activation Analysis - Counting.
In: *Proceedings of the 2nd International Conference on Nuclear Methods in Environmental Research, Columbia, MO, 29-31 July 1974.* Edited by J.R. Vogt and W. Meyer (Univ. Missouri, Columbia, 1974) Vol. 1 (CONF-740701) 69-80

Becker, R.R., A. Veglia and E.R. Schmid.
Instrumental Neutron Activation Analysis of Standard Biological Materials.
Radiochem. Radioanal. Lett. **19** (1974) 343-354

Behne, D. and F. Diel.
Relations between carbohydrate and trace element metabolisms investigated by neutron activation analysis.
In: Nuclear Activation Techniques in the Life Sciences. Proceedings of a Symposium held at Bled, Yugoslavia, 10-14 April 1972 (IAEA, Vienna, 1972) 407-414

Behne, D. and P.A. Matamba.
Trocknung und Veraschung biologischer Proben bei der neutronenaktivierungs-analytischen Spurenelementbestimmung.
Fresenius' Z. Anal. Chem. **274** (1975) 195-197

Bek, F., J. Janoušková and B. Moldan.
Determination of Manganese and Strontium in Blood Serum Using the Perkin-Elmer HGA-70 Graphite Furnace.
Chem. Listy **66** (1972) 867-875
Engl. translation: At. Absorpt. Newsl. **13** (1974) 47-48.

Bergström, J. and P.O. Wester.
The Effect of Dialysis on the Arsenic Content of Blood and Muscle Tissue from Uraemic Patients.
In: Replacement of Renal Functions. Symposium held in Lyon, June 1966. Edited by D.N.S. Kerr (Excerpta Medica Foundation, Amsterdam, 1967) (Proceedings of the European Dialysis and Transplant Association, 3) (International Congress Series, 131) 38-40.

Berkson, J.
Do Radioactive Decay Events Follow a Random Poisson-Exponential?
Int. J. Appl. Radiat. Isot. **26** (1975) 543-549

Bethard, W.F., R.A. Schmitt and D.A. Olehy.
The Quantitation of Magnesium, Manganese, Zinc, and Copper in Blood by Neutron Activation Analysis.
In: Radioaktive Isotope in Klinik und Forschung V. Edited by K. Fellinger and R. Höfer (Urban & Schwarzenberg, München, 1962) 222-236.

Bethard, W.F., D.A. Olehy, and R.A. Schmitt.
The Use of Neutron Activation Analysis for the Quantitation of Selected Cations in Human Blood.
In: 3e Colloque International de Biologie. L'Analyse par Radioactivation et ses Applications aux Sciences Biologiques, Saclay, Septembre 26-28, 1963. Edited by D. Comar (Presses Univ., Paris, 1964) 379-392.

Biloen, P., J. Dorrepaal and H.B. van der Heijde.
Instrumental Activation Analysis Using High-Resolution Gamma-Spectrometry and Automated Data Analysis.
Anal. Chem. **45** (1973) 288-292.

Bolotin, H.H., M.G. Strauss and D.A. McClure.
Simple Technique for Precise Determinations of Counting Losses in Nuclear Pulse Processing Systems.
Nucl. Instrum. Methods **83** (1970) 1-12

Bolton, B.A. and Ph. K. Hopke.
The use of Instrumental Neutron Activation Analysis for the Determination of Arsenic Concentrations in Poultry.
J. Radioanal. Chem. 25 (1975) 299-302.

Borg, D.C., R.E. Segel, P. Kienle and L. Campbell.
Selective Radioactivation and Multiple Coincidence Spectrometry in the Determination of Trace Elements in Biological Material. Measurement of Manganese.
Int. J. Appl. Radiat. Isot. **11** (1961) 10-29

Bowen, H.J.M.
The determination of manganese in biological material by activation analysis, with a note on the gamma spectrum of blood.
J. Nucl. Energy **3** (1956) 18-24

Bowen, H.J.M. and D. Gibbons.
Radioactivation Analysis. (Clarendon, Oxford, 1963) 295 pp.

Bowen, H.J.M.
A Standard Biological Material for Elementary Analysis.
In: Proceedings of the Society for Analytical Chemistry Conference, Nottingham, July 19-23, 1964. Edited by P.W. Shallis (Heffer, Cambridge, 1965) 25-31

Bowen, H.J.M.
Trace Elements in Biochemistry. (Academic Press, London, 1966) 241 pp.

Bowen, H.J.M.
The Biochemistry of the Trace Elements.
In: Nuclear Activation Techniques in the Life Sciences. Proceedings of a Symposium held at Bled, Yugoslavia, 10-14 April 1972 (IAEA, Vienna, 1972) 393-405.

Bowen, H.J.M.
Problems in the elementary analysis of standard biological materials.
J. Radioanal. Chem. **19** (1974) 215-226.

Bowen, H.J.M.
The Use of Reference Materials in the Elemental Analysis of Biological Samples.
At. Energy Rev. **13** (1975) 451-477

Bowen, H.J.M. *(Reading, U.K.).*
personal communication, 1976.

Boyd, G.E.
Method of Activation Analysis.
Anal. Chem. **21** (1949) 335-347

Braun, T., W.S. Lyon and E. Bujdoso.
Literature growth and decay: an activation analysis resume.
Anal. Chem. **49** (1977) 682A-686A

Bresesti, A.M., M. Bresesti, A. Rota, R.A. Rydin and L. Lesca.
Threshold Reactions Excitation Functions Intercalibrated in a Pure Fission Spectrum.
Nucl. Sci. Eng. **40** (1970) 331-338

Brune, D., K. Samsahl and P.O. Wester.
A Comparison between the Amounts of As, Au, Br, Cu, Fe, Mo, Se, and Zn in Normal and Uraemic Human Whole Blood by Means of Neutron Activation Analysis.
Clin. Chim. Acta **13** (1966) 285-291.

Brune, D. and O. Landström.
Freezing Technique in Neutron Activation Analysis.
Radiochim. Acta **5** (1966) 228-230.

Brune, D.
Transfer of Activities from Container Material to Sample in Neutron Activation Analysis.
Radiochim. Acta **5** (1966) 14-16.

Brune, D. and B. Bivered.
Epithermal Neutron Activation Analysis of Elements present in Trace Quantities in Biological Materials.
Anal. Chim. Acta **85** (1976) 411-414

Bruninx, E.
The Evaluation of Errors caused by Flux Inhomogeneities in Thermal Neutron Activation Analysis.
Anal. Chim. Acta **60** (1972) 207-217.

Bruninx, E.
The Accurate Determination of Major Components in Ga_xSe_y by means of Instrumental Neutron Activation.
Anal. Chim. Acta **67** (1973) 17-28.

Brøndum, L. and J.D. Monrad.
Statistisk forsøgsplanlægning I: Varians- og regressionsanalyse. (Teknisk Forlag, København, 1972) 363 pp.

Buono, J.A., J.C. Buono and J.L. Fasching.
Simultaneous Determination of Al, V, Mn and Cu from Neutron-activated Saline Matrices by Precipitation with Poly-5-vinyl-8-hydroxyquinoline.
J. Radional. Chem. **36** (1977) 353-358

Butt, E.M., R.E. Nusbaum, T.C. Gilmour, S.L. Didi and S. Mariano.
Trace Metal Levels in Human Serum and Blood.
Arch. Environ. Health **8** (1964) 52-57.

Byrne, A.R. and A. Vakselj.
Rapid Neutron Activation Analysis of Arsenic in a Wide Range of Samples by Solvent Extraction of the Iodide.
Croat. Chem. Acta **46** (1974) 225-235.

Byrne, F.P.
The Analyst and Accuracy.
In: Accuracy in Trace Analysis. Proceedings of the 7th IMR Sumposium, held at Gaithersburg, Md., 7-11 October 1974. Edited by P.D. LaFleur. Vol. 1 (USGPO, Washington D.C., 1976) (National Bureau of Standards Special Publication 422) 123-126.

Calamand, A.
Cross-Sections for Fission Neutron Spectrum Induced Reactions.
In: Handbook on Nuclear Activation Cross-Sections. Edited by D. Brune and J.J. Schmidt. IAEA Technical Reports Series 156 (1974) 273-324.

Cali, J.P., J. Mandel, L. Moore and D.S. Young.
A Referee Method for the Determination of Calcium in Serum.
(USGPO, Washington, D.C., 1972) (National Bureau of Standards Special Publication 260-36) 136 pp.

Cali, J. Paul.
The NBS Standard Reference Materials Program: An Update.
Anal. Chem **48** (1976) 802A-818A

Campero, A., F.M. Graber and H.R. Lukens.
Neutron Activation Analysis of Biological Materials.
In: Proceedings of the International Conference on the Utilization of Research Reactors and Reactor Mathematics and Computation, Mexico City, 2-4 May 1967. CNM-R-2 Vol. 1, 346-356.

Campion, P.J., J.E. Burns and A. Williams.
A code of practice for the detailed statement of accuracy.
National Physical Laboratory (H.M.S.O., London, 1973) 52 pp.

Campion, P.J.
Procedures for Accurately Diluting and Dispensing Radioactive Solutions.
(Bureau International des Poids et Mesures, Sevres, 1975) (Monographie BIPM-1) 32 pp.

Carpenter, B.S.
Lithium Determination by the Nuclear Track Technique.
J. Radioanal. Chem. **19** (1974) 233-234

Challenger, F. *(Nottingham, U.K.).*
personal communication, 1976.

Chan, C.C.Y.
Improvement in the Fluorimetric Determination of Selenium in Plant Materials with 2,3-Diaminonaphthalene.
Anal. Chim. Acta **82** (1976) 213-215.

Chandra, S.V., P.K. Seth and J.K. Mankeshwar.
Manganese Poisoning: Clinical and Biochemical Observations.
Environ. Res. **7** (1974) 374-380

Chattopadhyay, A. and R.E. Jervis.
Multielement Determination in Market-Garden Soils by Instrumental Photon Activation Analysis.
Anal. Chem. **46** (1974) 1630-1639

Christoffersen, P., E. Damsgaard, K. Heydorn, N.A. Larsen, B. Nielsen and H. Pakkenberg.

