IRI-report: 133-79-02

A ROUTINE CHROMIUM DETERMINATION IN BIOLOGICAL MATERIALS; APPLICATION TO VARIOUS REFERENCE MATERIALS AND STANDARD REFERENCE MATERIALS

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*) This report also contains contributions to:

- Research Coordination Meeting for the Coordinated Research Programme on Comparative Methods for the Study of Trace Elements in Human Nutrition, IAEA Headquarters, Vienna, Austria, 2 - 4 November 1977;
- 5th Symposium on Recent Developments in Activation Analysis, St. Catherine College, Oxford, England, 17 - 21 July 1978.

1 INTRODUCTION

Chromium is an important element in various biological systems, since it is an essential element, but can also be toxic under certain conditions. Chromium exists in biological materials in different forms, of which the biochemical and analytical characteristics are not fully understood. Moreover, many chromium levels of physiological interest are so low - generally in the ppb-region - that problems may arise in an adequate determination. This does not only involve the question of sensitivity and accuracy, but also the risks of contamination. Furthermore, several investigators have pointed out that during sample treatment and analysis chromium may be lost, particularly when present in biological active form with a low molecular weight [1-3].

Neutron activation analysis and atomic absorption spectrometry in principle meet the requirements of sensitivity and accuracy. However, in practice the accurate determination of chromium in biological materials still meets many problems. For instance, in intercomparison studies the reported chromium values easily differ by one or two, and sometimes even by three orders of magnitude [4-10].

At the Interuniversity Reactor Institute at Delft, The Netherlands a sensitive and accurate destructive neutron activation analysis procedure for chromium in biological materials has been developed, and is now being routinely used *). This chromium determination is a part of a larger multi-element scheme for the determination of 12 elements in biological materials [11,12].

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*) Part of this work was performed in the frame of Research Agreement No 1834 CF with the IAEA, Vienna, Austria.

2 GENERAL OUTLINE OF THE PROCEDURE AND SOME CHARACTERISTICS

2.1 Sample preparation

Since chromium is a rather abundant element in the natural and man-made environment, special attention has to be paid to contamination prevention. To minimize contamination, sample handling and sample treatment are performed within a laminar-flow hood placed inside a low dust-level room. This room is accessible via a system of air locks and is kept at an overpressure via a supply of air filtered over an absolute filter. Inside the room all sources of dust and contamination are avoided as much as possible. Utensils li knives, scissors, and tweezers - which come into contact with the sample - are made of titanium. Further details on contamination pre vention are given elsewhere [13].

In order to enable high integrated neutron fluxes, biological samples are usually freeze-dried to reduce the pressure build-up du to radiolysis during irradiation. The freeze-drying is performed at $-25^{o}C$ and 10^{-5} atm for 24 to 48 hours. The weight of the dried sample normally ranges from 200 to 300 mg. If desired, biological samples can also be irradiated in the wet state (cfr. Section 2.2).

For the irradiation of the samples - as well of standards and standard reference materials - quartz vials are used. Depending on the expected chromium content of the samples, normal quartz (e.g. Vitreosil [14]) or synthetic quartz (e.g. Spectrosil [14] and Suprasil [15]) are used. The inner diameter and wall thickness of the vials amount to about 7 mm and 1 mm respectively; the internal volume is about 2.5 ml.

2.2 Irradiation

The samples are irradiated in the nuclear reactor of the Inter-university Reactor Institute in a neutron flux of approximately

10^{13} n/cm^{2}s. The irradiation time is limited by the pressure build-up in the quartz vials due to radiolysis of biological materials. Although quartz vials may withstand internal pressures of over 80 atmospheres - depending upon wall thickness and diameter - the pressure build-up should be limited to 10 to 20 atmospheres at room temperature. This is necessary in order to minimize loss of irradiated materials when the vials are opened (cfr. Section 2.3).

Irradiations of wet and fresh biological materials can be extende to 9 hours without large risks of damage to the vial. Freeze-drying reduces the pressure build-up, resulting in a maximum irradiation peri up to 48 hours. Before irradiating new types of biological materials, the pressure build-up as a function of the irradiation time is checkec in a special system. A sketch of this system given in Figure 1. Foi most lyophilized biological samples of 200 to 300 mg packed in 2.5 ml quartz vials, ir. 'iation periods of 12 to 24 hours can be maintained.

Each irradiation batch consists of six items, generally four samples, one liquid multi-element standard, and one aliquot of (stand reference material or an empty vial as a blank. Two 4-mg pieces of zinc metal serve as flux monitors. The insertion of a (standard) reference material - e.g. Bowen's Kale [16] or NBS SRM 1577 Bovine Liver [17] - is used for analytical quality control purposes.

2.3 Post-irradiation chemical separations

The irradiated samples are allowed to cool for two days, resulting in a substantial reduction of activity level, mainly due to sodium-24 decay. Before opening the vials, the outside surface contamination is removed by boiling the vials in aqua regia for 20 minutes and by rinsing twice with running water. The vials are then cooled in liquid nitrogen, wrapped in paper tissue, and loosely packed in an open

polyethylene bag. In a pneumatic press the packed vials are broken. The sample, quartz splinters and paper tissue are removed from the polyethylene bag and processed together.

Prior to destruction 1 ml carrier solution is added, containing about 100 - 2000 µg of each of the trace elements of interest. The samples are mineralized by charring with 15 ml of concentrated sulfuric acid at a temperature of 200 to 250°C (the time required depends on the type of sample) and by oxidation with a 30-percent hydrogen-peroxide solution added at a rate of 0.5 ml/minute. The volatile elements (bromine, arsenic, mercury, antimony, selenium, and tin) are removed via distillation at 210°C with concentrated hydrobromic acid (47 percen added at a rate of 1 ml/minute for 15 minutes. The wet destruction and hydrobromic-acid distillation are performed in an automated apparatus described earlier [11,12]. In the automated apparatus six samples may be processed simultaneously.

