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Analysis of Selenium in Body Fluids: A Review

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I. Introduction

Obtaining analytically meaningful and biologically interpretable data for trace elements in biomedical investigations is a tedious task and requires dedicated efforts through a multidisciplinary approach by analytical chemists and health science investigators.[1] Therefore, Morrison[2] has evaluated the effectiveness of a number of the more popular trace element analytical techniques in meeting the needs of the life scientists.

There is a rather narrow range of adequacy of several essential elements such as Se in most organisms. Smaller concentrations result in different abnormalities because of pertinent specific biochemical changes. Higher concentrations result in toxicity.[3] Although it is firmly established that Se is an essential trace element,[4,5] initially, interest in Se was caused by its potential toxicity.[6] Selenium intoxications (selenosis) have been reported in recent years in seleniferous regions,[7,8] but a more important problem is Se deficiency in several geographical areas. Keshan and Kashin–Beck diseases are directly associated with Se deficiency in areas of low Se.[5,9,10] Low Se status may be associated with an increased risk of cancer[5,11–13] or with patients receiving total parenteral nutrition.[14] Another important problem related to Se deficiency is its association with an increased risk of ischemic heart disease as the epidemiological studies[5,15,16] have shown. Also, Se deficiency can accelerate the progression of liver disease in chronic alcoholism by decreasing the protective activity of the peroxidase against lipoperoxidation of intercellular membranes.[17,18] There are many other diseases in which low Se status has been reported, among them the acquired immunodeficiency syndrome.[19] The Food and Nutrition Board of the National Research Council[20] has recommended a dietary Se allowance of 0.87 μg kg^{-1} or, with rounding, 55 and 70 μg day^{-1} for the reference North American adult female and male, respectively. It is clear, however, that due to variations in the amount of Se in the soil of different geographical areas, the daily intake by a local population may be above or

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below the recommended limits, and this will give large differences in the concentration found in human fluids such as urine.[21,22]

Selenium supplementation has been assayed in areas low in Se. Supplementation of the diet with sodium selenite,[9] and organic and inorganic Se species,[23] was effective in reducing Se deficiency. Also, Se supplementation has been used in cancer prevention,[13,24] as well as in patients receiving long-term parenteral nutrition,[25] and in patients with phenylkethonuria.[26]

Accurate analytical data are essential for any retrospective or prospective studies relating Se status to health and disease, for establishing appropriate Se intake and/or supplementation guidelines, and for the monitoring of environmental and occupational exposure. Thus, there is increasing interest in the techniques available for assessing Se status in humans, including the establishment of levels in human fluids.[5,27,28] Also, the use of these techniques can contribute to determining the metabolic pathway of Se. Selenium is somewhat unique among trace elements in that several good and independent analytical methods have been developed. Some authors have reviewed the analytical procedures for Se determination in urine[21] and several other human fluids.[29]

Little attention has been dedicated to the form of Se occurring in body fluids and how the different forms may reflect the Se status. Selenium can be found in the body in a range of oxidation states, from Se(VI) to Se(-II), which constitute several chemical Se species.[30] The main Se compounds present in body fluids, such as blood, milk, semen, and others are the seleno amino acids which form the selenoproteins. Although several selenoproteins have been isolated and identified,[31,32] only the metabolic function of glutathione peroxidase (GSH-Px) is known. The importance of Se is associated with the activity of this enzyme.[33,34] The residue of selenocystine, which is found in GSH-Px and several other Se-containing proteins, is the predominant form of Se in biological tissues.[35-37] However, urinary Se species differ from the rest of several organic (seleno amino acids and trimethylselenonium ion) and inorganic (selenite and selenate) species. Alkylation of selenide contributes to the elimination of Se and to regulating the body burden. Progressive methylation yields the the hydrophobic and volatile dimethyl selenide, followed by the water-soluble and nontoxic trimethylselenonium ion ($TMSe^+$) which is eliminated by urine.[38] $TMSe^+$ contributes ~7% of the Se excreted in urine by people on normal diets, but it can become the major urinary metabolite in human urine after intake of high amounts of Se.[8,39-42]

In the present paper, we review the suitable analytical methods for Se determination in human body fluids that were published from 1975 to 1993. However, depending on the relevance of the work, we also include some methods from earlier years. This review article has been divided into four sections: 1) sampling and storage; 2) sample treatment; 3) determination procedure; and 4) quality control and reference material.

A. Sampling and Storage

Analytical results are significantly affected by the homogeneity of the samples and by storage procedures. In general, small aliquots of a sample taken from the bulk material are used, often after long-term storage. These can be representative only if sampling and storage procedures are systematic. Sampling and storage procedures depend on the type of fluid that is to be analyzed.

1. Urine

Representative samples of urine can only be obtained by a 24 h urine collection. As with most urine compounds, Se exhibits diurnal variations as a result of variations in drinking patterns.[21] Some authors[43] have recommended the urine samples be taken from the second micturition of the day and expressing the result in micrograms per gram ($\mu g\ g^{-1}$) or micrograms per mole ($\mu g\ mol^{-1}$) of creatinine.

In sharp contrast to most elements[2] the sampling, storage, and preparation steps in the determination of Se in urine are essentially free from contamination problems.[44] The determination of elements such as Se, in the micrograms per milliliter ($\mu g\ mL^{-1}$) or micrograms per gram ($\mu g\ g^{-1}$) range can be performed in an ordinary analytical or clinical laboratory.[45] Cornelis et al.[46] emphasized the importance of the method of sampling and storage of urine. They advised sampling as soon as possible into an appropriate ultrapure container. Usually precleaned polyethylene, polypropylene, or teflon containers are used to prevent contamination or adsorption losses.[44] These containers must be washed thoroughly with detergent and H_2O_2, soaked overnight in diluted nitric or sulfuric acid, and rinsed several times with Milli-Q water. Two hours after sampling no Se losses could be found.[46] Urine stored at 4 °C in polyethylene bottles suffer losses of 0, 2, 3, and 7% after 12, 24, 48, and 72 h, respectively.[47] The storage of urine can produce losses by adsorption[48,49] depending on the composition of the container walls and the pH of the solution. For polyethylene containers the losses from a 1 $\mu g\ mL^{-1}$ Se solution during 2 week storage accounted for 8.3 and 2% at pH values of 7 and 3.8, respectively.

In order to prevent bacterial growth and especially to minimize adsorption losses in urine samples, toluene,[50-52] formaldehyde,[53,54] hyrochloric acid,[55,56] nitric acid,[57,58] sulfuric acid,[42,59] or benzoate[60] can be used. The samples must be stored in a refrigerated or frozen form until the moment of analysis.