Concentrations of Arsenic, Manganese and Selenium in Peripheral Nervous Tissue of Patients with Uraemia and a Control Group.
In: Dialysis and Renal Transplantation. Proceedings of the European Dialysis and Transplant Association held in Stockholm, June 1969. Edited by D.N.S. Kerr et al. Vol. 6 (Williams and Wilkins, Baltimore, Md, 1969) 198-202.

Coleman, R.F., F.H. Cripps, A. Stimson and H.D. Scott.
The trace element content of human head hair in England and Wales and the application to forensic science.
Atom No. 123 (1967) 12-22.

Comar, D., C. le Poec, M. Joly and C. Kellershohn.
L'analyse par radioactivation. Application a la determination de l'iode, du mercure, du cuivre, du manganese et du zinc dans les milieux biologiques.
Bull. Soc. chim. Fr. (1962) 56-71.

Cotzias, G.C., S.T. Miller and J. Edwards.
Neutron Activation Analysis: The stability of manganese concentrations in human blood and serum.
J. Lab. Clin. Med. **67** (1966) 836-849

Cotzias, G.C., P.S. Papavasiliou, E.R. Hughes, L. Tang and D.C. Borg.
Slow Turnover of Manganese in Active Rheumatoid Arthritis Accelerated by Prednisone.
J. Clin. Invest. **47** (1968) 992-1001.

Covell, D.F.
Determination of Gamma-Ray Abundance Directly from the Total Absorption Peak.
Anal. Chem. **31** (1959) 1785-1790

Currie, L.A.
Limits for Qualitative Detection and Quantitative Determination. Application to Radiochemistry.
Anal. Chem **40** (1968) 586-593.

Currie, L.A.
On the Interpretation of Errors in Counting Experiments.
Anal. Lett. **4** (1971) 873-882.

Currie, L.A.
The Limit of Precision in Nuclear and Analytical Chemistry.
Nucl. Instrum. Methods **100** (1972) 387-395.

Dalager, P. *(Vedbæk, Denmark).*
unpublished work, 1969

D'Amico, D.J. and H.L. Klawans.
Direct Microdetermination of Manganese in Normal Serum and Cerebrospinal Fluid by Flameless Atomic Absorption Spectrophotometry.
Anal. Chem. **48** (1976) 1469-1472.

Dams, R., J. Billiet and J. Hoste.
Neutron Activation Analysis of F, Sc, Se, Ag and Hf in Aerosols Using Short-lived Isotopes.
Int. J. Environ. Anal. Chem. **4** (1975) 141-153.

Damsgaard, E.
Simultaneous Determination of Manganese, Arsenic and Selenium in Normal Human Blood Serum by Neutron Activation Analysis.

Paper presented at the 2nd Symposium on the Recent Developments in Neutron Activation Analysis, Cambridge, 28 June - 1 July 1971.

Damsgaard, E., K. Heydorn and B. Rietz.
Determination of Vanadium in Biological Materials by Neutron Activation Analysis.
In: Nuclear Activation Techniques in the Life Sciences. Proceedings of a Symposium held in Bled, 10-14 April 1972 (IAEA, Vienna, 1972) 119-128.

Damsgaard, E. and K. Heydorn.
Arsenic in Standard Reference Material 1571 Orchard Leaves.
Risø-M-1633 (1973) 7 pp.

Damsgaard, E. and K. Heydorn.
Why Interference Test?
Risø-M-1814 (1975) 10 pp.

Damsgaard, E. and K. Heydorn.
Simultaneous Determination of Arsenic, Copper, Manganese, Selenium, and Zinc in Biological Materials by Neutron Activation Analysis.
Risø Report No. 326 (1976) 24 pp.

Damsgaard, E. and K. Heydorn.
Sources of Variability for the Single-Comparator Method in a Heavy-Water Reactor.
Paper presented at the 5th Symposium on Recent Development in Activation Analysis, Oxford, 17-21 July 1978.
Risø-M-2141 (1978) 15 pp.

Davydov, M.G. and A.P. Naumov.
Optimization in Quantitative Activation Analysis.
Sov. At. Energy **40** (1976) 499-500.
Atomnaya Energiya **40** (1976) 417

Debertin, K. and U. Schötzig.
Limitations of the pulser method for pile-up corrections in Ge(Li)-spectrometry.
Nucl. Instrum. Methods **140** (1977) 337-340

de Bruin, M., S.S. Then, P. Bode and P.J.M. Korthoven.
A simple dead-time stabilizer for gamma-ray spectrometers.
Nucl. Instrum. Methods **121** (1974) 611-613.

de Corte, F., A. Speecke and J. Hoste.
Reactor Neutron Activation Analysis by a Triple Comparator Method.
J. Radioanal. Chem. **3** (1969) 205-215.

de Jorge, F.B., L.C.G. Ferreira and C.P. Tilbery.
Vanadio na Amiotrofia de Charcot-Marie.
Rev. Bras. Med. **27** (1970) 303-307.

de Soete, D., R. Gijbels and J. Hoste.
Neutron Activation Analysis. (Chemical Analysis, Vol. 34). Edited by P.J. Elving and I.M. Kolthoff. (Wiley-Interscience, London, 1972) 836 pp.

Dickson, R.C. and R.H. Tomlinson.
Selenium in Blood and Human Tissues.
Clin. Chim. Acta **16** (1967) 311-321.

Dickson, R.C. and R.H. Tomlinson.
Instrumental Radioactivation Analysis of Selenium in Biological Materials.
Int. J. Appl. Radiat. Isot. **18** (1967) 153-159.

Diplock, A.T., C.P.J. Caygill, E.H. Jeffery and C. Thomas.
The Nature of the Acid-Volatile Selenium in the Liver of the Male Rat.
Biochem. J. **134** (1973) 283-293.

Doisy, E.A., Jr.
Micronutrient Controls on Biosynthesis of Clotting Proteins and Cholesterol.
In: Trace Substances in Environmental Health VI. Edited by D.D. Hemphill. Proceedings of a Symposium held in Columbia, Mo., 13-15 June 1972 (University of Missouri, Columbia, Mo, 1973) 193-199.

Dols, T.J. and B.H. Armbrecht.
Assessment of Analytical Method Performance Characteristics: Systematic Error.
J. Assoc. Off. Anal. Chem. **60** (1977) 940-945.

Eckhoff, N.
Optimal Neutron Activation Analysis.
Dissertation (Ph.D.), Kansas State Univ., 1968 (University Microfilms, Ann Arbor, Mich.) 196 pp.

Eckhoff, N.D.
A Study of the Variance-Covariance Matrix for Gamma Ray Spectra.
Nucl. Instrum. Methods **68** (1969) 93-102.

Eckschlager, K.
Errors, Measurement and Results in Chemical Analysis.
Edited by R.A. Chalmers. (Van Nostrand, London, 1969) 155 pp.

Eckschlager, K.
Criterion for Judging the Acceptability of Analytical Methods.
Anal. Chem. **44** (1972) 878-879

Eckschlager, K.
Characteristics for Judging the Reliability of Analytical Methods.
Collect. Czech. Chem. Commun. **39** (1974) 1426-1430.

Eisenhart, C.
The Reliability of Measured Values - Part I Fundamental Concepts.
Photogrammetric Eng. **18** (1952) 542-554; 558-565.

Eisenhart, C.
Expression of the Uncertainties of Final Results.
Science **160** (1968) 1201-1204.

Erdtmann, G. and O. Aboulwafa.
Selective Chemical Separation Procedures for Activation Analysis II.
Separation of As by Column Extraction with Tin (II) Ethylxanthate. Determination in Titanium Dioxide of High Sb Content and in Biological Materials.
Fresenius' Z. Anal. Chem. **272** (1974) 105-114.

Fahr, Egon.
Instrumentelle Analytik: Technologische Spielerei oder Hilfswissenschaft für Naturwissenschaften und Medizin.
Fresenius' Z. Anal. Chem. **281** (1976) 1-8

Fedoroff, M.
Contribution au Calcul des Conditions Optimales d'Irradiation et de Mesure de la Radioactivite en Analyse par Activation.
Nucl. Instrum. Methods **91** (1971) 173-187

Fedoroff, M.
Calcul des Conditions Optimales en Analyse par Activation.
J. Radioanal. Chem. **15** (1973) 435-449.

Fernandez, A.A., C. Sobel and S.L. Jacobs.
Sensitive Method for the Determination of Submicrogram Quantities of Manganese and its Application to Human Serum.
Anal. Chem. **35** (1963) 1721-1724.

Fiorino, J.A., J.W. Jones and S.G. Capar.
Sequential Determination of Arsenic, Selenium, Antimony, and Tellurium in Foods via Rapid Hydride Evolution and Atomic Absorption Spectrometry.
Anal. Chem **48** (1976) 120-125.

Fisher, R.A., H.G. Thornton and W.A. Mackenzie.
The Accuracy of the Plating Method of Estimating the Density of Bacterial Populations.
Ann. Appl. Biol. **9** (1922) 325-359.

Flynn, F.V., K.A.J. Piper, P. Garcia-Webb, K. McPherson and M.J.R. Healy.
The Frequency Distributions of Commonly Determined Blood Constituents in Healthy Blood Donors.
Clin. Chim. Acta **52** (1974) 163-171.

Fourie, H.O. and Max Peisach.
Loss of Trace Elements During Dehydration of Marine Zoological Material.
Analyst **102** (1977) 193-200

Fritze, K. and R. Robertson.
Instrumental and Radiochemical Neutron Activation Analysis Techniques for Protein Bound Trace Metals in Human Serum.
J. Radicanal. Chem. **1** (1968) 463-473.

Fukai, R.
Intercalibration of Analytical Methods on Marine Environmental Samples.
Progress Report No. 13 (IAEA, Monaco, 1976) 26 pp.

Furr, A. Keith, B.A. Roscoe, S.C. Kitchings and T.F. Parkinson.
VAT-69 A Software System for Gamma Spectroscopy.
Trans. Am. Nucl. Soc. **28** (1978) Suppl. 1, 27-29.

Gallorini, M., M. DiCasa, R. Stella, N. Genova and E. Orvini.
Multielement Trace Analysis by Atomic Absorption Spectrometry and Neutron Activation Analysis in Biological Matrices.
J. Radioanal. Chem. **32** (1976) 17-23.