Subsequently, the sulfuric acid is evaporated to dryness at 400°C, using a thermo-regulated aluminium-metal block with cavities for the destruction/distillation vessels. To the residue 15 ml of a 70-percent perchloric acid solution and 40 mg of chromium (added as $CrCl_3$ solution) are added. At a temperature of 200 ± 5 °C dry hydrogen-chloride gas is passed through the perchloric acid medium at a rate of 90 ml/minute for 10 minutes. The chromium distills over as chromyl chloride (CrO_2Cl_2) and is collected in a receptacle with 5 ml of a 10-percent hydroxylammonium chloride solution in order to reduce the hexavalent chromium to the less volatile trivalent state. The distillation yield is over 96 percent after 10 minutes of distillation. A good decontamination from interfering radionuclides (mainly sodium-24, potassium-42, phosphorus-32, and molybdenum-99) is obtained. The shape of the distillation vessel - given in Figure 2 - and the constancy of the temperature and the hydrogen-chloride gas flow proved to be crucial

in this separation. For the chromyl-chloride distillation a separate sixfold distillation unit has been developed. Special attention has been paid to the safe use of hydrogen-chloride gas, which is obtained from a gas cylinder. After termination of the distillation, all pipes and tubes are flushed with dry nitrogen gas in order to minimize the corrosion due to hydrogen chloride. For the hydrogen-chloride gas flow control, six special corrosion-resistant rotameters have been inserted in the feeding pipes. The rotameters are mounted in an air-tight box in order to ensure containment in case of break or leakage of the rotameters and/or valves. A picture of the apparatus is given in Figure 3

Figure 4 shows the position of the chromium distillation step in the routine separation scheme [11] being used at the Interuniversity Reactor Institute at Delft for the analysis of trace elements in biological materials.

2.4 Counting and gamma-spectrum evaluation

The distillate fractions resulting from the chromium distillation are transferred to a counting vial and the volume is made up to 20 ml. The 319.8-keV gamma ray of chromium-51 is measured in an $1\frac{1}{4}$" well of a 3" x 3" NaI(Tl) scintillation crystal coupled to a 128-channel pulse-height analyzer. The entire scintillation crystal is shielded by a 8-cm thick layer of lead. The gain setting amounts to about 6 keV per channel. The counting times range from 1,000 to 10,000 seconds, dependi upon the activity of the fractions.

Since the gamma spectra are not complex after chemical separation, a relatively simple peak area determination and error estimation is use After subtraction of the background - as determined by measuring an empty vial - the resulting spectrum is smoothed using a five-points smoothing procedure [23]. After smoothing, the nett peak area is determined via subtraction of the underlying background. If the computer

cannot find a significant nett peak, an upper limit for detection is calculated. Under standard working conditions (12 hours of irradiation in a neutron flux of 1×10^{13} n/cm^{2}s, a 2-week decay period, and a counting period of 10,000 s in the well of a 3" x 3" NaI(Tl) scintillation crystal), the detection limit amounts to about 0.002 µg of chromium.

A more detailed treatment of the error in the peak-area determination is presented in Appendix I.

2.5 Calibration

From the standard in each run (Cf. Section 2.2) an ETZR-value (elemento-zinc-ratio) is calculated. The ETZR-value is the ratio of the activity of the standard (expressed as the decay-corrected activity p mg of element) to the activity of the zinc flux monitor (expressed as the decay-corrected zinc-65 activity per mg of zinc). A discussion of the advantages of the calibration via ETZR-values is given elsewhere [18]. The ETZR-values for each irradiation run are checked with the corresponding averages of ETZR-values calculated from a number of preceding irradiations. The deviation should not be more than 10 percent For routine analysis the calibration of the activities is carried out with average ETZR values.

As standards 0.5-ml aliquots of a freshly prepared 4-M hydrochloric acid/4-M nitric acid solution are used. This solution contains known amounts of all elements which are of interest in the automated routine separation scheme, being used at the Interuniversity Reactor Institute [11]. Chromium in the stock solution is added as ammonium dichromate, resulting in a concentration of 6.6 µg of chromium per ml.

2.6 The $^{54}Fe(n,\alpha)^{51}Cr$ reaction

Since most biological materials may contain iron - sometimes up to a few hundred ppm - a correction should be applied for the chromium-51

contribution due to the $^{54}Fe(n,\alpha)^{51}Cr$ reaction. This reaction is induced by fast neutrons and, consequently, the contribution will depend on the fast-neutron contribution at the site of the irradiation facility in the nuclear reactor.

For the determination of this correction, samples of spectrographically pure iron [19] were irradiated at the usual irradiation facility in the nuclear reactor of the Interuniversity Reactor Institu In order to minimize the production of chromium-51 due to the (n,γ)-re tion on traces of chromium present in the iron, the irradiations were carried out in a 1-mm thick cadmium-metal cladding. Since cadmium depresses the thermal neutron flux in the irradiation facility, the chromium standards were irradiated in separate runs in the same irradi tion position. All irradiations of iron samples and chromium standards were related to each other by applying iron wires as flux monitors and measuring the manganese-54 activity induced by a (n,p)-reaction on iro After irradiati n the iron samples and chromium standards were chemica processed and counted for chromium-51 activity (Cf. Section 2.3 and 2.4). A set of four experiments yielded for the correction factor an average value with a 95-percent confidence interval of 14 ± 3 μg of "apparent chromium" per gram of iron present in the sample. This factor was used i all chromium determinations described in this report.

2.7 Blank value

In addition to contamination during sample handling (Cf. Section 2.1), the blank value in the determination of chromium may be caused by two factors:

- (A) Contamination in the automated apparatus due to "tailing effects" from previously separated samples and standards;
- (B) Trace-element contribution from the irradiation vial to the sample.

"Tailing effects"

Possible tailing effects in the automated apparatus are minimized as follows: All removable parts (destruction/distillation vessel, receptacle) are thoroughly cleaned in an ultrasonoric bath of an aqueous detergent solution at 80°C for 30 minutes and subsequently ultra-sonorically treated with a 30% NaOH solution with 2% H_2O_2 at 80°C for 30 minutes. Finally, the parts are rinsed twice with tap water.

The distillation head, which cannot be removed, is cleaned after every distillation by a "blank" distillation. This implies that the chromium distillation is completely repeated, however, without a sample After a blank distillation, tailing effects amount to less than 0.01 percent, as was found by various tracer experiments. When very low chromium levels are expected, two blank distillations are performed successively before the low-chromium samples are distilled.

Trace-element contribution from the irradiation vial

The contribution of trace elements from the container walls to the trace-element levels of the sample being analyzed is negligible for most trace elements of the routine separation scheme [11]. However, in the determination of chromium in various types of biological materials, a blank correction will be necessary.

One of the main problems is not only the value of the blank itself, but also the high variability of the blank value. In order to minimize the variability, irradiation vials are made from quartz tubes purchased as one large batch from the supplier. However, since the process of breaking of the irradiated vials is not quite reproducible, fractures of variable areas may be leached out during the chemical processing (destruction with concentrated sulfuric acid, distillation with hydrobromic acid and hydrogen-chloride gas in perchloric acid medium). This may enhance the blank variability.

The average level of the blank value due to the contribution from the irradiation vial may be kept low by using pure synthetic quartz - like Spectrosil [14] and Suprasil [15] - and by applying rigorous cleaning procedures. The procedure for the manufacture and cleaning of the vials is as follows: The quartz tubes of 1-meter length are cut into 14 sections. The sections are sealed with an oxygen/acetylene flame from a titanium burner, so that one-end open 65-mm long vials result. These vials are cleaned in four steps: (1) etching in a 5-percent hydrofluoric acid solution at room temperature during 15 hours (this corresp to a removal of a layer of approximately 25 µg of silica), (2) standing in aqua regia for 6 days, (3) rinsing with de-ionized, double quartz-distilled water, and (4) internal cleaning with steam of 100°C from a quartz steam generator for 3 minutes.