No selenium is lost when either milk or whole blood is evaporated to dryness at 100–150 °C[61] but about 30% is lost from the urine due to the presence of several volatile forms of the selenium.[62] To avoid losses of Se in body fluids on drying,[63] samples are treated at low temperature (75 °C),[64] 30 °C overnight,[65] or dried under vacuum dessicator with $Mg(ClO_4)_2$.[66,67]

2. Blood

Versieck[68] has reviewed the collection and preparation of human blood plasma and serum for trace element analysis. A great deal of the inconsistencies in the final results of some trace elements may be ascribed to unsuspected contamination of the samples with exogenous material during their collection and preparation. However, there are no serious problems due to the contamination of the samples for Se analysis in body fluids.

In general, blood samples were obtained by standard venepuncture techniques from the antecubital vein[69,70] using plastic syringe[47] or in sick persons via catheter[71-73] or heal stick.[72] Behne et al.[74] have concluded that changes in posture can also be responsible for a genuine alteration in the concentration of Se serum, due to different water flows in the blood vessels. They suggest that the effects of posture on serum element levels should be prevented by means of standardized sampling procedures. Sampling must be done in the morning, after each subject has abstained from food and drink, other than water, for at least 12 h.[75] In one balance study,[76] the blood sample collection was done in the afternoon (2−3 hours after lunch). In order to avoid contamination, Versieck[73] recommends using a catheter through which 20 to 40 mL of blood is allowed to pass before a sample aliquot is taken. This may be an unacceptably large volume with chronically ill patients. Most authors[69,71,77,78] utilize a more realistic sample size of 10 mL of whole blood, yielding only 3 or 4 mL of serum. However, other authors[72] have considered that 0.5 to 1 mL of blood is enough. When analysis of Se in blood cells such as platelets or leucocytes is performed, larger volumes (450 mL) of blood must be sampled.[79]

When analyzing selenium in whole blood, erythrocytes, or plasma, it is necessary to add an anticoagulant such as sodium heparin,[43,76,80-82] lithium heparin,[83] EDTA,[83,84] or sodium citrate.[85] There were no differences in the results of selenium analyses of blood samples preserved with EDTA or heparin as anticoagulant.[83] Heparin used as anticoagulant reversibly interacted with a major selenoprotein in human plasma. The choice of anticoagulant is therefore important in studies of Se distribution in plasma.[32,86] Red blood cells were separated by centrifugation at 2500−3000 rpm and the plasma was removed for subsequent assay of selenium.[80,82] The buffy coat was removed and discarded,[80,82] and the red cells were washed several times in cold isotonic saline solution and then resuspended to an hematocrit of approximately 40%[80] or in isotonic saline solution to reconstitute the original volume.[82,83] Platelets and leucocytes were separated from whole blood by centrifugation at 3300*g*. Afterward, the concentrate of platelets must be resuspended and recentrifugated in order to purify it.[79,87] Serum samples were obtained without coagulants and allowed to clot[77,78,88,89] and centrifuged for 20 min,[90] or 2 h[91] within collection. The samples with visible hemolysis were excluded from the following measurements.[89] Selenium in blood serum is mainly protein bound, and it may be precipitated with trichloroacetic acid and redissolved in ammonia solution.[92]

The problems associated with glass tubes and anticoagulants suggested that clean, dry, plastic tubes of polystyrene,[47] polyethylene,[72] polycarbonate,[83] or polypropylene[84] can be used for sample collection and storage.[91] The plastic tubes were immersed in HNO_3[91] or H_2SO_4,[93] diluted for 24 h after washing with detergent and rinsed three times with redistilled water. All material utilized must be previously washed with acid and checked afterward in order to evaluate the contamination or desorption.[91] The blood with EDTA as an anticoagulant was filtered through a 125 μm nylon screen, hemolyzed by repeated freezing and thawing, and stored in polypropylene vials.[84] With this treatment, no change in blood Se concentrations was observed in the samples for at least 3 years.[84]

The samples can be stored refrigerated (2 °C) for a maximum of 8 days;[70,81] storage at −20 to −30 °C was needed for longer periods.[70] Many authors have utilized a temperature of congelation of −20 °C,[69,91,94-100] although others have been used such as −15[101] or −70 °C.[102] Also, sterilization with γ irradiation with 2.5 Mrad of ^{60}Co has been utilized.[84] Preliminary analysis of untreated serum and tissue showed the presence of ^{24}Na, ^{38}Cl, ^{19}O, and ^{23}Ne contaminants which can interfere in the Se determination by NAA. To reduce the oxygen content of the samples, specimens were lyophilized prior to irradiation. Because serum samples contain approximately 7 times more sodium and 10 times more chlorine than tissues, dialysis was necessary prior to lyophilization to eliminate the contaminants ^{19}O, ^{23}Ne, ^{24}Ne, and ^{38}Cl in the neutron activation analysis.[103]

Other authors prefer the lyophilization techniques[104,105] or desiccation and irradiation (at 5 Mrads) without any previous treatment,[106] which are used as a previous step in the method of analysis (NAA). No loss was observed in freeze-drying but Se was lost from whole blood and other tissues in oven drying at 120 °C.[61] Behne et al.[107] have studied the changes in the elemental content of blood serum samples due to drying (90 °C/3 days) and freeze-drying, combined with ashing (active O_2) procedures. Although Se is capable of forming volatile compounds, no differences could be detected between both procedures.

3. Milk

Milk samples must be collected via mechanical pump according to the standard procedures described in the IAEA/WHO document.[108] All sample collection equipment must be plastic or polypropylene[109] and acid washed to prevent Se contamination. Care should be taken when sampling mature human milk for the estimation of Se concentration.[110] Although Smith et al.[110] did not find differences in Se content throughout the day, samples must be collected from different feeds during the same day. Also, when possible various samples were collected during the same feed.[111]

Milk or colostrum were freeze-dried[112-114] or frozen[115-118] in liquid nitrogen[110] or in solidified carbon dioxide[119] immediately after sampling. Then, the samples were stored at 14,[109] at 18,[120-122] at −20,[110] at −70 °C,[116-118] or kept in an ice bath.[123] After thawing the samples were heated to 40 °C and carefully mixed before analysis.[121]

4. Semen and Other Human Fluids

Human semen was kept at ~37 °C until delivery to the laboratory. Upon receipt, spermatozoa were separated from seminal plasma by centrifugation at 680g for a 15 min period. Aliquots of either specimen were then transferred to the polypropylene containers and stored at −20 °C until assay. Seminal plasma or spermatozoa not used for analysis were pulled and stored at −20 °C for use as unassayed controls and for precision studies.[124] Saeed et al.[92] centrifuged the semen at 1000 rpm for 10 min, immediately after sampling. The supernatant fluids were then kept frozen until required. Samples of amniotic fluid were placed in plastic vials, stored at −15 °C, and lyophilized before analysis.[125]

In conclusion, sampling procedures depend on the type of fluid that is to be analyzed. Body fluids must be sampled according to the standard procedures using plastic containers to prevent adsorption losses. These containers must be throughly cleaned with diluted nitric acid in order to eliminate possible Se adsorbed. Anticoagulants such as heparin or EDTA must be added when whole blood or plasma are going to be analyzed. Centrifugation is used to separate plasma or serum and ultracentrifugation is necessary for separating platelets and leucocytes from whole blood. Refrigeration can be used to store blood and urine samples for a few days. But most authors prefer to store the samples of body fluids frozen to −20 °C. Storage with lyophilization or desiccation techniques constitutes a previous step in NAA.