Gaudry, A., B. Maziere, D. Comar and D. Nau.
Multi-element Analysis of Biological Samples after Intense Neutron Irradiation and Fast Chemical Separation.
J. Radioanal. Chem. **29** (1976) 77-87.

Giovanetti, S., Q. Maggiore and R. Malvano.
Neutron Activation Analysis of Trace Elements in Plasma from Normal Subjects and from Chronic Uraemic Patients.
In: Nuclear Activation Techniques in the Life Sciences. Proceedings of a Symposium held in Amsterdam, 8-12 May 1967. (IAEA, Vienna, 1967) 511-515.

Girardi, F., G. Guzzi and J. Pauly.
Reactor Neutron Activation Analysis by the Single Comparator Method.
Anal. Chem. **37** (1965) 1085-1092.

Gladney, Ernest S.
Copper Determination in Standard Materials by Neutron Activation and Srafion NMRR Anion-Exchange Resin.
Anal. Chim. Acta **91** (1977) 353-356.

Gofman, J.W., O. de Lalla, G. Johnson, E.L. Kovich, O. Lowe, W. Martin, D.L. Piluso, R.K. Tandy, F. Upham, R. Weitzel and D. Wilbur.
Chemical Elements of the Blood of Man.
In: Biology and Medicine Semiannual Report. Edited by J.H. Lawrence. UCRL-9897 (1961) 1-26.

Gofman, J.W., O.F. de Lalla, G. Johnson, E.L. Kovich, O. Lowe, D.L. Piluso, R.K. Tandy and F.Upham.
Chemical Elements of the Blood of Man.
In: Biology and Medicine Semiannual Report. Edited by J.H. Lawrence. UCRL-10211 (1962) 62-68.

Gofman, J.W., O.F. de Lalla, E.L. Kovich, O. Lowe, W. Martin, D.L. Piluso, R.K. Tandy and F.Upham.
Chemical Elements of the Blood of Man.
Arch. Environ. Health **8** (1964) 105-109.

Goldsmith, J.R., M. Deane, J. Thom and G. Gentry.
Evaluation of Health Implications of Elevated Arsenic in Well Water.
Water Res. **6** (1972) 1133-1136.

Gordadze, G.P.
Generalized Criterion of Choice of the Optimum Time Regime for Carrying out Activation Analysis. (In Russian).
Soobsch. Akad. Nauk Gruz. SSR **78** (1975) 581-584.

Görner, W. and G. Höhnel.
An Automatic Life Time Correction in Multi-channel Counting of Short-Lived Nuclides.
Nucl. Instrum. Methods **88** (1970) 193-195.

Gottschalk, G.
Standardisierung quantitativer Analysenverfahren IV
Anwendung von Standardverfahren.
Fresenius' Z. Anal. Chem. **278** (1976) 1-12.

Graber, F.M., H.R. Lukens and K. Heydorn.
The determination of Tb, Er, Yb, and Y by Neutron Activation Analysis.
Trans. Am. Nucl. Soc. **9** (1966) 87.

Grafflage, B., G. Buttgereit, W. Kübler and H.M. Mertens.
Die Messung der Spurenelemente Chrom und Mangan in Serum mittels Flammenloser Atomabsorption.
Z. Klin. Chem. Klin. Biochem. **12** (1974) 287-293.

Greiner, A.C., S.C. Chan and G.A. Nicholson.
Human Brain Contents of Calcium, Copper, Magnesium, and Zinc in some Neurological Pathologies.
Clin. Chim. Acta **64** (1975) 211-213.

Guinn, V.P., M. Di Casa, J.J.M. de Goeij and D.R. Young.
Neutron Activation Analysis Studies of Marine Biological Species and Related Marine Sediments.
In: Proceedings of the 2nd International Conference on Nuclear Methods in Environmental Research held in Columbia, Mo., 29-31 July 1974. Edited by J.R. Vogt and W. Meyer. (NTIS, Springfield, Va., 1974) (CONF-740701) 24-31.

Guinn, V.P., M.A. Purcell and W.W. Wadman.
The Measurement of Vanadium in Oceanographic Environmental Samples by Neutron Activation Analysis with Pre-irradiation Separation.
In: Measurement, Detection and Control of Environmental Pollutants. Proceedings of a Symposium held in Vienna, 15-19 March 1976 (IAEA, Vienna, 1976) 407-412.

Guzzi, G. and J. Cuypers.
GRETEL, A Computer Program for Gamma Ray Spectrometry with Ge(Li) detectors.
Eur 5117e (1974) 74 pp.

Guzzi, G., R. Pietra and E. Sabbioni.
Determination of 25 elements in biological standard reference materials by neutron activation analysis.
J. Radioanal. Chem. **34** (1976) 35-57.

Gysel, H.
Unbewusste individuelle Schätzungs-Anomalien beim Wägen und ihre Auswirkungen auf die Genauigkeit von Mikro-analysen.
Mikrochim. Acta No. 3 (1953) 266-282.

Haas, W.E., R. Hofmann and J.A. Martin.
Ortsabhängigkeit des Ansprechvermögens bei Ge-Li-detektoren.
Radiochem. Radioanal. Lett. **19** (1974) 207-216.

Hagenfeldt, K., L.O. Plantin and E. Diczfalusy.
Trace Elements in the Human Endometrium 2. Zinc, copper and manganese levels in the endometrium, cervical mucus and plasma.
Acta Endocrinol. **72** (1973) 115-126.

Hahn, H.K.J., R.V. Williams, R.E. Burch, J.F. Sullivan and E.A. Novak.
Determination of serum selenium by means of solvent extraction combined with activation analysis.
J. Lab. Clin. Med. **80** (1972) 718-722.

Hambidge, K.M. and W. Droegenmueller.
Changes in Plasma and Hair Concentrations of Zinc, Copper, Chromium, and Manganese During Pregnancy.
Obstet. Gynecol. **44** (1974) 666-672.

Hamilton, E.I. and M.J. Minski.
Spark source mass spectrometric sensitivity factors for elements in different matrices.
Int. J. Mass Spectrom. Ion Phys. **10** (1972) 77-84.

Hamilton, E.I and M.J. Minski.
Abundance of the chemical elements in man's diet and possible relations with environmental factors.
Sci. Total Environ. **1** (1973) 375-394.

Hansen, P.G. *(Århus, Denmark).*
unpublished work, 1964.

Harrison, S.H., P.D. La Fleur and W. H. Zoller.
Evaluation of Lyophilization for the Preconcentration of Natural Water Samples prior to Neutron Activation Analysis.
Anal. Chem. **47** (1975) 1685-1688.

Haven, M.C., G.T. Haven and A.L. Dunn.
Simultaneous Determination of Calcium, Copper, Manganese, and Magnesium by Neutron Activation Analysis.
Anal. Chem. **38** (1966) 141-143.

Hegde, B. G.C. Griffith and E.M. Butt.
Tissue and Serum Manganese Levels in Evaluation of Heart Muscle Damage.
Proc. Soc. Exp. Biol. Med. **107** (1961) 734-737.

Heinonen, J. and O.Suschny.
The Reliability of Neutron Activation Analysis for Trace Elements in Biological Materials.
In: Nuclear Activation Techniques in the Life Sciences. Proceedings of a Symposium held in Bled, Yugoslavia, 10-14 April 1972 (IAEA, Vienna, 1972) 155-173.

Hertogen, J., J. De Donder and R. Gijbels.
Experimental Data on Photopeak Integration Methods in Activation Analysis.
Nucl. Instrum. Methods **115** (1974) 197-212.

Hevesy, G. and Hilde Levi.
The Action of Neutrons on the Rare Earth Elements.
K. Dan. Vidensk. Selsk., Mat.-Fys. Medd. XIV,5 (1936) 3-34.

Heydorn, K., J. Lippert and P. Theodorsson.
The Radioactivity in the Risø District. Measurements up to 1st April, 1957.
AERE Lib/Trans 790 (1959) 132 pp.

Heydorn, K.
Production of Radioisotopes in Denmark.
Ingeniøren, Int. Ed. **4** (1960) 78-87.

Heydorn, K.
Experiences with the Irradiation of Ammonium Bromide.
In: Production and Use of Short-lived Radioisotopes from Reactors. Proceedings of a Symposium held in Vienna, 5-9 November 1962. Vol. 1 (IAEA, Vienna, 1963) 123-139.

Heydorn, K.
Geometrical Effects on the Response of the National Physical Laboratory Ionization Chamber Type 1383 A.
Int. J. Appl. Radiat. Isot. **18** (1967) 479-483.

Heydorn, K.
Use of the National Physical Laboratory Ionization Chamber Type 1383A in Neutron Activation Analysis.
J. Radioanal. Chem. **10** (1972) 245-256.

Heydorn, K. and K. Nørgård.
Analysis of Precision of Activation Analysis Method.
J. Radioanal. Chem. **15** (1973) 683-693.

Heydorn, K. and M.E. Wacks.
Increased Sensitivity in the Non-Destructive Determination of Lead via Pb-207m.

Paper presented at the 3rd Symposium on Recent Developments in Neutron Activation Analysis, Cambridge, 2-4 July 1973.

Heydorn, K.
Præcision og Nøjagtighed i Neutron-Aktiveringsanalyse.
Paper presented at the 4de Nordiske Sporelement- og Mikrokjemi-Konferanse Sandefjord, 21-23 November 1973.

Heydorn, K. and R.M. Parr.
Quality Control Procedures with Real Samples and Actual Results.
In: Trace Elements in Relation to Cardiovascular Diseases.
IAEA-157 (1973) 186-188.

Heydorn, K., B. Kruse and V.P. Guinn.
Losses of Mercury from Irradiated and Unirradiated Polyethylene Vials.
Paper presented at the 4th Symposium on Recent Developments in Neutron Activation Analysis, Cambridge, 4-7 August 1975.

Heydorn, K., E. Damsgaard, N. Horn, M. Mikkelsen, I. Tygstrup, S. Westermark and J. Weber.
Extra-Hepatic Storage of Copper. A Male Foetus Suspected of Menkes' Disease.
Humangenetik **29** (1975) 171-175.

Heydorn, K., E. Damsgaard, N. Horn, M. Mikkelsen and I. Tygstrup.
Trace Element Metabolism in Children with Menkes' Syndrome.
Risø-M-1852 (1976) 19 pp.