At regular intervals, in the irradiation runs (Cf. Section 2.2), an aliquot of reference material is replaced by an empty vial for the measu ment of the blank value. This empty vial passes through the same proced as the samples, including the handling in the laminar-flow bench in the low dust-level room (Cf. Section 2.1). Under normal working conditions of irradiation (Cf. Section 2.2) and counting (Cf. Section 2.4), the blank value of Spectrosil vials is below the detection limit of about 0.002 µg of chromium (Cf. Section 2.4). In order to enable a more accurate measurement of the blank value, empty vials were irradiated for 100 hours, chemically processed and counted. Spectrosil vials, cleaned according the procedure described above, exhibit an average blank value and standard deviation of 0.0004 ± 0.0004 µg of chromium. In order to investigate the effect of the hydrofluoric-acid etching, for some vials the etching step in the cleaning procedure was omitted. For those vials an average and standard deviation of 0.0017 ± 0.0012 µg of chromium was obtained.

Finally, it should be noted that the blank value for cleaned and hydrofluoric-acid etched Spectrosil vials is in exceptional events larger

than the detection limit of 0.002 µg of chromium. This may be due to an uneven distribution of the traces of chromium over the quartz tube. In some cases also a contamination of the vials - either prior to irradiation or during the chemical processing ("tailing effects") - may be responsible for it.

All results for the samples and reference materials are corrected for the blank contribution. If only an upper limit for the blank is established, the correction is performed with $\frac{1}{2}U \pm \frac{1}{2}U$, where U stands for the upper limit of detection.

2.8 Precision, accuracy, limits of detection and determination

Precision

The overall precision of the method depends on the amount of chromium in the sample to be analyzed. For the (a priori) overall precision, a formula is derived in section I.2 of Appendix I. Using this formula, the overall precision for the nett amount of chromium in the sample was calculated as a function of the gross amount of chromium and tabulated in Table 1. The terms nett and gross refer to data in which the corrections for the blank contribution and the apparent-chromium contribution are applied or are not applied respectively. As may be seen from Table 1, there are roughly three regions of chromium amounts with different characteristics:

- Above 0.1 µg of chromium: The precision is a constant fraction (10 percent) of the amount of chromium in the sample. The precision is mainly determined by the analytical variation.
- Between 0.1 and 0.01 µg of chromium: In addition to the analytical variation, the influences of counting statistics, variable blank contribution, and uncertainty in the correction for the apparent-chromium contribution become noticeable. The coefficient of variation, related to the gross amount of chromium, ranges from 10 percent (at the

level of 0.1 µg of chromium) to 16 - 19 percent (at the level of 0.01 µg of chromium).

- Below 0.01 µg of chromium: The analytical variation becomes negligible. The influence of the poor counting statistics and - to a lesser extent - the influence of the variation due to the variable blank contribution and the uncertainty in the correction for the apparent chromium are predominant. These factors cause a precision, which is (almost) no function of the amount of chromium in the sample. For normal samples (iron levels below 200 ug) the precision ranges from 0.0012 to 0.0016 ug of chromium.

The estimated (a priori) precision was tested by analyzing a set of eleven 200-250 mg aliquots of NBS SRM 1577 Bovine Liver [17]. The avera nett chromium concentration found was 0.096 ppm, which corresponds to a nett chromium amount per sample of about 0.021 µg. The gross amount of chromium per sample was about 0.023 ug, including the blank value of 0.001 µg and an amount of apparent chromium of 0.0008 µg. For the nett chromium amount per sample a precision - expressed as standard deviation - of 0.0027 µg of chromium was calculated, using the formulas of Appendix I. The actual precision - again expressed as standard deviation - for the chromium measurements in the Bovine Liver amounted to 0.0026 µg of chromium, being in good agreement with the calculated one.

Detection and determination limits

The detection limit for the gross amount of chromium is in the order of 0.002 µg of chromium (Cf. Section 2.4). Assuming no iron is present in the sample, a gross value of 0.002 µg of chromium will correspond to about 0.001 µg of nett chromium, since the blank value is also about 0.001 µg of chromium. According to Table 1, for a gross amount of 0.002 µg, the precision for the nett amount is 0.0012 µg, indicating that a reliable

chromium determination is not feasible. Therefore, we define here a determination limit, being the amount of nett chromium, which is 4 times the precision. This implies that in 95 percent of all cases the nett value has an error of less than ± twice the precision, resulting in a reproducibility error of 50 percent or less. From Table 1 it may be derived that for the usual samples (iron content below 200 µg per sample) the determination limit is about 0.0055 µg of chromium.

Accuracy

The accuracy of the method developed may be affected by a number of factors. The most important ones are:

- ETZR values used for calibration. Normally the average of 15 ETZR values is used for calibration. Since a single ETZR value shows a coefficient of variation of about 0.10, the 95-percent confidence interval for the average of 15 ETZR values amounts to ± 5.5 percent (Viz. $0.10 * 15^{-\frac{1}{2}} * 2.13 * 100$, where 2.13 is the t-factor for the 95-percent confidence interval for N-1=14).
- Blank correction. For the correction of the blank a value of 0.001 µg of chromium is used. Since the average blank value may lie between 0.0 µg and 0.002 µg (detection limit), a possible maximum systematic error of ± 0.001 µg may be involved. For sake of calculations, this value is considered as a 95-percent confidence interval.
- Apparent-chromium correction. The 95-percent confidence interval in the correction factor amounts to $\pm 3 * 10^{-6}$ (Cf. Section 2.6). For iron amounts of 200 µg, 400 µg and 600 µg per sample this corresponds to a maximum systematic error of ± 0.0006 µg, ± 0.0012 µg, and ± 0.0018 µg of chromium respectively.

Conclusively, it can be said that the maximum error consists of two types of contributions: (a) a constant amount of chromium (expressed

in µg) due to the uncertainty in the correction for the blank and the apparent chromium; (b) a relative amount (expressed as a percentage of the nett chromium amount in the sample) due to an uncertainty in the average ETZR. Assuming that the errors show a Gaussian-distribution character and are independent of each other, the 95-percent confidence interval for the overall error may be calculated by the usual procedure (squaring, summing, and taking the square root).