B. Sample Treatment

The main factor that influences the choice of sample preparation is the instrumental method chosen. Other important factors, such as type of body fluids or concentration levels, must be considered. In many analytical methods for Se determination in body fluids, a previous treatment of the sample is necessary to preconcentrate the analyte and/or eliminate interferences in its final determination. Only in neutron activation analysis, electrothermal atomic absorption spectrometry, and X-ray fluorescence techniques, can the previous treatment be eliminated.[126] A representative aliquot must be taken from body fluid. To minimize the sedimentation that occurs in urine and ensure constant sampling, urine was treated with NH_4OH and formaldehyde and allowed to stand for 24 h before analyzing. If sedimentation occurred, a suspension was produced by vigorous shaking. There is no statistical difference between treated and untreated urine samples.[54]

In the majority of the reported methods, the sample treatment is for the elimination of organic matter. The inconsistent results for Se determination in various body fluids can basically be attributed to incomplete conversion of native forms of Se or loss of Se during the oxidation of organic matter. With this treatment, selenides, organoselenium compounds, and elemental selenium (if present) are oxidized to selenite or selenate. Thus, these methods give the total Se content of the sample rather than the concentrations of specific Se-containing compounds. Prior separation procedures of Se compounds are necessary to speciation studies of Se. Gel chromatography has been employed to separate different fractions of selenoproteins[127] present in plasma, obtaining recoveries of 95−102%. Cation-exchange chromatography has been used to determine $TMSe^+$ and other Se compounds,[128] selenomethionine (SeMet),[129,130] and $TMSe^+$ [131] in human urine. Also, anion-exchange column can be used for determining seleno amino acids, $TMSe^+$, and selenite in urine or serum samples.[53,54]

This oxidative step can be carried out via dry ashing or acid digestion, but usually, acid digestion is recommended to minimize losses by volatilization.[132] Several conditions of acid digestion have been studied. Significant losses of Se have been observed in heating the sample with acid mixture (HNO_3−$HClO_4$) above 200−210 °C.[133,134] When the final digestion temperature was decreased from 210 to 125 °C, the blood Se concentration was 20% lower.[135] Also using the latter mixture, heating at 170 °C over 5 h 30 min produced large losses of Se. If the duration was less than 3 h 40 min the digestion would not be complete. Therefore, the duration of 4 h 30 min was chosen in the recommended procedure.[91] The loss of Se due to incomplete decomposition is considerably more serious than volatilization losses.[135] If the fuming temperature is controlled at 200−220 °C, and the fumes are prevented from running away from the flask, losses are only about 1% from 0.2 μg of Se.[136] Heating with an acid mixture (HNO_3−$HClO_4$) at 210 °C appears to be a most convenient method of decomposing the body fluids: The matrix was completely destroyed and no significant losses could be observed (except some urine samples).[133] However, procedures that include a digestion with HNO_3-H_2SO_4-$HClO_4$ mixtrure at maximum temperature of 310 °C have been recommended. The temperature was slowly raised to 140, 220, 250, 310 °C, and held at each of these temperatures for 15 min before the next increase. The final temperature was held 20 min and the final volume of digest was 0.5 mL.[137] With this sample mineralization, a grade destruction optimum was found to be efficient for the subsequent determination of Se with HG-AAS.[137−139] Geahchan and Chambon[140] carried out a study about the digestion time using different proportions of the mixture $HClO_4$−HNO_3. They concluded that 30 min after $HClO_4$ fumes no longer appear and 7.5/2.5 (HNO_3-$HClO_4$) are the optimum conditions. Although no losses of Se occur when digests in concentrated HNO_3, $HClO_4$, or H_2SO_4 are vigorously boiled,[62] appreciable losses can occur when allowed to evaporate to dryness. Losses of ^{75}Se in wet digestion (HNO_3−$HClO_4$) were observed at the end of the procedure when an excess of acid was evaporated. The addition of $MgCl_2$ to the digestion mix prevented the escape of ^{75}Se and thus permitted the total evaporation without any loss of Se.[132]

Acid digestion in an open procedure at atmospheric pressure and subsequent Se reduction shows no significant differences in relation to closed bomb-digestion under pressure[141−142] when a similar acid mixture is utilized. But the pressure decomposition[67,142] fails in so far as the matrix is not completely mineralized. Thus, difficulties are encountered not only in polarography[143] but also in EAAS as HG-AAS[144] on account of high background or strong foaming of the solution.[67] An apparatus for the programmed wet decomposition of organic samples has been developed. A large number of different sample matrices in a relatively short period can be carried out which is extremely useful for routine analysis.[145]

Nève et al.[146] have compared three wet digestion methods for the decomposition of biological materials for the determination of total Se and Se(VI). They recommended the Ihnat's wet digestion technique,[147] based on the use of $HNO_3-H_2SO_4-HClO_4$ mixture for the determination of total Se content. However, they did not find a reliable method of digestion for differentiation of Se(IV) and Se(VI) in biological materials. Complete oxidation of urine Se to Se(IV) requires use of HNO_3-HClO_4 and other mixtures such as $HNO_3-H_2O_2$, $H_2SO_4-H_2O_2$, or $HNO_3-H_2SO_4$ as well as continuous combustion and oxygen flask methods are not suitable.[148,149] This is due, at least in part, to the presence of $TMSe^+$ and SeMet in the human fluids (principally urine) which resists oxidation except with HNO_3-HClO_4.[150,151] $TMSe^+$ ion is not digested by concentrated HNO_3 and in this instance the digestion temperature should be raised (220−230 °C) by the addition of $HClO_4$.[152] Nitric acid can only be recommended for the predigestion for GC determination of milk samples[153] and other biological materials,[154] where $TMSe^+$ is not present. Also, the use of a mixture of $HClO_4$ and H_2SO_4 with sodium molybdate as a catalyst for the wet digestion of organic matter results in a very good recovery of Se in ED-XRF[155,156] and in electrochemical methods.[157−159]