Heydorn, K.
Cross-Section of the Reaction Br-81(n,p) Se-81m for Reactor Fast Neutrons.
Radiochim. Acta **23** (1976) 170-171

Heydorn, K., P.Z. Skanborg, R. Gwozdz, J.O. Schmidt and M.E. Wacks.
Determination of Lithium by Instrumental Neutron Activation Analysis.
J. Radioanal. Chem. **37** (1977) 155-168.

Heydorn, K.
Quality Assurance of Computer Programs for Photopeak Integration in Activation Analysis.
Trans. Am. Nucl. Soc. **28** (1978) Suppl. 1, 7-8

Heydorn, K. and B. Wanscher.
Application of Statistical Methods to Activation Analytical Results Near the Limit of Detection.
Fresenius' Z. Anal. Chem. **292** (1978) 34-38.

Heydorn, K., E. Damsgaard, N.A. Larsen and B. Nielsen.
Sources of Variability of Trace Element Concentrations in Human Serum.
In: Nuclear Activation Techniques in the Life Sciences 1978 (IAEA, Vienna, 1979) 129-142.

Hinton, C.L.
Food Additive Control in the United Kingdom.
(FAO, Rome, 1960) 52 pp.

Hirsch, Roland F.
Analysis of Variance in Analytical Chemistry.
Anal. Chem. **49** (1977) 691A-700A.

Hislop, J.S.
Some application at Harwell of nondestructive gamma photon activation analysis. AERE-R 7646 (1974) 17 pp.

Hoede, D., R. Massée and H.A. Das.
The Determination of Selenium in Dry Organic Material by Thermal Neutron Activation and Extraction.
Radiochem. Radioanal. Lett. **23** (1975) 379-384.

Hoffman, G.L., P.R. Walsh and M.P. Doyle.
Determination of a Geometry and Dead Time Correction Factor for Neutron Activation Analysis.
Anal. Chem. **46** (1974) 492-496.

Hofstader, R.A., O.I. Milner and J. H. Runnels.
Analysis of Petroleum for Trace Metals.
In: Advances in Chemistry Series 156. Edited by Robert F. Gould (American Chemical Society, Washington, 1976) 189 pp.

Holden, N.E. and F.W. Walker.
Chart of the Nuclides. Eleventh Edition. (General Electric, Schenectady, 1973)

Hoste, J., J. Op de Beeck, R. Gijbels, F. Adams, P. Van den Winkel and D. De Soete.
Instrumental and Radiochemical Activation Analysis. (Butterworths, London, 1971) 148 pp.

How, S.W. and S. Yeh.
Studies of Endemic Chronic Arsenical Poisoning 2. Histopathology of Arsenical Cancer with Special Reference to Bowen's disease.
National Taiwan University, Institute of Pathology Report No. 14 (1963) 75-105.

Huysmans, K., R. Gijbels and J. Hoste.
Experimental Comparison of Multi-Channel Analyzer Dead-Time Corrections for Short-Lived Radionuclides.
J. Radioanal. Chem. **20** (1974) 51-69

International Agency for Research on Cancer *(Lyon).*
Arsenic and Inorganic Arsenic Compounds.
In: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Vol. 2: Some Inorganic and Organometallic Compounds (WHO, Geneva, 1973) 48-69.

International Commission on Radiological Protection.
Report of the Task Group on Reference Man.
ICRP Publication No. 23 (Pergamon Press, Oxford, 1975) 480 pp.

Isenhour, T.L. and G.H. Morrison.
A Computer Program to Optimize Times of Irradiation and Decay in Activation Analysis.
Anal. Chem. **36** (1964) 1089-1092.

Ivanov, I.N., V.V. Filippov and A.S. Shtan'.
Calculation of Optimum Times in Activation Analysis.
In: Radiation Technology, no. 3. Proceedings of the Institute of Radiation Technology. Edited by A.S. Shtan'. (Israel Program for Scientific Translations, Jerusalem, 1971) (AEC-tr-7138) 84-88.

Ivanov, I.N.
Application of Computers for the Selection of the Optimum Method and for Automated Data-Processing in Instrumental Activation Analysis.
J. Radioanal. Chem. **15** (1973) 451-465.

Iwashima, K., M. Fujita and N. Yamagata.
Determination of Nanogram Amounts of Arsenic in Aerosols by Neutron Activation.
Radioisotopes (Tokyo) **25** (1976) 443-447.

Iwataki, N. and K. Horiuchi.
Concerning the Upper Limits of Arsenic in Blood, Urine and Feces among Normal Japanese Population.
Osaka City Med. J. **5** (1959) 209-217.

Jacobi, S., K. Letz and G. Schmitz.
DERAN: Ein Rechenprogramm zur Identifizierung radioaktiver Nuklide in gemessenen Gammaspektren.
KFK 2447 (1977) 84 pp.

Jacobson, A., S. Brar, T. Fields, I.G. Fels, E. Kaplan, P. Gustafson, Y.T. Oester.
Practical Application of Neutron Activation Analysis for the Determination of Protein Bound Metals in Blood Serum.
J. Nucl. Med. **2** (1961) 289-296.

Johansen, O. and E. Steinnes.
Determination of cadmium and zinc in kidney and liver tissue by neutron activation analysis.
In: Trace elements in relation to cardiovascular diseases. IAEA-157 (IAEA, Vienna, 1973) 51-56.

Junod, E.
Correction of Counting Losses in Gamma-Spectrometry of Short-lived Radionuclides.
J. Radioanal. Chem **20** (1974) 113-134.

Kaiser, H.
Zur Definition von Selektivität, Spezifität, und Empfindlichkeit von Analysenverfahren.
Fresenius' Z. Anal. Chem. **260** (1972) 252-260.

Kamemoto, Y. and S. Yamagishi.
Re-activation Analysis of Magnesium.
Nature **201** (1964) 65-66.

Kanabrocki, E.L., T. Fields, C.F. Decker, L.F. Case, E.B. Miller, E. Kaplan and Y.T. Oester.
Neutron Activation Studies of Biological Fluids: Manganese and Copper.
Int. J. Appl. Radiat. Isot. **15** (1964) 175-190.

Kanabrocki, E.L , L.F. Case, L. Graham, T. Fields, E.B. Miller, Y.T. Oester and E. Kaplan.
Non-Dialyzable Manganese and Copper Levels in Serum of Patients with Various Diseases.
J. Nucl. Med. **8** (1967) 166-172.

Karin, R.W., J.A. Buono and J.L. Fasching.
Removal of Trace Elemental Impurities from Polyethylene by Nitric Acid.
Anal. Chem. **47** (1975) 2296-2299.

Kasperek, K., H. Schicha, V. Siller, L.E. Feinendegen and A. Höck.
Trace-Element Concentrations in Human Serum; Diagnostic Implications.

In: Nuclear Activation Techniques in the Life Sciences. Proceedings of a Symposium held in Bled, Yugoslavia, 10-14 April 1972 (IAEA, Vienna, 1972) 517-526.

Kato, T., N. Sato and N. Suzuki.
Multielement Photon Activation Analysis of Biological Materials.
Anal. Chim. Acta **81** (1976) 337-347.

Kehoe, R.A., J. Cholak and R.V. Story.
A Spectrochemical Study of the Normal Ranges of Concentrations of Certain Trace Metals in Biological Materials.
J. Nutr. **19** (1940) 579-592.

Kendall, M.G. and A. Stuart.
The Advanced Theory of Statistics. Distribution Theory. Vol. 1, 3rd ed. (Charles Griffin, London, 1969) 439 pp.

Kendall, M.G. and A. Stuart.
The Advanced Theory of Statistics. Inference and Relationship. Vol. 2, 3rd ed. (Charles Griffin, London, 1973) 723 pp.

Kimura, I., K. Kobayashi and T. Shibata.
Measurements of Average Cross Sections for some Threshold Reactions for Neutrons with Fission-type Reactor Spectrum.
J. Nucl. Sci. Technol. **8** (1971) 59-69.

Kirzhner, M.A., N.A. Kryzhenkova and A.A. Kist.
Potentialities of preliminary concentration for the analysis of matrices into aluminum and vanadium by the neutron activation method.
Sov. At. Energy **37** (1974) 1277-1278
Atomnaya Energiya **37** (1974) 498.

Kishi, H., K. Oikawa and Y. Hashimoto.
A simple method of designing irradiation-, cooling-, and counting time in multi-element neutron activation analysis.
Bunseki Kagaku **25** (1976) 519-523.

Koch, O.G. and G.A. Koch-Dedic.
Handbuch der Spurenanalyse. Vol. 1-2. 2te Auflage (Springer, Berlin, 1974) 1597 pp.

Kucera, E.T. and R.R. Heinrich.
Multielement Trace Analysis of Coals, Ashes and Related Materials from Coal-Treatment Facilities by Instrumental Neutron Activation Analysis.
J. Radioanal. Chem. **32** (1976) 137-150.

LaFleur, P.D.
Standard Reference Materials for the Determination of Trace Elements in Environmental Samples.
In: Comparative Studies of Food and Environmental Contamination. Proceedings of a Symposium held at Otaniemi, Finland, 27-31 Aug. 1973 (IAEA, Vienna, 1974) 489-496.

Larsen, Elfinn *(Risø, Denmark).*
personal communication, 1975

Larsen, N.A., B. Nielsen, H. Pakkenberg, P. Christoffersen, E. Damsgaard and K. Heydorn.

Neutron Activation Analysis of Arsenic, Manganese and Selenium Concentrations in Organs of Uraemic and Normal Persons.
In: Nuclear Activation Techniques in the Life Sciences. Proceedings of a Symposium held in Bled, Yugoslavia, 10-14 April 1972 (IAEA, Vienna, 1972) 561-568.

Larsen, N.A., H. Pakkenberg, E. Damsgaard and K. Heydorn.
Topographical Distribution of Arsenic, Manganese, and Selenium in the Normal Human Brain.
J. Neurol. Sci. **42** (1979) 407-416.

Lauwerys, R., J.-P. Buchet, H. Roels, A. Berlin and J. Smeets.
Intercomparison Program of Lead, Mercury, and Cadmium Analysis in Blood, Urine, and Aqueous Solutions.
Clin. Chem. **21** (1975) 551-557.