The accuracy of the method developed was tested with eleven aliqu of NBS SRM Bovine Liver 1577 [17]. This is the material with the lowe certified chromium level commercially available sofar. The measured av value and its 95-percent confidence interval amounted to 0.096 ppm and 0.008 ppm respectively. Using the above-mentioned figures for the contributions to the error, a 95-percent confidence interval for the maximum possible overall error was calculated to be $\pm$ 0.0015 µg of chromium. For an average sample weight of 220 mg, this corresponds to a value of $\pm$ 0.007 ppm. The certified value for the chromium level in NBS SRM Bovine Liver 1577 is 0.088 $\pm$ 0.012 ppm [17]. Taking into accou the error limits - and especially those in the certified level - no conclusive answer can be given as to the presence of a maximum error of $\pm$ 0.007 ppm in our measurements. Nevertheless, the results do not give an indication of a substantial systematic error.

3 DETERMINATION OF CHROMIUM IN VARIOUS REFERENCE MATERIALS AND STANDARD REFERENCE MATERIALS

Additional to the NBS SRM 1577 Bovine Liver, chromium was also determine in a number of reference materials and standard reference materials. The results obtained are listed in Table 2. The chromium levels found agree well with the ranges of "best values", "overall values", and/or "probable values", which are reported in the literature, or may be derived from it.

However, since the chromium values given by various authors for the very same material show sometimes substantial differences, no additional conclusions as to the accuracy of the method developed may be drawn.

In the determination of chromium in NBS SRM 1569 Brewer's Yeast some problems were encountered. Applying the method as developed, an average value and 95-percent confidence interval of 1.12 ± 0.08 ppm of chromium was found, being about half of the certified value [20]. A closer examination showed that an appreciable fraction of the chromium-51 induced in the Brewer's Yeast was retained in the perchloric acid residue. No significant increase of the distillation yield was observed in repeated distillations after addition of extra amount of carrier. This led us to the conclusion that a considerable fraction of the chromium in NBS SRM 1569 Brewer's Yeast is present in a relatively "non-reactive" state. Filtration of the perchloric acid medium showed that the non-reac chromium was indeed mainly associated with suspended particles. A rough analysis of NBS SRM 1569 Brewer's Yeast showed a silica content and an alumina content in the order of ½ and 1 percent respectively.

In another experiment the remaining perchloric acid was evaporated and the dry residue fused with a mixture of 100 µg of chromium carrier, sodium carbonate and sodium nitrate. The resulting melt was dissolved in hydrochloric acid and evaporated to dryness. After adding perchloric acid and 40 mg of chromium carrier, the liquid was again submitted to distillation in hydrogen chloride gas. Now an average and 95-percent confidence interval of 1.01 ± 0.05 ppm (average of nine aliquots) were found in the distillate. Combination of this value with the value found earlier in the direct treatment (1.12 ± 0.08) yielded an overall chromium level and 95-percent confidence interval of 2.13 ± 0.13 ppm of chromium, being in excellent agreement with the certified value of 2.12 ± 0.05 ppm. Further experiments with NBS SRM 1569 Brewer's Yeast are described in a separate paper [21].

The above-mentioned phenomenon of the occurrence of "non-reactive" chromium in the perchloric acid medium was the first indication that the dissolution procedure [11] for irradiated materials is not complete, at least not for chromium. Therefore, the influence of the repetition of the chromium distillation after fusion with alkali was investigated for other (standard) reference materials, some of which contain appreciable amounts of silica, alumina, and/or calcium phosphate. Also some other brands of Brewer's Yeast were enclosed in this investigation. The resu are listed in Table 2 and Table 3. The results show that the occurrenc of non-reactive chromium only happens with the NBS SRM 1569 Brewer's Yeast. One straightforward distillation step for all other materials leads to a good yield, at least higher than 90-percent and generally h than 95-percent. Conclusively, the behaviour of chromium in NBS SRM 15 Brewer's Yeast is not typical for biological materials, not even for brewer's yeast.

4 CONCLUDING REMARKS AND DISCUSSION

The determination limit under standard working conditions (0.0055 µg of chromium for samples containing up to 200 µg of iron) is sufficiently low to enable an adequate chromium determination in many biological materials of interest. The precision of the method developed (expressed as coefficient of variation) ranges from 25% at the level of 0.0055 µg to 10% for amounts of 0.1 µg chromium or larger. The method developed does not show any appreciable systematic error for samples containing chromium amounts above 0.020 µg per sample.

Since the counting statistics mainly determine the precision and the limits of detection and determination, improvements may be obtained by increasing the counting period. Calculations, using the formulas of Appendix I, show that doubling and quadrupling of the standard

counting period of 10,000 s will result in a determination limit of 0.0039 µg and 0.0033 µg of chromium respectively. Further increase of the counting period is not only impractical, but also ineffective for a substantial reduction of the determination limit. If no problems arise with the pressure-build-up (Cf. Section 2.2), also the irradiation period may be increased to improve the determination limit. Doubling of the irradiation period to 24 hours will reduce the determination limit to respectively 0.0033 µg, 0.0029 µg, and 0.0027 µg of chromium for counting periods of 10,000 s, 20,000 s, and 40,000 s respectively. It should be stressed that these calculations - as well as all other calculations - are based upon a number of assumptions (Cf. Appendix I). Especially for small amounts of chromium to be determined discrepancies between the assumptions and the actual situation may occur.

Another aspect to be discussed is the freeze-drying procedure used (Cf. Section 2.1). Recently it was suggested that chromium may be lost by volatilization upon freeze-drying [10]. The possibility of a chromium loss upon freeze-drying of human liver material was recently studied by the authors. No substantial and significant loss, neither for chromium nor for any of the other eleven elements studied could be detected, as indicated in Figure 6. A more detailed discussion is presented elsewhere [22]. In cases that there is not sufficient certainty about the behaviour of chromium during freeze-drying, the samples may be irradiated in wet state, however, for shorter periods. For many biological materials irradiation periods up to 9 hours are feasible, resulting in an increased determination limit of 0.0063 µg of chromium. For quite a few biological materials this limit is still adequate.

Finally, since our chromium determination is a part of a larger multi-element scheme, the method can easily be extended to the determination of eleven to twelve other relevant trace elements in biological

materials [11]. This is of interest in studies, in which a number of trace elements - including chromium - have to be determined in the same sample, viz. in the case of the IAEA/WHO Coordinated Research Programme on Trace Elements in Relation to Cardiovascular Diseases [9]. This programme requires the determination of the elements chromium, copper, cadmium, zinc, and selenium in human autopsy materials.

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!/ J.J.M. de Goeij, K.J. Volkers, P.S. Tjioe, "A search for losses of ch and other trace elements during lyophilization of human liver tissue" Anal. Chim. Acta, 109 (1979) 139 - 143.

3/ P.J.M. Korthoven, "Resolf, a computer program for analysis of gamma s IS-1811, Iowa State University, Ames, Iowa, USA (1968).