However, other authors[59,160] point out that the digestion of urine and most biological materials for the determination of Se does not require the use of $HClO_4$. Also, overlapping peaks of unknown sign in the gas chromatogram[153] were produced when the sample was digested with HNO_3-HClO_4, consequently the measurement of the peak height was made more difficult. No difference was found in digestion efficiency between $HNO_3-H_2SO_4$ mixture and a $HNO_3-H_2SO_4-HClO_4$ mixture.[59,160] Therefore, the H_3PO_4 digestion procedure for the fluorimetric determination of body fluids has been proposed in order to eliminate the need for $HClO_4$, thus increasing the safety and convenience of the determination considerably.[161] The $HNO_3-H_3PO_4-H_2O_2$ method of sample digestion can be recommended as an effective alternative to HNO_3-HClO_4 to those who wish to avoid the use of the latter.[162,163] The use of nitric acid alone, gives many interferences and erroneous results because of incomplete mineralization of some organic Se compounds.[83,137,146,151,152,164] However, the significance of these earlier observations arises in only a small fraction of the total Se under normal conditions. Thus, suitability of the use of $HNO_3-H_2O_2$ system in the analysis of urinary Se depends on the actual quantitative significance of trimethylselenonium ion.[165] The unmodified $HNO_3-H_2SO_4$,[83] HNO_3,[151] and $HNO_3-K_2S_2O_8$[166] digestion procedures adopted for open digestion of the urine samples which were adequate for gas chromatography[151] and AAS[83,167] proved inadequate for the cathodic stripping voltammetric determination of Se.[168] However, a modified procedure using $HNO_3-H_2SO_4$ mixtures or $HNO_3-K_2S_2O_8$ enables adequate digestion of the sample material and retention of Se in a state amenable for determination of the element in most sample materials.[168] Watkinson[169] pointed out that all residual nitric acid must be removed if reduction of selenate to selenite is to be complete. Urea,[154] hydrochloric acid,[170] or formic acid[171,173] was added, and the samples were heated to decompose and remove any residual HNO_3.

Some authors[173,174] indicate that the acid digestion procedures do not often completely digest lipids in the sample. In order to eliminate lipids, some authors have extracted the digested samples with chloroform[171,172] or cyclohexane.[175] Due to the difficulty in the digestion process, recoveries of SeMet from blood were always lower than selenite. The presence of acid-resistant organic Se compounds in the erythrocytes of whole blood might explain the different behavior of blood and plasma toward acid mineralization.[135]

A few papers have recommended dry ashing which can be combined with acid digestion. Treatment of the sample with $Mg(NO_3)_2$ and HNO_2 in a programmable temperature muffle furnace has proved to be efficient.[176−181] These procedures are valid alternatives to more common destruction methods (often including $HClO_4$). Thus, Mattos et al.[181] have preferred dry ashing with respect to wet ashing on the basis of sensitivity, precision, rapidity, and cost in HG-AAS determination. However, Drabek and Kalouskova[132] have observed significant losses and low precision after dry ashing with HNO_3 and $Mg(NO_3)_2$. Recently, Wang and Pan[64] have utilized ashing (480 °C) in the determination of Se in whole blood by differential pulse polarography and catalysis. By standard addition techniques it was experimentally determined that 3% Se is lost in the ashing.[64] Some authors[67] have developed a method in which Se is evolved from organic materials, after adding a mixture with silicic acid, by combustion in oxygen under dynamic conditions. While concomitant elements that form sparingly volatile oxides remained in the ash, selenium dioxide volatilizes and condenses on a cold finger, whence it is delivered off with HCl or HNO_3 by boiling under reflux. The isolated Se is determined by HG-AAS or by differential pulse cathodic stripping voltammetry.

Recently a revolution in sampling digestion of organic and inorganic matrices has occurred with the introduction of the microwave oven. It is found to be faster, more controlled, more elegant, and more amendable to automation than conventional open-beaker, reflux, and closed vessel pressurized techniques.[45,182,183] A quartz high-pressure digestion tube has been employed in the determination of Se in human whole blood by computerized flow constant−current stripping at carbon fiber[184] or EAAS.[185,186] The pressure decomposition[187] in closed systems fails in so far as the matrix is not completely mineralized. Thus, difficulties are encountered not only in polarography[143] but also in EAAS as well as in HG-AAS[144] because of high background or strong foaming of the solution.[97] The high-pressure decomposition device made of Cr-Ni-Mo steel has been developed, in which organic samples can be burned in oxygen up to 90 bar.[188] A high temperature/pressure ashing at a temperature of 320 °C and pressure up to 100 bars is used to complete sample decomposition. This automatic decomposition is applicable to some biological samples.

To summarize, acid digestion is a destructive treatment which altered the original Se compounds present in the biological fluid. Thus, previous separation such as, gel filtration chromatography or ion exchange chromatography, of Se compounds is necessary for speciation studies. Acid digestion have two important problems: losses of Se due to incomplete mineralization and volatilization. Conditions of digestion treatment must be chosen as a function of body fluid and determination procedures. Different acid mixtures ($HNO_3-H_2SO_4-HClO_4$) have been used for acid mineralization. Maybe HNO_3-HClO_4 is the most adequate for urine samples with $TMSe^+$ because this ion resists oxidation except with that acid mixture. The use of H_3PO_4 acid can be a good alternative method to eliminate the need for $HClO_4$, which improves the Se determination in body fluids in terms of safety and convenience. Also, dry ashing combined with acid digestion with $HNO_3-Mg(NO_3)_2$ is a valid alternative. The use of microwave must be improved for complete mineralization.

II. Determination Procedures

A. Spectrofluorimetry and Spectrophotometry

1. Spectrofluorimetry

Spectrofluorimetric measurements utilize the fluorescence of the piazselenoles derived from selenite. All original species of Se present in the sample must be converted to selenite. An acid-digestion step is initially required for this technique. It has been reported that the extended boiling of selenite in $HClO_4$ may convert up to 60% of selenite to selenate.[189] Compounds of molybdenum added to the acid oxidant mixture for catalytic purposes can induce possible precipitation of 2,3-diaminonaphthalene (DAN) in the presence of sulfate, oxidation of DAN by molybdate, leading to loss of sensitivity.[140] Also, the process of purifying the molybdic acid increases the time of analysis.[190] Thus, the digested sample is heated with HCl to reduce the selenate to selenite. Then, piazselenole is derived from a reaction with DAN. The derivative is extracted from the aqueous phase with a hydrophobic solvent such as cyclohexane and measured in a fluorescence spectrometer with excitation wavelength set at 360 nm and emission wavelength at 520 nm.[189] Analytical aspects, such as sample treatment, detection limit, precision, and recoveries, corresponding to the main papers published about fluorimetric Se determination in body fluids, are presented in Table 1.