Levine, R.J. and R.E. Olson.
Blood Selenium in Thai Children with Protein-Calorie Malnutrition.
Proc. Soc. Exp. Biol. Med. **134** (1970) 1030-1034.

Levstek, M., L. Kosta, M. Dermelj and A.R. Byrne.
Vanadium Determination in Biological Materials by the Use of Preconcentration.
In: Nuclear Activation Techniques in the Life Sciences. Proceedings of a Symposium held in Bled, Yugoslavia, 10-14 April 1972. (IAEA, Vienna, 1972) 111-117.

Liebscher, K. and Hamilton Smith.
Essential and Nonessential Trace Elements. A method of determining whether an element is essential or nonessential in human tissue.
Arch. Environ. Health **17** (1968) 881-890.

Linnig, F.J., J. Mandel and J.M. Peterson.
A Plan for Studying the Accuracy and Precision of an Analytical Procedure.
Anal. Chem. **26** (1954) 1102-1110.

Lowe, D. Armstrong.
A Guide to International Recommendations on Names and Symbols for Quantities and on Units of Measurement.
Progress in Standardization, No. 2 (World Health Organization, Geneva, 1975) 314 pp.

Lukens, H.R., L.A. Weinrich and V.P. Guinn.
Activation Analysis of Blood Serum.
General Atomic Report GA-6658 (1965) 52 pp.

Lukens, H.R., F.M. Graber and D.M. Settle.
Halogen Determination Errors Due to Radiolysis and Recoil in Neutron Activation Analysis.
Trans. Am. Nucl. Soc. **9** No.1 (1966) 88.

Mahoney, J.P., K. Sargent, M. Greland and W. Small.
Studies on Manganese I:
Determination in Serum by Atomic Absorption Spectrophotometry.
Clin. Chem. **15** (1969) 312-322.

Maletskos, C.J., M.D. Albertson, J.C. Fitzsimmons, M.R. Masurekar and C.W. Tang.
Sampling and Sample Handling of Human Tissue for Activation Analysis.

In: Trace Substances in Environmental Health - IV. Proceedings of the 4th Annual Conference held at Columbia, 23-25 June 1970. Edited by D.D. Hemphill. (University of Missouri, Columbia, Mo, 1971)367-377.

Malikova, T.A.
Influence of Hydrotherapy on the Concentration of Some Trace Elements in Serum and Blood from Patients with Residual Symptoms of Contagious Hepatitis.
Vopr. Kurortol., Fizioter. Lech. Fiz. Kult. **35** (1970) 212-217.

Malissa, H. and J. Rendl.
Information Theory in Analytical Chemistry. Basic Terms. Definitions and Interpretations. Edited by I.L. Marr.
Talanta **22** (1975) 597-600.

Malissa, H.
Problems of Standard Qualities and Standards in Environmental Analysis.
Fresenius' Z. Anal. Chem. **282** (1976) 407-416.

Mandel, J. and R.C. Paule.
Interlaboratory Evaluation of a Material with Unequal Numbers of Replicates.
Anal. Chem. **42** (1970) 1194-1197.

Mandel, J.
The Analysis of Interlaboratory Test Data.
ASTM Stand. News **5** No.3 (1977) 17-20.

Marinov, V.M., M.S. Lazarova, M.I. Mihajlov and D. Apostolov.
On the possibility of multiple utilization of Bowen's Kale for neutron activation analysis of biological materials.
Radiochem. Radioanal. Lett. **31** (1977) 191-206.

Maxia, V., S. Meloni, M.A. Rollier, A. Brandone, V.N. Patwardhan, C.I. Waslien and S.E. Shami.
Selenium and Chromium Assay in Egyptian Foods and in Blood of Egyptian Children by Activation Analysis.
In: Nuclear Activation Techniques in the Life Sciences. Proceedings of a Symposium held in Bled, 10-14 April 1972 (IAEA,Vienna, 1972) 527-550.

Maziere, B., A. Gaudry, W. Stanilewicz and D. Comar.
Possibilites et limites de l'analyse par activation neutronique multielementaire d'echantillons biologiques avec ou sans separation de la matrice activable.
J. Radioanal. Chem. **16** (1973) 281-296.

Maziere, B., A. Gaudry, J. Gros and D. Comar.
Biological Sample Contamination Due to Quartz Container in Neutron Activation Analysis.
Radiochem. Radioanal. Lett. **28** (1977) 155-164.

McConnel, K.P., W.L. Broghamer,Jr., A.J. Blotcky and O.J. Hurt.
Selenium Levels in Human Blood and Tissues in Health and in Disease.
J. Nutr. **105** (1975) 1026-1031.

McFarren, E.A., R.J. Lishka and J.H. Parker.
Criterion for Judging Acceptability of Analytical Methods.
Anal. Chem. **42** (1970) 358-365.

Meinke, W. Wayne.
Sensitivity Charts for Neutron Activation Analysis.
Anal. Chem. **31** (1959) 792-795.

Meinke, W.W. and B.F. Scribner *(editors).*
Trace Characterization. Chemical and Physical. National Bureau of Standards, Monograph 100. (USGPC, Washington D.C. 1967) 580 pp.

Meinke, W.W.
The ultimate contribution of nuclear activation to analysis.
J. Radioanal. Chem. **15** (1973) 419-433.

Meloni, S., V. Caramella-Crespi, M.T. Ganzerli-Valentini and P. Borroni.
Accurate Determination of Arsenic and Mercury in Reference Materials by Destructive Neutron Activation Analysis.
Radiochem. Radioanal. Lett. **25** (1976) 117-128.

Merten, D. *(I.A.E.A., Vienna).*
personal communication, 1971

Merten, D., L.A. Currie, J. Mandel, O. Suschny and G. Wernimont.
Intercomparison, Quality Control and Statistics.
In: Standard Reference Materials and Meaningful Measurements (USGPO, Washington, D.C., 1975) (National Bureau of Standards Special Publication 408) 804-811.

Mertz, D.P., R. Koschnick, G. Wilk and K. Pfeilsticker.
Untersuchungen über den Stoffwechsel von Spurenelementen beim Menschen. I. Serumwerte von Co, Ni, Ag, Cd, Cr, Mo, Mn.
Z. Klin. Chem. Klin. Biochem. **6** (1968) 171-174.

Midgley, D.
Criterion for Judging the Acceptability of Analytical Methods.
Anal. Chem. **49** (1977) 510-512

Miller, D.O. and J.H. Yoe.
Spectrophotometric Determination of Manganese in Human Plasma and Red Cells with Benzohydroxamic Acid.
Anal. Chim. Acta **26** (1962) 224-229.

Miller, G.E. and L. Sangermano.
Geometry Effects in Small Samples Analyzed by NAA.
Trans. Am. Nucl. Soc. **21** Suppl.3 (1975) 27 28.

Milner, O.I., J. R. Glass, J.P. Kirchner and A.N. Yurick.
Determination of Trace Metals in Crudes and Other Petroleum Oils. Analysis for Iron, Nickel, Vanadium, and Copper.
Anal. Chem, **24** (1952) 1728-1732.

Mitchell, J.W.
Ultrapurity in Trace Analysis.
Anal. Chem. **45** (1973) 492A-500A.

Mizuike, A.
Separations and Preconcentrations.
In: Trace Analysis Physical Methods. Edited by G.H. Morrison (Interscience, New York, 1965) 103-159.

Moldenhawer, F. and B. Uhlig.
Aktivitätsverluste beim Umgang mit wässrigen J-131-Lösungen.
Isotopenpraxis **2** (1966) 333-338.

Molokhia, M.M. and B. Portnoy.
Neutron Activation Analysis of Trace Elements in Skin. VI. Manganese in Psoriasis, Vitiligo and Other Dermatoses.
Br. J. Dermatol. **89** (1973) 273-278.

Morrison, George H. *(editor).*
Trace Analysis Physical Methods (Interscience New York, 1965) 582 pp.

Morrison, G.H. and N.M. Potter.
Multielement Neutron Activation Analysis of Biological Material Using Chemical Group Separations and High Resolution Gamma Spectrometry.
Anal. Chem. **44** (1972) 839-842.

Morss, S.G., H.R. Ralston and H.S. Olcott.
Selenium Determination in Human Serum Lipoprotein Fractions by Neutron Activation Analysis.
Anal. Biochem. **49** (1972) 598-601.

Mosulishvili, L.M., M.A. Kolomi'tsev, V.Yu. Dundua, N.I. Shonia and O.A. Danilova.
Multielement Standards for Instrumental Neutron Activation Analysis of Biological Materials.
J. Radioanal. Chem. **26** (1975) 175-188.

Muzzarelli, R.A.A. and R. Rocchetti.
Atomic-Absorption Determination of Manganese, Cobalt and Copper in Whole Blood and Serum, with a Graphite Atomizer.
Talanta **22** (1975) 683-685.

Nadkarni, R.A. and W.D. Ehmann.
Determination of Trace Elements in the Reference Cigarette Tobacco by Neutron Activation Analysis.
Radiochem. Radioanal. Lett. **2** (1969) 161-168.

Nadkarni, R.A. and W.D. Ehmann.
Determination of Trace Elements in Biological Standard Kale by Neutron Activation Analysis.
J. Radioanal. Chem. **3** (1969) 175-185.

Nadkarni, R.A. and W.D. Ehmann.
Trace Element Determination in Biological Materials by Neutron Activation Analysis.
In: Trace Substances in Environmental Health - IV. Proceedings of a Symposium held at Columbia, 23-25 June 1970. Edited by D.D. Hemphill. (University of Missouri, Columbia,1971) 407-419.

Nadkarni, R.A. and G.H. Morrison.
Multielement Instrumental Neutron Activation Analysis of Biological Materials.
Anal. Chem. **45** (1973) 1957-1960

Nadkarni, R.A.
Re-evaluation of Arsenic Concentration in the Biological Standards.
Radiochem. Radioanal. Lett. **19** (1974) 127-134.

National Bureau of Standards.
Catalog of NBS Standard Reference Materials 1975-76 edition (USGPO, Washington, D.C., 1975) (National Bureau of Standards Special Publication, 260) 87 pp.