Table 1

Calculated precision in microgram of chromium for the nett amount of chromium in the sample as a function of the gross amount of chromium and various amounts of iron in the sample*)

gross amount of chromium per sample in µg	coefficient of variation S_c due to counting statistics**)	precision in µg of chromium***)			
		0 µg of Fe	200 µg of Fe	400 µg of Fe	600 µg of F
0.001	1.09	0.0012	0.0012	0.0014	0.001
0.002	0.55	0.0012	0.0013	0.0014	0.001
0.005	0.22	0.0013	0.0014	0.0015	0.001
0.01	0.11	0.0016	0.0016	0.0017	0.001
0.02	0.057	0.0024	0.0024	0.0025	0.002
0.05	0.024	0.0053	0.0053	0.0053	0.005
0.10	0.013	0.010	0.010	0.010	0.01
0.20	0.0077	0.020	0.020	0.020	0.02
0.50	0.0041	0.050	0.050	0.050	0.05
1.0	0.0027	0.10	0.10	0.10	0.10
2.0	0.0018	0.20	0.20	0.20	0.20
5.0	0.0011	0.50	0.50	0.50	0.50
10.	0.00079	1.0	1.0	1.0	1.0

*) The terms nett and gross refer to respectively with and witho the corrections for the blank contribution and the apparent-c contribution.

**) Calculated with Formula 1 and Formula 2 as given in Appendix

***) Calculated with Formula 3 as given in Appendix I.

Table 2

Chromium content of a number of standard reference materials and reference materials

type of material	average chromium value and 95-% confidence intervals in ppm, dry weight basis; number of replicates in parentheses.	fraction distilling over in the first chromyl-chloride distillation, in percent; number of replicates in parentheses.
Bowen's Kale	0.46 ± 0.04 (15)	95.3 ± 0.6 (4)
NBS Bovine Liver 1577	0.096 ± 0.008 (11)	98.9 ± 0.5 (2)
NBS Orchard Leaves 1571	2.9 (2)	98.4 ± 0.1 (2)
NBS Brewer's Yeast 1569	1.12 ± 0.08*) (10)	52.8 ± 3.0 (10)
IAEA Copepod MA-A-1	0.23 ± 0.03 (11)	> 93 (2)
IAEA Seaplant SP-M-1	4.2 ± 0.2 (12)	97.4 ± 0.7 (2)
IAEA Oyster MA-M-1	0.65 ± 0.35 (6)	> 98.5 (2)
IAEA Wheat Flour V-2/1	0.011 ± 0.003 (5)	-
IAEA Calcinated Animal Bone A-3/1	980 ± 120 (4)	90.7 ± 1.7 (4)
BCR Single Cell Protein	0.31 ± 0.01 (20)	> 98.9 (4)

*) After an alkaline fusion and a second chromylchloride distillation a value of 2.13 ± 0.13 ppm was obtained.

Table 3

Chromium content of a number of brewer's yeasts

type of material	average chromium value and 95-% confidence intervals in ppm, dry weight basis; number of replicates in parentheses.	fraction distilling over in the first chromyl-chloride distillation, in percent; number of replicates in parentheses.
NBS Brewer's Yeast 1569	1.12 ± 0.08 *) (10)	52.8 ± 3.0 (10)
Brewer's yeast,		
- commercial, Dutch	0.27 ± 0.03 (6)	> 98.3 (4)
- commercial, Belgian	0.32 ± 0.10 (5)	93.5 ± 1.6 (5)
- research grade	0.41 ± 0.04 (6)	> 97.7 (6)
- research grade, siliconated	0.57 ± 0.04 (5)	> 98.7 (5)

*) After an alkaline fusion and a second chromylchloride distillation a value of 2.13 ± 0.13 ppm was obtained.

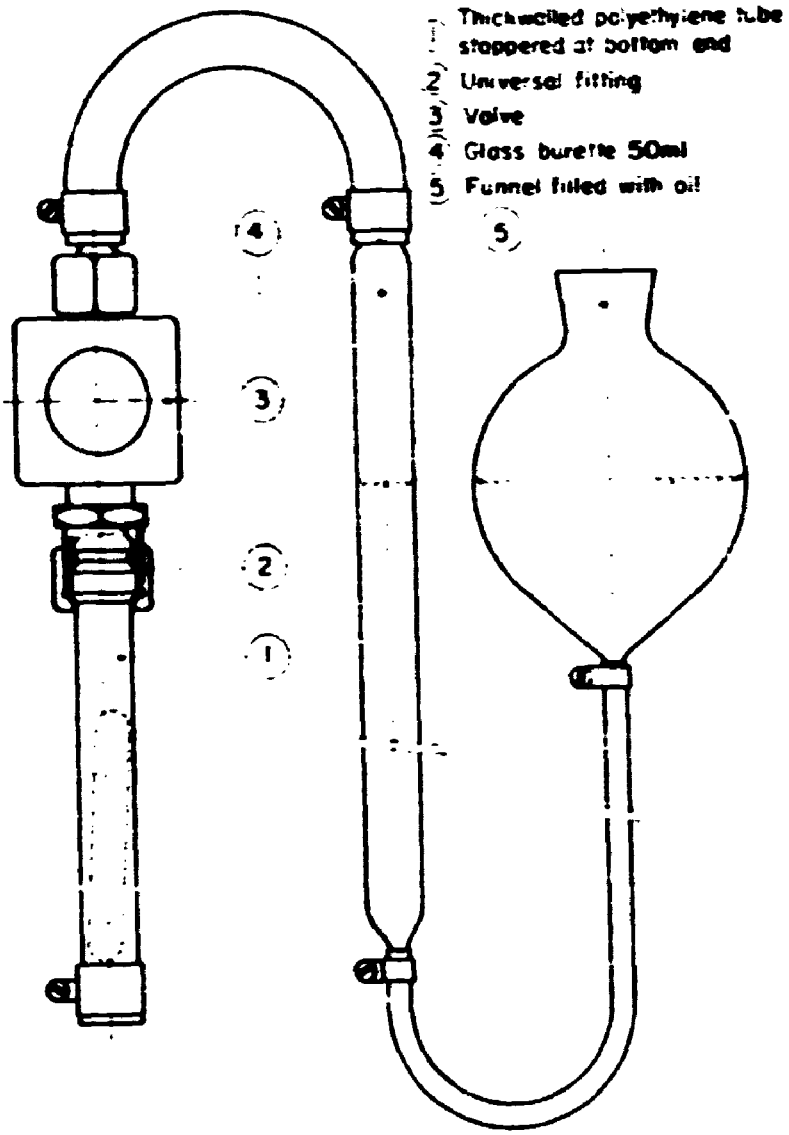

Figure 1

Measuring device for the estimation of the pressure build-up in the vial after irradiation.