There are a few authors[194,204,205] who did not perform the reduction step. At 310 °C there was no difference between the results with and without the reduction step. This can be explained by the thermal instability of selenate.[137] Generally, in this step, HCl (4−6 M) is the reducing agent used but some laboratories reported the use of H_2O_2[195,206] or hydroxylamine.[136,155,196] Several experiments have demonstrated that the rates of reduction with HCl were almost independent of the concentration of chloride in the range 2 to 5 M.[207] Some workers[170,208,209] indicate reduction at room temperature, but most[62,93,140,189,192] heat using different temperature/time. The dependence of the reaction rate on the hydrogen ion concentration is very marked. The temperatures needed to reach 99.9% reduction in about 30 min are 105, 85 and 65 °C for 4, 5 and 6 M hydrochloric acid, respectively.[207,210] This reduction step is critical as boiling of Se in a HCl medium exceeding 6 M (final) may result in losses due to the formation of volatile Se species.[148,211] To avoid possible losses of Se from hot HCl solutions, the hydroxylammonium chloride method has been used, which also reduces Fe(III) into Fe(II), so eliminating interference by the former and preventing high-temperature oxidization of DAN.[136]

Many authors[104,140,150,212] have indicated that the pH optimum in the formation of piazselenole is between 1−2 or certain values between 1 and 2.[134,136,148,169,170,174,179,189,190,204,205,213] Bayfield et al.[197] have studied the pH control with diverse indicators in the reaction mixture following acid digestion of samples and preceding formation of the piazselenole complex. Maximal fluorescence response is achieved by using methyl orange as an internal indicator to establish an initial pH of 3 and, after addition of the DAN reagent in 0.1 N HCl, a final pH of 1.8. However other authors[93,200] indicate that it is not necessary to control pH during the complexing step or to protect the DAN from light. The fluorescence slightly increased with increasing pH between 1.0 to 2.4. Under the conditions in the procedure a distinct pH maximum could not be demonstrated.[174] There is no agreement about the optimal temperature/time relation for the formation of DAN−Se complex; 50 °C/30 min[77,192] or 15 min,[134] 75 °C/10 min,[136] 60 °C/30 min[179] or 20 min,[194] 110 °C/30 min,[97] etc., have been proposed. The inclusion of a complex of cyclodextrins with surfactants can exhibit a significant synergistic enhancement effect on the fluorescence intensity.[214] The fluorescence intensity of 4,5-benzopiazselenole is ~30-fold greater in presence of the surfactant sodium dodecyl sulfate (SDS)/β-cyclodextrin (β-CD) than in aqueous solutions.[214] Most authors have used cyclohexane to extract the Se−DAN complex, however decahydronaphthalene (decalin),[77,80] *n*-hexane,[104] or toluene[196] have also been employed. A complete extraction of piazselenole into cyclohexane was achieved by vigorous manual shaking in 30 s.[174,204] Stability of extracted Se-DAN complex in contact with aqueous phase is good. Storing for 1 week would result in an increase of a fixed level of fluorescence signal in all cases, including the blank. However, such an apparent increase did not appear to affect the Se results.[93]

There are not too many interferences in the fluorimetric technique because separation processes are carried out on Se. It has been reported that 0.1 M sulfate in the digest decreased the recovery of Se by about 10%.[169] Nitrous acid would give an increased fluorescence through the formation of 2,3-naphthotriazole with DAN.[212] However, no interference was found for the concentration range 0.04−0.2 M except

Table 1. Determination of Selenium by Spectrophotometry and Spectrofluorimetry

sample	treatment	detection limit ($\mu g\ L^{-1}$)	RSD % between-assay (within-assay)	recovery %	ref
	Spectrofluorimetry				
body fluids	$HNO_3/HClO_4$ digestion; HCl reduction; DAN, cyclohexane extraction	0.04 μg	– (3)	100.3	191
serum plasma	$HNO_3/HClO_4$ digestion; HCl reduction; EDTA, DAN, cyclohexane extraction		– (6.98 ± 0.78)	98.1–99.4	192
blood	$HNO_3/HClO_4/H_2O_2$ digestion; EDTA, NaF, DAN, cyclohexane extraction	2			193
urine	$HNO_3/HClO_4$ digestion; HCl reduction; NH_2OH-HCl, EDTA, DAN, cyclohexane extraction	0.394	– (1.8–6.3)	99.4 (90–105)	140
plasma	$HNO_3/HClO_4$ digestion; EDTA, DAN, cyclohexane extraction	0.01 $\mu g\ g^{-1}$			194
blood milk urine	$HNO_3/HClO_4$ digestion; HCl reduction; DAN, cyclohexane extraction	0.2 ng		101.2 – 98.5	62
urine serum	$HNO_3/HClO_4$ digestion; HCl reduction; DAN, cyclohexane extraction		2.67 (2.93)	99.6	170
blood	$HNO_3/HClO_4/H_2SO_4/H_2O_2$ digestion; EDTA, DAN, cyclohexane extraction		9.22	91.3	195
body fluids	$HClO_4$ digestion; NH_2OH-HCl reduction; EDTA, 3,3′-diaminobenzidine tetrahydrochloride, toluene extraction	0.006 $\mu g\ g^{-1}$	– (12.8)	94	196
blood plasma serum urine milk	$HNO_3/HClO_4$ digestion; HCl reduction; EDTA, DAN, cyclohexane extraction		10 (2)	100 ± 2.2 (97–100)	93
blood	$HNO_3/HClO_4$ digestion; HCl reduction; methyl orange, DAN, cyclohexane extraction		2.3–3.3	96–103	197
serum	$HNO_3/HClO_4$ digestion; HCl reduction; EDTA, DAN, cyclohexane extraction		7.5 (5.4)	96–104	97
blood urine serum	$HNO_3/H_2SO_4/HClO_4/H_2O_2$ digestion; HCl reduction; DAN, cyclohexane extraction	0.45 ng	5–5.7 (4.2)	100 (97–101)	174
blood	$HNO_3/HClO_4$ digestion; HCl reduction; EDTA, DAN, cyclohexane extraction		– (3.6)		198
blood	$HNO_3/HClO_4$ digestion; HCl reduction; EDTA, NaF, DAN, cyclohexane extraction	0.005	2.0	87.3 ± 1.6	134
blood	$HNO_3/HClO_4$ digestion; NH_2OH–HCl reduction; DAN, cyclohexane extraction	1.2 $\mu g\ L^{-1}$ of cyclohexane		98.8	136
milk	$HNO_3/HClO_4$ digestion; HCl reduction; EDTA, DAN, cyclohexane extraction		5		199
urine plasma	$HNO_3/HClO_4$ digestion; HCl reduction; EDTA, DAN, cyclohexane extraction	10	4.2–5.8	90–96	200
serum	$NO_3^-/HClO_4$ digestion; HCl reduction; plasma DAN, cyclohexane extraction	7	5.94 (1.79–2.11)	90–112	201
	Spectrophotometry				
body fluids	HNO_3 digestion; 4-nitrodiaminobenzene; toluene extraction	3 $\mu g\ g^{-1}$		98–105	202
body fluids	KBH_4 reduction in H_2SO_4/tartaric acid; $AgNO_3$/arabic gum complexation	0.04 $\mu g\ g^{-1}$			203

a slight precipitation in the cyclohexane layer at the highest concentration.[174] Iron is the most likely metal to interfere with piazselenole formation.[212] The addition of EDTA, NaF or oxalate to eliminate this interference[62,134] has been proposed. A ratio of 1:1 EDTA to Fe is employed for masking.[170,174] With Fe(II) there may be a partial reduction of Se(IV) by the iron–EDTA complex, but there is no interference from Fe(II) itself.[212] In order to eliminate interferences such as Fe, some authors[134] have proposed a back-extraction in concentrated nitric acid and extraction again of complex DAN-Se in cyclohexane. Among the more common ions at 1 mM concentration, only Pd(II) and Sn(IV) interfered in the fluorimetric determination in the presence of oxalate or EDTA.[212]

The procedure developed by Alfthan[174] is especially suitable for serial operation with a daily (8 h) throughput of 25 samples in duplicate. Similar results were obtained by Whetter and Ullrey,[192] 40–80 determinations in an 8 h period. Another method allows the determination of 50 samples in 3 h.[201] The method proposed by Koh et al.[93] can handle 200 samples per batch and is applicable to a wide range of biological samples.