National Bureau of Standards.
Catalog of NBS Standard Reference Materials, Supplement 1977 (USGPO, Washington, D.C., 1977) (National Bureau of Standards Special Publication, 260) 32 pp.

Niedermeier, W., J. H. Griggs and R.S. Johnson.
Emission Spectrometric Determination of Trace Elements in Biological Fluids.
Appl. Spectrosc. **25** (1971) 53-56.

Nolkrantz, L. *(Hørsholm, Denmark).*
unpublished work, 1974.

Norton, Elinor F.
Chemical yield determinations in radiochemistry.
NAS-NS 3111 (1967) 95 pp.

Okada, M.
Optimum "Cooling Time" to Minimize Interfering Activity in Non-Destructive Activation Analysis.
Anal. Chim. Acta **24** (1961) 410-412.

O'Kelley, G.D. *(editor).*
Applications of Computers to Nuclear and Radiochemistry
Proceedings of a Symposium, Gatlinburg, Tenn., 17-19 October 1962.
NAS-NS 3107 (1962) 313 pp.

Olehy, D.A., R.A. Schmitt and W.F. Bethard.
Neutron Activation Analysis of Magnesium, Calcium, Strontium, Barium, Manganese, Cobalt, Copper, Zinc, Sodium, and Potassium in Human Erythrocytes and Plasma.
J. Nucl. Med. **7** (1966) 917-927.

Orvini, E., T.E. Gills and P.D. LaFleur.
Method for Determination of Selenium, Arsenic, Zinc, Cadmium, and Mercury in Environmental Matrices by Neutron Activation Analysis.
Anal. Chem. **46** (1974) 1294-1297.

Otoshi, T., T. Shiomi, K. Tomura and Y. Hashimoto.
Storage and simple preparation of the standard solutions for multi-element activation analysis.
Bunseki Kagaku **25** (1976) 620-625.

Panteliadis, C.
Beitrag zur Analyse von Spurenelementen bei Neugeborenen.
In: Spurenelemente in der Entwicklung von Mensch und Tier, vernachlässigte Elemente in der Säuglingsernährung, Symposium, Rottach-Egern, Germany 1974. Edited by K. Betke and F. Bidlingmaier (Urban und Schwarzenberg, München, 1975) 103-112.

Papavasiliou, P.S. and G.C. Cotzias.
Neutron Activation Analysis: The Determination of Manganese.
J. Biol. Chem. **236** (1961) 2365-2369.

Pařizek, J. and J. Beneš.
Methylated Radioselenium Compounds: their Synthesis, Metabolism and Practical Use.
In: Radiopharmaceuticals and Labelled Compounds. Proceedings of a symposium held in Copenhagen 26-30 March 1973. Vol. 2 (IAEA, Vienna, 1973) 141-144.

Parr, R.M.
On the need for radiochemical separations in multi-element neutron activation analysis of human tissues.
In: Trace Elements in Relation to Cardiovascular Diseases. IAEA-157 (1973) 143-148.

Payne, R.B.
The application of Standard Deviation calculated from successive Duplicates to Quality Control Data.
Clin. Chim. Acta **42** (1972) 263-266
ibid. **44** (1973) 276.

Peoples, S.A.
The Mechanisms of Action of Arsenicals in Feed on Performance and Health of Animals.
In: The Use of Drugs in Animal Feeds. (National Academy of Sciences, Publication 1679, Washington, 1969) 77-86.

Perkons, A.K. and R.E. Jervis.
Hair Individualization Studies.
In: Modern Trends in Activation Analysis. Proceedings of an International Conference at College Station, Texas, April 19-22, 1965. Edited by J.P. Guinn (Activation Analysis Research Laboratory, Texas A and M University, College Station, 1965) 295-303.

Petrenko, A.N. and B. Ya. Narkevich.
Selection of an Optimum Analytic Procedure During Instrument Activation Analysis.
Sov. At. Energy **35** (1973) 743-744.
Atomnaya Energiya **35** (1973) 121-125.

Pfrepper, G., C. Raitschev and M. Michailow.
Zur Bestimmung des Wirkungsquerschnittes der Reaktion Br-81 (n,p) Se-81m für Spaltneutronen.
Radiochim. Acta **23** (1976) 55-56.

Philippot, J.Cl.
Automatic Processing of Diode Spectrometry Results.
IEEE Trans. **NS-17** No. 3 (1970) 446-448.
CEA-TP-9730 (1970) 76 pp.

Pierce, J.O., A. Abu-Samra, D. Fehlauer, T. Clevenger and J. Vogt.
Elemental abundances in toenails, sugar and rice by neutron activation analysis and atomic absorption.
In: Trace elements in relation to cardiovascular diseases. IAEA-157 (1973) 103-112

Piotrowicz, S.R., J.L. Fasching, D.D. Zdankiewicz and R.W. Karin.
Geometry Factors and Flux Corrections in Neutron Activation Analysis.
Anal. Chem. **47** (1975) 2326-2328.

Plesch, R.
Die Überprüfung poissonverteilter Messwerte.
Fresenius' Z. Anal. Chem. **265** (1973) 114-121.

Pollack, L.R.
Recommended Delay in Counting after Neutron Activation.
Trans. Am. Nucl. Soc. **8** (1965) 325-326.

Pshetakovsky, I.L.
On diagnostic criteria of activity of Rheumatoid Arthritis. (In Russian).
Vrach. Delo **7** (1975) 93-96.

Quittner, P. and A. Montvai.
Determination of Optimum Schedule and Sensitivity for Non-Destructive Activation Analysis.
Acta Chim. Acad. Sci. Hung. **51** (1967) 371-380.

Quittner, P.
Gamma-Ray Spectroscopy with particular reference to detector and computer evaluation techniques. (Akademiai Kiado, Budapest, 1972) 111p.

Rancitelli, L.A. and T.M. Tanner.
Multielement Characterization of Atmospheric Pollutants by X-ray Fluorescence Analysis and Instrumental Neutron Activation Analysis. BNWL-SA-5610 (1976) 35 pp.

Ravnik, V., M. Dermelj and L. Kosta.
A Highly Selective Diethyldithiocarbamate Extraction System in Activation Analysis of Copper, Indium, Manganese and Zinc. Application to the Analysis of Standard Reference Materials.
J. Radioanal. Chem. **20** (1974) 443-453.

Reber, J.D. and J.K. Major.
Optimum Channel Width for Single Channel Analysis.
Nucl. Instrum. Methods **23** (1963) 162-164.

Reuter, F.W.
Numerical Matrix Correction Technique for the Measurement of Trace Elements in Plant Materials by X-ray Fluorescence Spectrometry.
Anal. Chem. **47** (1975) 1763-1766.

Rhead, W.J., E.E. Cary, W.H. Allaway, S.L. Saltzstein and G.N. Schrauzer.
The Vitamin E and Selenium Status of Infants and the Sudden Infant Death Syndrome.
Bioinorg. Chem. **1** (1972) 289-294.

Ricci, E.
Methodology for High-Flux Absolute Multielement Neutron Activation Analysis - Environmental Baselines by Analysis of Tree Rings.
Anal. Chim. Acta **79** (1975) 109-124.

Rietz, B. *(Roskilde, Denmark).*
unpublished work, 1972.

Robertson, D.E.
Contamination Problems in Trace-Element Analysis and Ultrapurification.
In: Ultrapurity Methods and Techniques. Edited by M. Zief and R. Speights. (Dekker, New York, 1972) 207-253.

Robertson, D.E. and R. Carpenter.
Neutron Activation Techniques for the Measurement of Trace Metals in Environmental Samples. NAS-NS-3114 (1974) 80 pp.

Rogers, V.C. and G.M. Sandquist.
Reduced Statistical Uncertainties in Counting Short-Lived Radioactive Isotopes.
Trans. Am. Nucl. Soc. **15** (1972) 154-155.

Roscoe, B.A. and A.K. Furr.
Algorithm for Computer Processing and Analysis of Gamma Ray Spectra for Large-Scale Laboratory Operations.
Nucl. Instrum. Methods **137** (1976) 173-178.

Rosenberg, R.J.
Evaluation of Error in the Instrumental Activation Analysis of Rocks.
In: Accuracy in Trace Analysis. Proceedings of the 7th IMR Symposium, held at Gaithersburg, Md., 7-11 October 1974 (USGPO, Washington, D.C., 1976) (National Bureau of Standards Special Publication 422) 1241-1256.

Ross, R.T. and J.G. Gonzalez.
The Direct Determination of Trace Quantities of Manganese in Blood and Serum Samples Using Selective Volatilization and Graphite Tube Reservoir Atomic Absorption Spectrophotometry.
Bull. Environ. Contam. Toxicol. **12** (1974) 470-474.

Rothman, L.D., S.R. Crouch and J.D. Ingle, Jr.
Theoretical and Experimental Investigation of Factors Affecting Precision in Molecular Absorption Spectrophotometry.
Anal. Chem. **47** (1975) 1226-1233.

Routti, J.T. and S.G. Prussin.
Photopeak Method for the Computer Analysis of Gamma-Ray Spectra from Semiconductor Detectors.
Nucl. Instrum. Methods **72** (1969) 125-142.

Ruf, H. and M. Friedrich.
Neutronen aktivierungsanalytische Bestimmung kleiner Np-237-Gehalte in Uran, Plutonium und Spaltprodukte enthaltenden Lösungsproben. KFK-2308 (1976) 15 pp.

Salbu, B., E. Steinnes and A.C. Pappas.
Multielement Neutron Activation Analysis of Fresh Water Using Ge(Li) Gamma Spectrometry.
Anal. Chem. **47** (1975) 1011-1016.

Samsahl, K., P.O. Wester and O. Landström.
An Automatic Group Separation System for the Simultaneous Determination of a Great Number of Elements in Biological Material. Recovery and Reproducibility Studies.
Anal. Chem. **40** (1968) 181-187.

Sasamoto, N., K. Koyama and S.I. Tanaka.
An Analysis Method of Gamma-Ray Pulse-Height Distributions Obtained with a Ge(Li) Detector.
Nucl. Instrum. Methods **125** (1975) 507-523.

Savitzky, A. and M.J.E. Golay.
Smoothing and Differentiation of Data by Simplified Least Squares Procedures.
Anal. Chem. **36** (1964) 1627-1639.