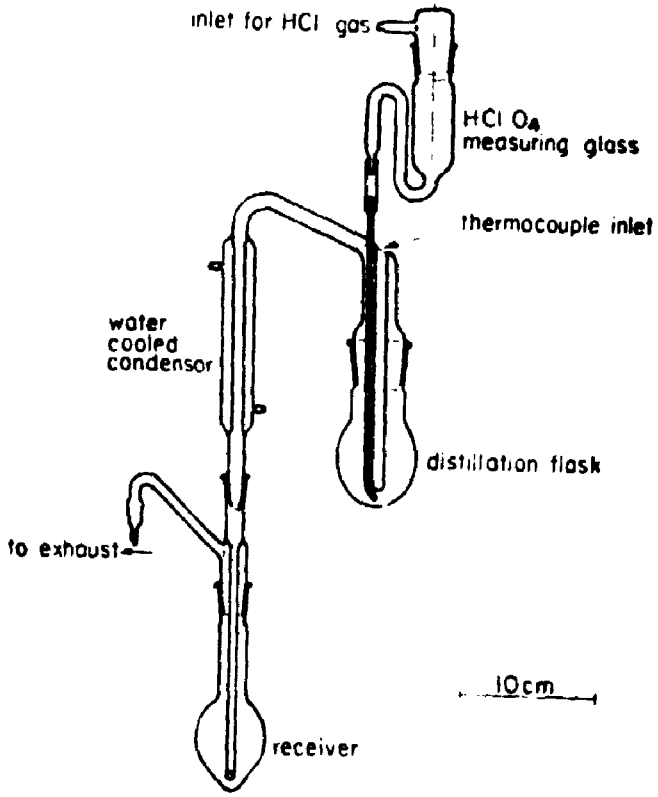

Figure 2

Distillation vessel for the distillation of chromium as chromylchloride [11].

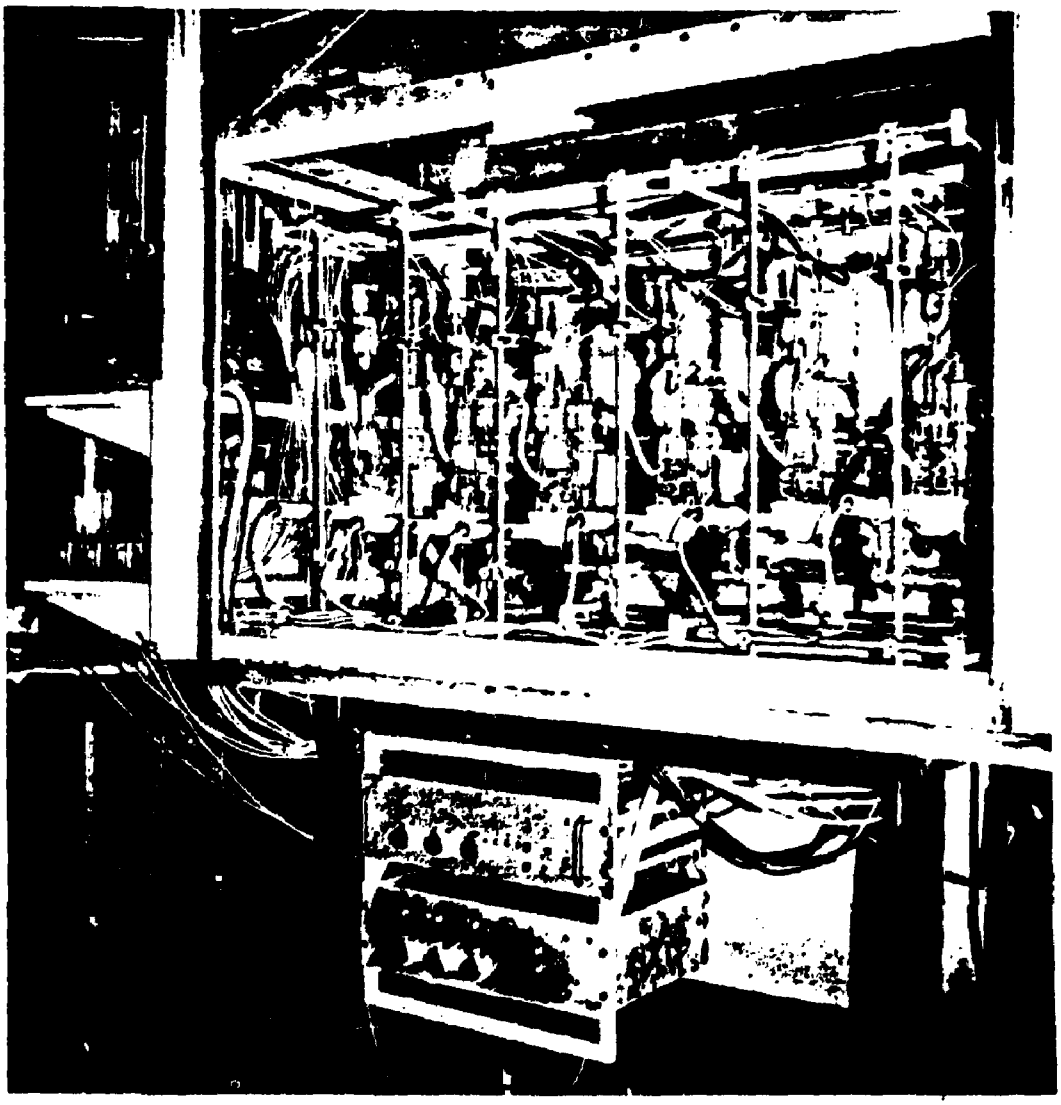

Figure 3

Apparatus for the simultaneous and automated distillation of chromium from six samples.