Most authors show a high recovery with values up to 95% (Table 1). In the method proposed by Tamari et al.,[134] low values (87.5 ± 1.6%) have been observed but the precision is good (1.6%). To increase the recovery of Se in this method,[134] one can use two extractions plus two back-extractions, which increases the recovery to 99.7%. However, this method

Table 2. Determination of Selenium by Flame Atomic Absorption Spectrometry

sample	treatment	flame	detection limit ($\mu g\ L^{-1}$)	RSD %	recovery %	ref
urine	electrolytic deposition on a Pt filament (−1.0 V vs Ag/AgCl)	air−C_2H_2	5	10		219
blood	$HNO_3/HClO_4/H_2O_2$ digestion	air−C_2H_2		4.8	95.7−102	221
biological material	DDTC/MIBK extraction	air−C_2H_2	<100			222
urine	HNO_3/H_2SO_4 digestion; HCl reduction; $NaBH_4$	H_2−N_2[a]	2.0 ng	2	98	223
blood	$HNO_3/HClO_4$ digestion; antifoam; HCl reduction; $NaBH_4$	air−C_2H_2[a]	1.5 ng	3.5−6.2	95	224

[a] Hydride generation is used to introduce the sample in the flame.

is not recommended because it is too time-consuming and provides more opportunities for contamination. Alfthan[174] obtained a recovery of 98−101% for selenite and selenomethionine, values higher than those obtained for selenomethionine-enriched milk powder.[215]

Most methods for fluorimetric Se determination in urine analyze total Se. Recently, an analytical methodology for separation and determination of $TMSe^+$ and other Se compounds from human urine by cation exchange chromatography and fluorimetry has been developed.[216] The urine samples, adjusted to pH 2.2−2.4, were applied to a Dowex 50W-X4 column and then eluted with 4 M HCl. Five major Se-containing fractions were found. After applying the first fraction to an AG1-X8, it was separated further into two subfractions, an unknown peak and a minor peak of selenite.

2. Spectrophotometry

All spectrofluorimetric methods can be considered spectrophotometric methods too. The substantial difference between the two methods is the much poorer sensitivity, 50−1000 times, of the spectrophotometric technique. A review on spectrophotometric methods for determining Se and other trace elements in milk is presented.[217] The method proposed by Bem[202] is essentially the same as that proposed by Shimoshi.[153] This method can be applied to samples containing Hg, Cd, As, or Pb, obtaining a good recovery but the detection limit is 3 $\mu g\ g^{-1}$ much higher than in spectrofluorimetric methods. A simple and indirect spectrophotometric method for determination of Se with 2-mercaptoethanol has been developed. This reactant reduces Se(IV) to Se(0) and its excess can form a zerovalent Se complex that shows a maximum absorption at 380 nm.[218] The major interferent, As(III), can be oxidized to As(V) with H_2O_2 prior to the analysis, and the excess of H_2O_2 can be boiled off before the addition of 2-mercaptoethanol. Also, Fe(III) and Cu(II) can be removed by precipitation as their hydroxides precipitate in the working pH of between 10 and 13.

Recently, a UV spectrophotometric method based on hydride generation has been developed for determination of Se in biological samples such as urine, hair, yeast, or rice. Selenium was reduced to H_2Se by potassium borohydride and then reacted with $AgNO_3$−arabic gum to form selenide, which was measured at 246 nm. This method is simple and rapid and has a relatively low detection limit of 40 ng mL^{-1}.[203]

B. Atomic Absorption Spectrometry

Several authors[219−221] have developed methods for determining Se in body fluids by flame (air−C_2H_2 or H_2−N_2) AAS (Table 2). Electrochemical preconcentration has been described[222] in order to improve the sensitivity of the determination of Se by flame AAS. The sample is electrolyzed for 2 min at −1.0 V vs Ag/AgCl electrode and Se is deposited on a platinum spiral filament. Afterward the Se deposited on the filament is efficiently atomized by the hot flame (air−C_2H_2).

Many authors prefer more sensitive techniques. Two different methods for the determination of Se in the sub nanogram per milliliter range by AAS have been established by using the graphite furnace technique (EAAS) and the hydride generation technique (HG-AAS).[225] By comparing both techniques we can observe that the best absolute sensitivity and detection limit are obtained with the graphite furnace technique, typical values being 50 and 100 pg, respectively. The hydride generation technique allows a wider linear range of determination[164,225] and therefore offers the most favorable relative sensitivity and detection limit, 0.8 an 0.25 ng mL^{-1}, respectively.[226] In spite of the fact that the samples need to be 10-fold larger in the hydride generation technique, both procedures work well on microscale.[164] Although the graphite furnace technique permits the avoidance of predigestion treatment, the time required for an individual determination is between 1.5 and 2 min for the hydride technique and between 3 and 4 min for the graphite furnace techniques.[227] Furthermore, the EAAS method is too expensive, as the lifetime of the graphite tubes is short due to the high atomization temperature required.[228] Therefore, HG-AAS has been recommended for routine analysis;[226,228] 100−150 duplicate digestions can easily be accomplished within a working week.[226] Other authors[229] indicate that up to 80 samples ca be digested during the night and measured the next day. The relatively poor precisions, losses by volatilization and the interferences are the principal problems of both methods. The Se determination in body fluids by HG-AAS and EAAS has been correlated (r = 0.94) satisfactorily.[162,225]