Scheving, L.E., J.E. Pauly, E.L. Kanabrocki and E. Kaplan.
A 24-hour Rhythm in Serum Copper and Manganese Levels of Normal and Adrenal Medullectomized Adult Male Rats.
Texas Rep. Biol. Med. **26** (1968) 341-347.

Schicha, H., L.E. Feinendegen, K. Kasperek, H. . Klein and V. Siller.
Non-Homogeneous but Parallel Distribution of Essential Trace Elements in Multiple Adjacent Samples of Single Livers, Measured by Neutron Activation Analysis.
Beitr. Pathol. **141** (1970) 227-232.

Schramel, P.
Determination of Light Metals in the International Biological Standard by Flameless Atomic-Absorption Spectrometry.
Anal. Chim. Acta **67** (1973) 69-77.

Schrauzer, G.N., W.J. Rhead and G.A. Evans.
Selenium and Cancer: Chemical Interpretation of a Plasma "Cancer Test".
Bioinorg. Chem. **2** (1973) 329-340.

Schrauzer, G.N.
Selenium and Cancer: A Review.
Bioinorg. Chem. **5** (1976) 275-281.

Schrauzer, G.N., D.A. White and C.J. Schneider.
Cancer Mortality Correlation Studies III and IV.
Bioinorg. Chem. **7** (1977) 23-34 and 35-56.

Schroeder, H.A., J.J. Balassa and I.H. Tipton.
Abnormal Trace Metals in Man: Vanadium.
J. Chronic Dis. **16** (1963) 1047-1071.

Schroeder, H.A. and J.J. Balassa.
Abnormal Trace Elements in Man: Arsenic.
J. Chronic Dis. **19** (1966) 85-106.

Schroeder, H.A., D.V. Frost and J.J. Balassa.
Essential Trace Metal in Man: Selenium.
J. Chronic Dis. **23** (1970) 227-243.

Schroeder, H.A. and A.P. Nason.
Trace Element Analysis in Clinical Chemistry.
Clin. Chem **17** (1971) 461-474.

Schüttelkopf, H. and H. Fessler.
Schnelle Radiojodabtrennung aus Abwasserproben und Bestimmung der I-129 Konzentration durch Neutronenaktivierung.
KFK-2024 (1974) 36 pp.

Schwarz, K. and D.B. Milne.
Growth Effects of Vanadium in the Rat.
Science **174** (1971) 426-428.

Scott, R.O. and A.M. Ure.
Some Sources of contamination in trace analysis.
Proc. Soc. Anal. Chem. **9** (1972) 288-293.

Seeling, W., F.W. Ahnefeld. W. Dick, R. Dölp, L. Fodor. E. Schmitz and R. Hohage.
Das Verhalten der Übergangselemente Cr, Mn, Co, Ni, Cu und Zn im Serum und Urin polytraumatisierter Intensivpatienten.
In: Spurenelemente in der Entwicklung von Mensch und Tier, vernachlässigte Elemente in der Säuglingsernährung. Symposium, Rottach-Egern, Germany 1974. Edited by K. Betke and F. Bidlingmaier (Urban und Schwarzenberg, München, 1975) 159-177.

Shields, W.R., T.J. Murphy, E.L. Garner and V.H. Dibeler.
Absolute Isotopic Abundance Ratio and the Atomic Weight of Chlorine.
J. Am. Chem. Soc. **84** (1962) 1519-1522.

Simesen, M. *(Copenhagen, Denmark).*
unpublished work, 1970

Simonits, A., F. De Corte and J. Hoste.
Zirconium as a Multi-isotopic Flux Ratio Monitor and a Single Comparator in Reactor-Neutron Activation Analysis.
J. Radioanal. Chem. **31** (1976) 467-486.

Smith, Hamilton.
Arsenic in Biological Tissue.
Its Estimation by Activation Analysis: Part 2.
J. Forensic Med. **9** (1962) 143-149.

Smith, G.W. and D.A. Becker.
Preparation of an NBS Biological Standard Reference Material for Trace Element Analysis.
In: Nuclear Activation Techniqees in the Life Sciences. Proceedings of a Symposium held in Amsterdam, 8-12 May 1967 (IAEA, Vienna, 1967) 197-207.

Speecke, A., J. Hoste and J. Versieck.
Sampling of Biological Materials.
In: Accuracy in Trace Analysis. Proceedings of the 7th IMR Symposium held at Gaithersburg, Md., 7-11 October 1974 (USGPO, Washington, D.C., 1976) (National Bureau of Standards Special Publication 422) 299-310.

Standard Reference Material 1571, Orchard Leaves.
Certificate of Analysis (National Bureau of Standards, Washington, D.C.) (Revised October 1, 1971) 3 pp.

Standard Reference Material 1571, Orchard Leaves.
Certificate of Analysis (National Bureau of Standards, Washington, D.C.) (Revised August 15, 1976) 5 pp.

Standard Reference Material 1571, Orchard Leaves.
Certificate of Analysis (National Bureau of Standards, Washington, D.C.) (Revised August 31, 1977) 4 pp.

Standard Reference Material 1577, Bovine Liver.
Certificate of Analysis (National Bureau of Standards, Washington, D.C., 1972) 3 pp.

Steenbuch, Fl.
Risø-rapporter. Vejledning i den formelle udformning.
Risø-M-764 (Revised 1974) 23 pp.

Steinnes, E.
Determination of Traces of Selenium in Biological Tissue by Neutron Activation.
Int. J. Appl. Radiat. Isot. **18** (1967) 731-734.

Steinnes, E., O.R. Birkelund and O. Johansen.
Determination of Trace Elements in Biological Materials by Neutron Activation and Extraction with TOPO (tri-octyl-phosphine oxide).
J. Radioanal. Chem. **9** (1971) 267-272.

Steinnes, E.
Some Neutron Activation Methods for the Determination of Minor and Trace Elements in Rocks. *Thesis (Institutt for Atomenergi, Kjeller, 1972)* 23 pp.

Steinnes, E.
Development of a group separation scheme for the determination of trace elements in biological tissue by neutron activation analysis.
In: Trace elements in relation to cardiovascular diseases. IAEA-157 (1973) 149-154.

Steinnes, E.
A two-group separation scheme for the determination of eleven trace elements in biological material by neutron activation analysis.
Anal. Chim. Acta **78** (1975) 307-315.

Sterlinski, S.
Analysis of Digital Data from a Multichannel Pulse Height Analyzer on Gamma Ray Total Absorption Peaks in Activation Analysis.
Anal. Chem. **40** (1968) 1995-1998.

Sterlinski, S.
General Formulas for Calculation of Savitzky and Golay's Filter Weights and Some Features of these Filters.
Nucl. Instrum. Methods **124** (1975) 285-287.

Sterlinski, S. and W. Hammer.
Simple Methods for Dead-Time and Pile-Up Corrections in Analytical Gamma-Ray Spectrometry. Advantages and Disadvantages.
J. Radioanal. Chem. **31** (1976) 235-257.

Stevenson, P.C.
Processing of Counting Data. NAS-NS 3109 (1966) 167 pp.

Strain, W.H., C.G. Rob, W.J. Pories, R.C. Childers, M.F. Thompson, Jr., J.A. Hennesen and F.M. Graber.
Element Imbalances of Atherosclerotic Aortas.
In: Modern Trends in Activation Analysis. Proceedings of the 1968 International Conference held at Gaithersburg, Md., 7-11 October 1968. Edited by J.R. DeVoe and P.D. LaFleur. Vol. 1 (USGPO, Washington, D.C., 1969) (National Bureau of Standards Special Publication 312) 128-133.

Stærkind, G.
Neutronfluxforhold i DR 2's termiske kolonne, november 1970.
Risø-M-1327 (1970) 14 pp.

Sutarno, R. and G.H. Faye.
A measure for assessing certified reference ores and related materials.
Talanta **22** (1975) 675-681.

Taczanowski, S.
On the Application of the Binomial Distribution Statistics for the Estimation of Precision of Activation Analysis.
Radiochem. Radioanal. Lett. **16** (1974) 65-72.

Taczanowski, S.
Integration of Small Photopeaks Lying on the High Background in Ge(Li) Spectrometry.
Nucl. Instrum. Methods **123** (1975) 409-410.

Thiers, R.E.
Contamination in Trace Element Analysis and its Control.
In: Methods of Biochemical Analysis. Edited by D. Glick. Vol. 5. (Interscience, New York, 1957) 273-335.

Thompson, M. and R.J. Howarth.
The Rapid Estimation and Control of Precision by Duplicate Determinations.
Analyst **98** (1973) 153-160.

Thompson, M. and R.J. Howarth.
Duplicate Analysis in Geochemical Practice I. Theoretical Approach and Estimation of Analytical Reproducibility.
Analyst **101** (1976) 690-698.

Tjell, J.C.
... resultat af interkalibrering.
Slam Nyt (Laboratoriet for Teknisk Hygiejne, D.t.H., Lyngby) Nr. 10 (1974) 11 pp.

Tobias, C.A. and R.W. Dunn.
Analysis of Microcomposition of Biological Tissue by Means of Induced Radioactivity.
Science **109** (1949) 109-113.

Tölg, G.
Spurenanalyse der Elemente: Zahlenlotto oder exakte Wissenschaft?
Naturwissenschaften **63** (1976) 99-110.

Tominaga, H., M. Dojyo and M. Tanaka.
Effect of Spectrum Smoothing on Peak Area Determinations.
Nucl. Instrum. Methods **98** (1972) 69-76.

Tomov, T., V. Gadzhokov and Kh. Popov.
Reduction of Experimental Error in Activation Analysis without Chemical Processing.
Dokl. Bolg. Akad. Nauk **19** (1966) 469-472.

Tsirlin, Yu. A., A. Ya. Gel'fman and E.P. Mokhir.
Some Problems in Establishing the Optimum Conditions for the Radioactivation Analysis of Binary Systems.
J. Anal. Chem. USSR **21** (1966) 901-903.

Tugsavul, A., D. Merten and O. Suschny.
The reliability of low-level radiochemical analysis. Results of Intercomparisons organized by the Agency during the period 1966-1969. (IAEA, Vienna, 30 March 1970) 52 pp.