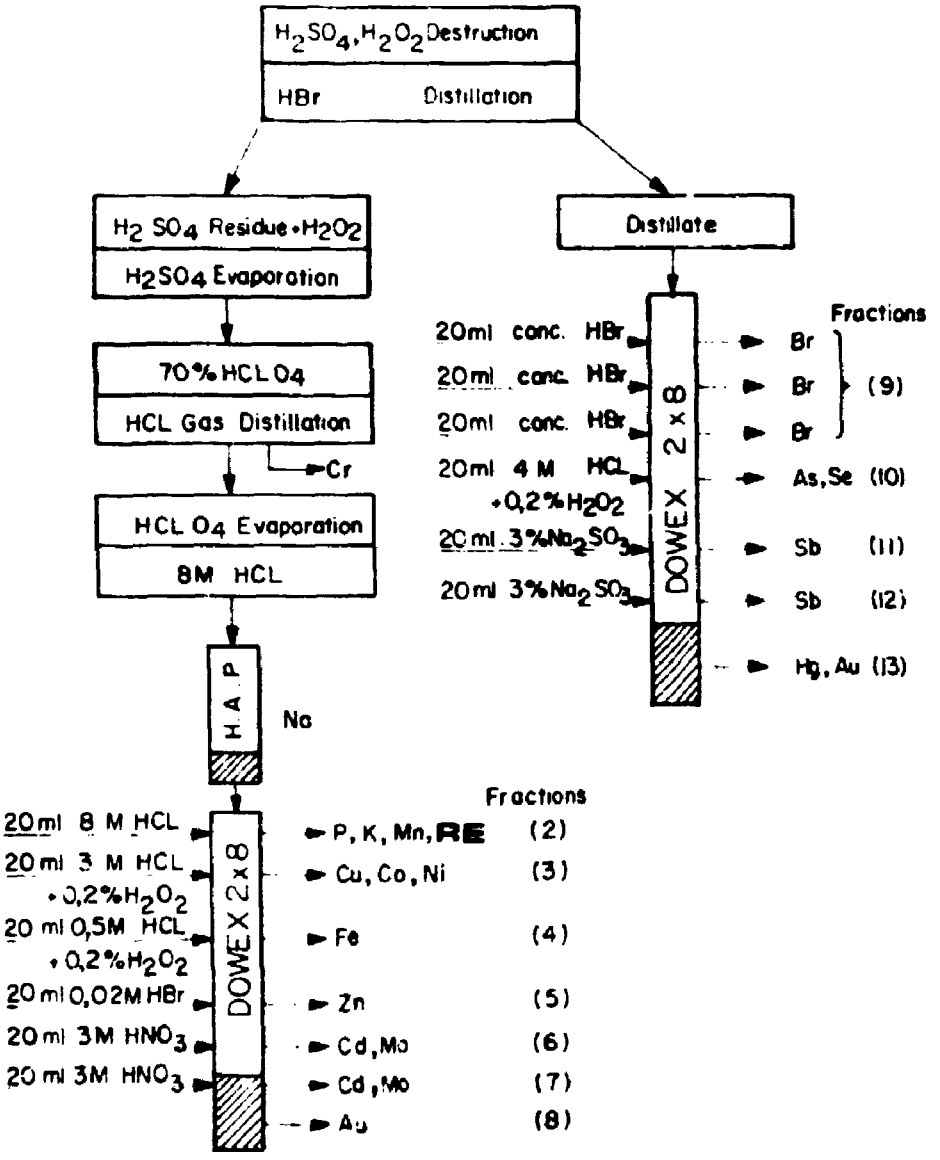

Figure 4

Separation scheme for the analysis of trace amounts of arsenic antimony, bromine, cadmium, chromium, cobalt, copper, gold, iron, mercury, molybdenum, nickel, selenium, and zinc in biological materials [11].

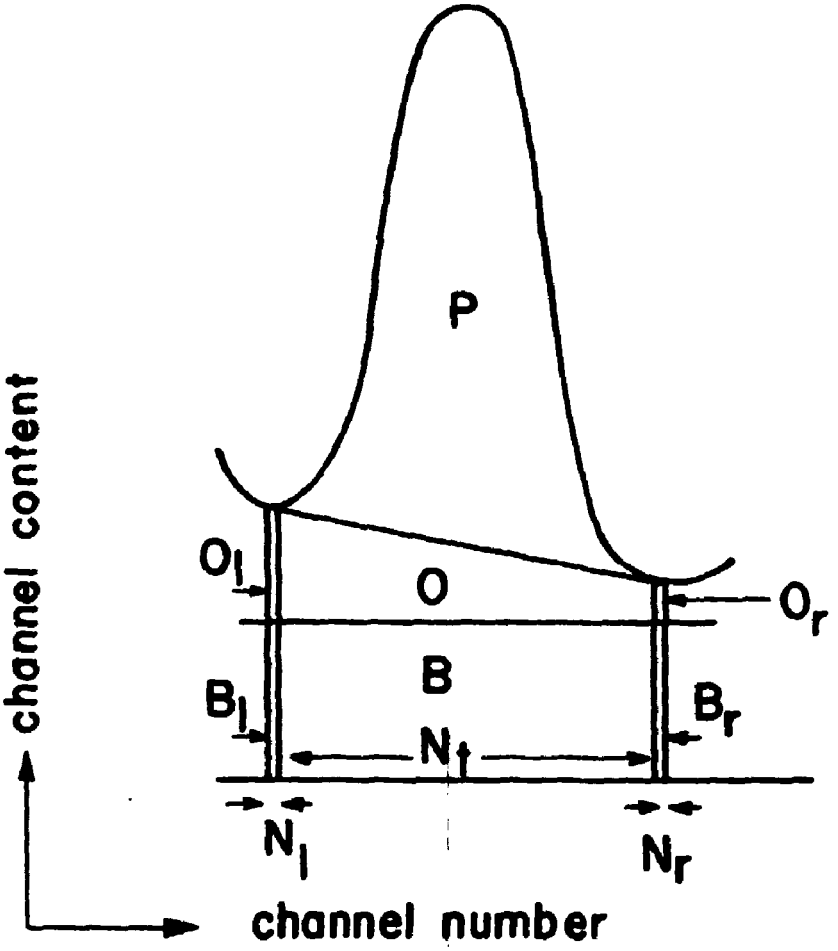

Figure 5

Schematic presentation of the chromium-51 peak in the gamma spectrum.