1. EAAS

Selenium is analytically one of the most difficult elements in graphite furnace AAS. Table 3 shows the main analytical characteristics of direct Se determination by electrothermal AAS. Different oxidation states present in urine can exhibit substantially different thermal stabilities depending upon the matrix modifier used.[162,265] The addition of metal ions such as Cd, Sb, KIO_3, KI, Tl, Mn, Zn, Zr, and Th to the Se sample is beneficial due to the refractory selenides formed thus increasing the signal.[266] Mercury oxide[267] and salts of nickel nitrate[17,91,129,130,151,230-233,234,236,237,241,245,246,252,260,266,268-273] or chloride[225,274] are commonly used in preventing the volatilization of organically bound Se during the ashing stage. Oster and Prellwitz[225] proposed an original treatment with graphite tube immersed in a $Ni(NO_3)_2$ solution. Hughet et al.[237] dilute the serum sample in an albumin solution and add $Ni(NO_3)_2$. However, the absorption signal in absence of Ni is completely removed at concentrations of albumin higher than 35 g L^{-1}.[237] Other authors[164] confirm that nickel allows thermal pretreatment temperatures of up to 1200 °C. However, when a nickel modifier (10 and 25 μg Ni) was used some signal depression of up to 25% in serum samples was observed.[164,220] Also, Cu,[92,164,233,240] Mo,[260,266] Ag,[92,233,266] or Rh,[235,275] as well as Ni/Pt,[253,276,277] Ni/Mg,[278] Ni/Pd,[251] Pd/Mg,[199,257,279] Ir/Mg,[263] Cu/Pd,[280] Cu/Fe,[164] Cu/Mg,[247,258,281] and Ag/Cu/Mg[248] mixtures have been employed as matrix modifiers. The addition of Cu and Fe had no stabilizing effect, whereas in the presence of Ni and Ag ions, the ashing temperatures could be raised to 1050 and 1250 °C respectively without losing Se.[233] García-Olalla et al.[282] have studied the effects of various single and mixed-metal chloride, sulfate, and nitrate. Among the metals studied (Pd, Hg, Cd, Ni, Cu, Mg, Ag) the best enhancement in the Se atomic absorption signal was obtained by the mixed pair Hg–Pd chloride.[282] No loss in activity was recorded when ashing temperatures were raised to 900, 1100, and 1300 °C in presence of Mo, Ni, and Ag, respectively.[233,266] Recently, it was shown that the palladium modifier produces a Se peak height signal of at least twice than produced by nickel modification.[255,283] Itai et al.[284] insert a porous carbon plate (PCP) into a graphite furnace and use Pd as matrix modifier. Without Pd and PCP no peaks are obtained with serum samples, and very low peaks with standard solutions. When only Pd is used, although peak heights appear, the absorbance time profile is considerably affected by the matrix, and the absorbance decreases as the concentration of Pd increases. This reduction is caused by NO_3^- and Cl^- in the Pd solution. Ammonium acetate (0.1M) minimized the negative effect of NO_3^- and Cl^-.[284] The Mg/Pd system, an universal modifier, has a substantial equalizing effect on the atomization temperature (1900–2100 °C).[279] Palladium alone has essentially the same stabilizing power as the mixture with magnesium nitrate.[279] The presence of a small amount of ascorbic acid ensures that maximum signal enhancement and analytical precision are obtained.[254,255,261,283,285,286] Ascorbic acid reduces Pd^{+2} in solution to elemental Pd, so the mixing of the two solutions produces a precipitate to the metal; this can lead to the possibility of analyte loss. Automatic preinjection of the modifier (Pd) into the furnace was used to overcome these problems.[255,283] A measurable interference effect on the signal was only detected at a PO_4^{-3} concentration of 5.5 mmol L^{-1}, when a Pd solution was used as matrix modifier. Interference of Fe could not be observed with the method used.[262] Some authors[287,288] recommended the use of a "reduced" Pd modifier in which the Pd was either reduced chemically (e.g. H_2, ascorbic acid, or hydroxylamine hydrochloride), or thermally (treating the modifier at ca. 1270 K after injection into the furnace). Thus, Pd is mixed with ascorbic acid and ashed at 1200 °C before the determination by atomization at 2700 °C. Also, the authors found that when Pd was used the charring temperature could be elevated 700 K above that when Ni was used.

On the other hand, a study of the background absorption of whole blood and serum in EAAS has been carried out. If ashed whole blood is dissolved in HNO_3, the background absorption is very much lower than when HCl or NaCl is used.[289] In spite of the fact that some authors[164,252] use a previous dilution with HNO_3, other authors[266] have indicated the increase of Se(IV) volatility with this acid.

The EAAS technique was only used for plasma samples owing to the spectral interference using deuterium background correction, from iron and phosphate in whole blood and urine.[254,266,276,277,283,290,291] Maximum permissible ion/Se (wt/wt) ratios for Fe(II), Fe(III), and P(V) in determination of Se in various matrices have been established.[242] During the atomization step there are still severe interferences using D_2 background correction.[226] Iron at levels which would be commonly found in whole blood samples interfered significantly with the measurement of Se using the 196.0 nm analytical line and deuterium background correction. Not unexpectedly, the absorbances did not seem to be affected at 204.0 nm. The same interference trend was observed when using samples of plasma and serum, but the magnitude was different. In the case of whole blood the measured level of Se at 196.0 nm decreased as the added level of Fe increased.[292] Also, interferences were removed by adding EDTA;[240] but amounts greater than 40 mg resulted in the formation of a white precipitate, which reduced the sensitivity of the procedure. Saeed et al.[233,293] observed that when samples of whole blood or serum were ashed at 1050 °C in the absence of a stabilizing metal, and absorbances were measured at 204 nm, large positive signals were registered although no selenium was present. But at 196 nm, large negative absorption signals were recorded.[276] This overcompensation effect of the D_2 arc background corrector seems due to the presence of several iron absorption lines within the spectral band width.[273,290] When the volume of diluted sample is reduced (from 20 to 10 μL) no negative peak above background noise is obtained.[294] Calcium phosphate gives the same type of interference. Ce, Ni, Pd, Pt, W, and Zr depress these uncorrectable nonspecific signals significantly.[276] The sensitivity at 204 nm is not suitable for the direct determination of Se in whole blood.[276] These uncorrectable signals due to iron and phosphate at wavelengths below 220 nm[276,290] do not make the direct EAAS procedure recommendable for matrices rich in

Table 3. Determination of Selenium by Electrothermal Atomic Absorption Spectrometry