Turkstra, J., M.C.J. Van Rensburg and W.J. De Wet.
An Investigation into the different Computer Techniques available for the Analysis of Gamma-Ray Spectra obtained with a Lithium-drifted Germanium Detector. PEL-204 (1970) 42 pp.

Tyurnin, G.A. and L.A. Smakhtin.
Optimum Time Programming in Instrumental Activation Analysis.
Zavodskaya Laboratoriya **37** (1971) 1228-1230.
Ind. Lab. (Engl. Transl.) **37** (1972) 1574-1577.

Vanderborght, G.
Guidelines for Preparation of Texts of Dissertations for their Presentation and Publication. (Unisist, Paris, 1976) 18 pp.

Van der Linden, R., F. De Corte and J. Hoste.
Activation Analysis of Biological Material with Ruthenium as a Multi-Isotopic Comparator.
Anal. Chim. Acta **71** (1974) 263-275.

Vaño, E., L. Gonzalez, R. Gaeta and J.A. Gonzalez.
An empirical function which relates the slope of the Ge(Li) efficiency curves and the active volume.
Nucl. Instrum. Methods **123** (1975) 573-574.

Varcoe, F.T.
Activation Analysis of Trace Elements in Serum.
Thesis (Ph.D.) University of Florida, 1974 (University Microfilms, Ann Arbor, Mich.) 203 pp.

Versieck, J.M.J. and A.B.H. Speecke.
Contaminations Induced by Collection of Liver Biopsies and Human Blood.
In: Nuclear Activation Techniques in the Life Sciences. Proceedings of a Symposium held in Bled, Yugoslavia, 10-14 April 1972 (IAEA, Vienna, 1972) 39-49.

Versieck, J., A. Speecke, J. Hoste and F. Barbier.
Determination of Manganese, Copper and Zinc in Serum and Packed Blood Cells by Neutron Activation Analysis. Studies on the Metabolism of Trace Elements.
Z. Klin. Chem. Klin. Biochem. **11** (1973) 193-196.

Versieck, J., F. Barbier, A. Speecke and J. Hoste.
Manganese, Copper, and Zinc Concentrations in Serum and Packed Blood Cells during Acute Hepatitis, Chronic Hepatitis, and Posthepatitic Cirrhosis.
Clin. Chem. **20** (1974) 1141-1145.

Versieck, J., F. Barbier, A. Speecke and J. Hoste.
Influence of Myocardial Infarction on Serum Manganese, Copper, and Zinc Concentrations.
Clin. Chem. **21** (1975) 578-581.

Versieck, J., J. Hoste, F. Barbier, H. Michels and J. De Rudder.
Simultaneous Determination of Iron, Zinc, Selenium, Rubidium, and Cesium in Serum and Packed Blood Cells by Neutron Activation Analysis.
Clin. Chem. **23** (1977) 1301-1305.

Vijan, P.N., A.C. Rayner, D. Sturgis and G.R. Wood.
A Semi-automated Method for the Determination of Arsenic in Soil and Vegetation by Gas-Phase Sampling and Atomic Absorption Spectrometry.
Anal. Chim. Acta **82** (1976) 329-336.

Vinogradov, A.P.
The Geochemistry of Rare and Dispersed Chemical Elements in Soils, 2nd Edition.
Translated from Russian. (Consultants Bureau, New York, 1959) 209 pp.

Vis, R.D., P.M.A. Van der Kam and H. Verheul.
Elemental Trace Analyses in Serum using Proton-Induced X-ray Fluorescense.
Nucl. Instrum. Methods **142** (1977) 159-162.

Vozzhenikov, G.S.
Time Selection in Activation Analysis.
Sov. At. Energy **34** (1973) 143-144.
Atomnaya Energiya **34** (1973) 113-114.

Walsh, P.R., J.L. Fasching and R.A. Duce.
Losses of Arsenic during the Low Temperature Ashing of Atmospheric Particulate Samples.
Anal. Chem. **48** (1976) 1012-1014.

Watterson, J.I.W.
Optimisation of Irradiation and Decay Times in Nuclear Activation Analysis.
J. Radioanal. Chem. **26** (1975) 135-150.

Webb, J., K.A. Kirk. D.H. Jackson, W. Niedermeyer, M. E. Turner, C.E. Rackley and R.O. Russell.
Analysis by Pattern Recognition Techniques of Changes in Serum Levels of 14 Trace Metals after Acute Myocardial Infarction.
Exp. Mol. Pathol. **25** (1976) 322-331.

Welch, R.M. and W.H. Allaway.
Vanadium Determination in Biological Materials at Nanogram Levels by a Catalytic Method.
Anal. Chem. **44** (1972) 1644-1647.

Wester, P.O.
Trace Elements in Serum and Urine from Hypertensive Patients before and during Treatment with Chlorthalidone.
Acta Med. Scand. **194** (1973) 505-512.

Wilkinson, D.H.
A stable ninety-nine channel pulse amplitude analyser for slow counting.
Proc. Cambridge Philos. Soc. **46** (1950) 508-518.

Wilson, A.L.
Chemical species to be determined. Selectivity index.
Talanta **12** (1965) 701-702.

Wilson, A.L.
The Performance-Characteristics of Analytical Methods I.
Talanta **17** (1970) 21-29.

Wilson, A.L.
The Performance-Characteristics of Analytical Methods II.
Talanta **17** (1970) 31-44.

Wilson, A.L.
The Performance Characteristics of Analytical Methods III.
Talanta **20** (1973) 725-732.

Wilson, A.L.
The Performance Characteristics of Analytical Methods IV.
Talanta **21** (1974) 1109-1121.

Wiltink, W.F., J. Kruithof, C. Mol, G. Bos and H.G. van Eijk.
Diurnal and Nocturnal Variations of the Serum Iron in Normal Subjects.
Clin. Chim. Acta **49** (1973) 99-104.

Winefordner, J.D.
Quantitation in Analysis.
In: Trace Analysis. Edited by J. D. Winefordner. (Wiley, New York, 1976) (Chemical Analysis, Vol. 46) 435-447.

Winkel, P., B.E. Statland and H. Bokelund.
Faktorer af betydning for variationen af klinisk-kemiske kvantiteter.
Ugeskr. Læg. **137** (1975) 871-886.

Wood, E.C. and E.M. Ault.
Notes on the determination of copper in foods by High's method.
Analyst **74** (1949) 602.

Woods, M.J. and S.E.M. Lucas.
Calibration of the 1383A Ionization Chamber for I-125.
Int. J. Appl. Radiat. Isot. **26** (1975) 488-490.

Woolson, E.A. *(editor).*
Arsenical Pesticides. (American Chemical Society, Washington, 1975) (ACS Symposium Series, 7) 176 pp.

Wuyts, L., J. Smeyers-Verbeke and D.L. Massart.
Atomic Absorption Spectrophotometry of Copper and Zinc in Human Brain Tissue. A critical Investigation of Two Digestion Techniques.
Clin. Chim. Acta **72** (1976) 405-407.

Yang, T.H. and R.Q. Blackwell.
Nutritional and Environmental Conditions in the Endemic Blackfoot Area.
Formosan Sci. **15** (1961) 101-129.

Young, D.S.
Interpretation of Clinical Laboratory Data.
In: Accuracy in Trace Analysis. Proceedings of the 7th IMR Symposium held at Gaithersburg, Md., 7-11 October 1974 (USGPO, Washington, D.C. 1976) (National Bureau of Standards Special Publication 422) 109-122.

Yule, H.P.
Experimental Reactor Thermal-Neutron Activation Analysis Sensitivities.
Anal. Chem. **37** (1965) 129-132.

Yule, H.P.
Data Convolution and Peak Location, Peak Area, and Peak Energy Measurements in Scintillation Spectrometry.
Anal. Chem. **38** (1966) 103-105.

Yule, H.P.
Reactor Neutron Activation Analysis:
Instrumental Sensitivities in Six Matrix Materials.
Anal. Chem. **38** (1966) 818-821.

Yule, H.P.
Mathematical Smoothing of Gamma Ray Spectra.
Nucl. Instrum. Methods **54** (1967) 61-65.

Yule, H.P.
Computation of Lithium-Drifted Germanium Detector Peak Areas for Activation Analysis and Gamma Ray Spectrometry.
Anal. Chem. **40** (1968) 1480-1486.

Yule, H.P.
Computation of Experimental Results in Activation Analysis.
In: Modern Trends in Activation Analysis, Gaithersburg, Md., 7-11 October 1968. Edited by J.R. DeVoe and P.D. LaFleur. Vol. 2 (USGPO, Washington, D.C. 1969) (National Bureau of Standards Special Publication 312) 1155-1204

Yule, H.P.
Computer Data Reduction in Activation Analysis.
In: Activation Analysis in Geochemistry and Cosmochemistry. Proceedings of the NATO Advanced Study Institute, Kjeller, Norway, 7-12 September 1970. Edited by A.O. Brunfelt and E. Steinnes (Universitetsforlaget, Oslo, 1971) 145-166.

Zeisler, R., J.B. Cross and E.A. Schweikert.
Trace Elemental Analysis by Heavy Ion Induced X-ray Emission.
Anal. Chem. **48** (1976) 2124-2129

Zhernakova, T.V.
Relative Concentrations of Iron, Manganese, and Copper in Healthy Human Serum. (Translated from Russian)
Bull. Exptl. Biol. Med. **63** (1967) 608-609

Zief, M. and R. Speights *(editors).*
Ultrapurity Methods and Techniques. (Dekker, New York, 1972) 699 pp.

Zief, M. and J.W. Mitchell.
Contamination Control in Trace Element Analysis.
In: Chemical Analysis, Vol. 47. Edited by P.J. Elving (Wiley Interscience, New York, 1976) 262 pp.

Zikovsky, L.
The optimum Irradiation, Decay and Counting Times in Activation Analysis for Single and Double Counting and for different Minimization Functions.
J. Radioanal. Chem. **22** (1974) 165-172.

Zoller, W.H., G.E. Gordon, E.S. Gladney and A.G. Jones.
The Sources and Distribution of Vanadium in the Atmosphere.
In: Trace Elements in the Environment. A Symposium of the 162. Meeting of the American Chemical Society, Washington, D.C., 15. September 1971. Edited by E.L. Kothny (American Chemical Society, Washington, D.C., 1973) (Advances in Chemistry Series, 123) 31-47.

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