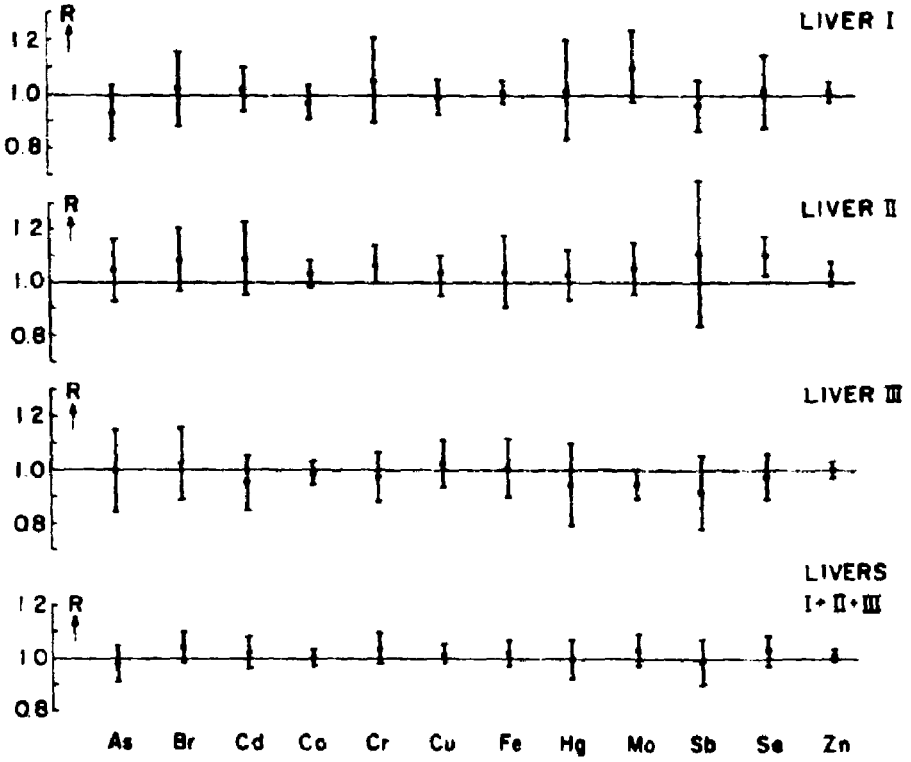

Figure 6

Ratios $\frac{\text{concentration measured in lyophilized aliquots}}{\text{concentration measured in wet aliquots}}$ and their 99-percent confidence intervals for a number of trace elements in human liver material [22].

Appendix I

I.1 Estimation of the error in the nett peak area determination

For the estimation of the error involved in the nett peak area determination, the following model may be used. Figure 5 depicts schematically the gross chromium-51 peak (P + O + B) as measured over N_t channels. After subtraction of the background B, the resulting spectrum is smoothed by a five-point smoothing procedure [23]. The nett peak area P is obtained by subtraction of the area O. This area is calculated on the basis of O_l and O_r, being the sum of the contents of a few channels N_l and N_r respectively at either side of the peak. The variance V_P in the nett peak area may be approximated by the formulas:

$$V_P = P + O + 2B + \left[N_t/(N_l + N_r)\right]^2 * (O_l + 2B_l + O_r + 2B_r) \qquad (1)$$

$$\text{coefficient of variation} = V_P^{\frac{1}{2}}/P \qquad (2)$$

Formula 1 takes into account that the variance in P is increased due to the simultaneous measurement and subsequent subtraction of the background B. Also is accounted for the fact that the variance in O is determined by the variance O_l and O_r, which in their turn are affected by the variance B_l and B_r, being the contents of the channels N_l and N_r respectively of the background B.

In the computer programme for the peak-area determination (Cf. Section 2.4) for O_l and O_r the centre of mass is taken, calculated of three successive channnels at the turning points on either side of the peak. Due to this way of calculation and furthermore due to the smoothing procedure, the statistical weight of O_l and O_r equals that of about 3.5 channels. Consequently $N_l = N_r = 3.5$. Also the value of O_l, O_r, B_l, and B_r in Formula 1 should refer to the contents of 3.5 channels.

As illustration, the coefficient of variation in the nett peak area as a function of the amount of chromium in the samples was calculated as follows: In a 10,000-second spectrum for a chromium standard with a known chromium content, being corrected for the contribution of the background, each channel content was multiplied with a factor F. To this new spectrum, a 10,000-second spectrum of a blank, not being corrected for the background, was added channel-wise. Due to a proper choice of the multiplicatio F for the standard spectrum, spectra could be composed correspondi various amounts of chromium analyzed under standard working conditi

From the composed spectra, the values of O, O_l, O_r, B, B_l, B_r P as a function of the amount of chromium are known. The value of N the composed spectra amounted to about 23. Inserting all these par meters in Formula 1 and Formula 2, an estimation of the coefficient variation in the nett peak area as a function of the (gross) amount chromium is obtained. The results are given in Table 1 (first and column). As may be seen, below 0.01 µg of chromium the coefficient variation rises sharply.

I.2 Estimation of the overall precision of the chromium determination

For the derivation of a formula for the estimation of the overall pre of the chromium determination developed, a number of assumptions have been made, viz.:

- The analytical errors show a Gaussian distribution, so that the usu error propagation rules may be used.
- A constant ratio between the thermal-neutron flux and the fast-neut flux, so that the correction factor for the apparent chromium does vary.
- No significant contamination, neither during sample handling nor during radiochemical processing.

- No contribution of radiochemical interferences from the sample to the background in the gamma spectrum.
- The variation of the blank (Cf. Section 2.7) contributes only once, viz. in the combination of a given sample with the blank value of a particular vial. For the correction an average blank value is used, being the same for all samples (N.B.: an error in the average blank value will not contribute to the precision, but to the systematic error).
- The variation in the "apparent chromium content" (Cf. Section 2.6) tributes only once, viz. in the correction for it where a measured concentration with a certain uncertainty is used.

The estimated precision R is given by the following formula:

$$R = \left[(S_c^2 + S_a^2 + S_s^2)\ G_{Cr}^2 \ + S_b^2\ G_b^2 \ + (14 * 10^{-6}\ G_{Fe}\ S_{Fe})^2 \right]^{\frac{1}{2}}$$

where:

R = Precision, expressed as standard deviation in µg of chromium, a correction for the blank and the apparent chromium.

S_c = Variation due to counting statistics only, expressed as coefficien of variation. S_c is calculated with Formula 1 of section I.1 of Appendix I. The calculated values are only valid for standard wor conditions, viz. (1) 12 hours of irradiation in a neutron flux of $1 * 10^{13}$ n/cm^{2}s, (2) a 2-week decay period after the end of irrad and (3) a 10,000-second counting period using the well of a 3" x NaI(Tl) scintillation crystal.

S_a = Variation in the chromium determination due to all sources of analytical errors (e.g. variations in neutron flux, chemical yiel and counting geometry) other than counting statistics, expressed coefficient of variation. From the chromium standard S_a was estin to be in the order of 0.10.

S_s = Variation in the calibration method, expressed as coefficient of variation. Since for calibration ETZR values are used, S_s represents

here the variation in the activity between flux monitors. This variation is normally in the order of 0.02.

G_{Cr} = Gross amount of chromium per sample, in µg of chromium. The gross amount of chromium includes: The natural amount of chromium in the sample, the chromium contribution from the blank, and the contribution of apparent chromium due to the presence of iron.

S_b = Variation in the chromium contribution from the blank, expressed as coefficient of variation. Since the blank value is below the detecti limit of 0.002 µg of chromium, we assume an average blank value of 0.001 µg of chromium and S_b equal to 0.5; thus, $\pm 2S_b$ covers the pos range of ≥ 0.000 µg to < 0.002 µg for the blank value

G_b = Average blank value, in µg of chromium.

G_{Fe} = Amount of iron per sample, in µg of iron.

S_{Fe} = Analytical variation, including counting statistics, in the determination of the iron content of the sample, expressed as coefficient of variation. Replicate measurements indicate S_{Fe} to be in the order of 0.12.