sample	treatment	background corrector	detection limit (μg L^{-1})	RSD % between-run (within-run)	recovery %	ref(s)
blood	oxygen flask combustion; cation exchange resin; HCl reduction; dithizone/CCl_4 extraction; $Ni(NO_3)_2$ modifier	D_2 lamp		2.0–4.2	96	230
blood	ash in O-filled flask; absorption in HCl; cation exchange column; dithizone/CCl_4 extraction; $Ni(NO_3)_2$ modifier			2–8.5	>93	231
plasma	$HNO_3/HClO_4/H_2O_2$ digestion; NH_2OH reduction; 4-chloro-1,2-diaminobenzene; toluene extraction		10			232
serum	dilution, Ni as matrix modifier	D_2 lamp	5 ng g^{-1}	4		233
blood						
serum	$HClO_4/H_2O_2$ digestion; $Ni(NO_3)_2$ modifier		50 μg g^{-1}		80–100	234
blood						
serum	$HNO_3/HClO_4/H_2SO_4/H_2O_2$ digestion; NH_2OH reduction; 4-chloro-1,2-diaminobenzene; toluene extraction; $Ni(NO_3)_2$ modifier		50	3.8	102–107	146
blood				4.7		
urine				7.3		
milk				4.0		
serum	HNO_3 digestion; urea, toluene; HCl reduction; 4-chloro-1,2-diaminobenzene, toluene extraction; $Ni(NO_3)_2$ modifier				105 ± 10	
urine					62 ± 7	
blood	$Rh(NO_3)_3$ dilution		7	5		235
serum	dilution, $NiCl_2$ as matrix modifier	D_2 lamp	11	5.7	100.4	225
serum plasma	HNO_3/Ni^{2+}	D_2 lamp	2.5	4.4	94.7–101	236
serum	dilution, albuminoid solution; $Ni(NO_3)_2$ modifier		11.9	5.7		237
blood	$HNO_3/HClO_4$ digestion; MIBK extraction			4.5–9.8		238
plasma	dilution, Ni^{2+} as matrix modifier	Zeeman	5	6.4		239
blood	$HNO_3/HClO_4$ digestion; HCl reduction; DAN, toluene extraction; $Cu(NO_3)_2$ modifier		5.3	5.6 (7.4)	99.9	240
serum	Triton X-100/HNO_3 dilution; Cu–Fe modifier	Zeeman L'vov platform	10	2.5		164
blood seminal fluid	Cl_3CCOOH precipitation; HNO_3 redissolution; guanidinium chloride as masking agent	D_2 lamp	0.94	3		92
urine	$HNO_3/Ni(NO_3)_2$	Zeeman	9		89–104	241
plasma	$Ni(NO_3)_2$ as matrix modifier		10	5.4		228
milk	$HNO_3/HClO_4/H_2SO_4/H_2O_2$ digestion; HCl reduction; EDTA, Cu^{2+} complexation; APDC-MIBK extraction	D_2 lamp	0.6	7.3	98.4	242
serum				6.0		
milk	$HNO_3/HClO_4/H_2SO_4/H_2O_2$ digestion; HCl reduction; Cu^{2+}, APDC-MIBK extraction	D_2 lamp			93.1	242
	$HNO_3/HClO_4/H_2SO_4/H_2O_2$ digestion; HCl reduction; EDTA, Ni^{2+}, APDC-MIBK extraction				82.1	
	$HNO_3/HClO_4/H_2SO_4/H_2O_2$ digestion; HCl reduction; EDTA, Cu^{2+}, NaDDC-MIBK extraction				95.1	
urine	cation exchange column separation; NH_2OH-HCl; dithizone/CCl_4 extraction		1 (SeMet)	3.7–6.2	82–102	129, 130
	HNO_3 digestion; $Ni(NO_3)_2$ modifier		0.5 (Se_r)	3.7–6.7	94–101	
milk	$HNO_3/HClO_4/H_2SO_4$ digestion; HCl reduction; EDTA, APDC-MIBK extraction; Cu^{2+} modifier	D_2 lamp		12.1		122
serum	Triton X-100/$Ni(NO_3)_2$		5.2	4.9	101	243
cerebrospinal fluid			0.75			
milk	$HNO_3/HClO_4$ digestion; HCl reduction; DAN, cyclohexane extraction organic Ag sulfonate/hydrocarbon oil		0.5	2		244
serum	HNO_3/H_2O_2 digestion; $Ni(NO_3)_2$ modifier			2.77		245
serum	Triton X-100 dilution; $Ni(NO_3)_2$ modifier	Zeeman	2	5		91
serum	Triton X-100 dilution; $Ni(NO_3)_2$ modifier	Zeeman	8	9.2–9.9		246
serum	Triton X-100, HNO_3 dilution; Cu–Mg modifier	Zeeman	5	7		247
plasma		D_2 lamp		(5)		
serum	Triton X-100 dilution; $Ag(NO_3)/Cu(NO_3)_2/Mg(NO_3)_2/HNO_3$ modifier	Zeeman L'vov platform	8.15	2.9 (1.8)	98 (Se_T) 100–103 (org. Se)	248
seminal plasma	HNO_3/HCl; Triton X-100; $Cu(NO_3)_2/Mg(NO_3)_2$ as matrix modifier	Zeeman L'vov platform	47.4	5.6 (4.7)		249
pig tissue	HNO_3/H_2O_2 digestion; Pd/$Mg(NO_3)_2$ modifier	Zeeman L'vov platform	1.4 ng g^{-1}	1.4	99 (92–112)	250

Table 3 (Continued)

sample	treatment	background corrector	detection limit (μg L^{-1})	RSD % between-run (within-run)	recovery %	ref(s)
blood	Triton X-100/$PdCl_2$/$Ni(NO_3)_2$ modifier	Zeeman	1.1		105.5 (95–117)	251
serum	Triton X-100/$Ni(NO_3)_2$/HNO_3 dilution	D_2 lamp	3.5	4.0 (3.4)	90–110	252
serum blood	Pt/Ni as matrix modifier		3	<10 (8)		253
serum	Triton X-100 dilution; $PdCl_2$/ascorbic acid as matrix modifier	D_2 lamp		2.7–5.7	95–102	254
blood	Pd/ascorbic acid as matrix modifier	Zeeman		5.9		255
milk	HNO_3/H_2SO_4 digestion, urea; Triton X-100; $PdCl_2$ as matrix modifier	D_2 lamp		20		256
milk	Triton X-100 dilution; Pd/$Mg(NO_3)_2$ modifier	Zeeman		18		199
blood	Triton X-100 dilution; HCl/Pd/$Mg(NO_3)_2$ as matrix modifier	D_2 lamp L'vov platform	10	2	99–102	257
serum blood	Triton X-100 dilution; Cu/Mg modifier	Zeeman L'vov platform	37 pg (sensitivity)	5.9 (5.7)	100	258
plasma serum	Triton X-100 dilution; $Cu(NO_3)_2$/$Mg(NO_3)_2$ as matrix modifier	Zeeman	37 pg (sensitivity)	5.7 (4.4)		259
serum	$TiCl_3$ reduction; APDC/MIBK extraction; Ni/Mo stabilizing agents		106 pg (sensitivity)	<5	95–114	260
plasma	Triton X-100 dilution; Pd/ascorbic acid as matrix modifier	D_2 lamp	5 ng g^{-1}	10.7		261
serum	Triton X-100 dilution; $PdCl_2$ modifier	D_2 lamp	0.03 ng	8.3 (3.8)	96.3–99.8	262
blood	oxygen combustion; Triton X-100 dilution; $(NH_4)_2IrCl_6$/$Mg(NO_3)_2$ as matrix modifier	Zeeman	35 pg (sensitivity)	peak surface: 2–3 peak height: 3–8		263
serum	Triton X-100; $Rh(NO_3)_3$ modifier	Zeeman		7.7		264

phosphate such as seminal fluids.[92] Also, interference by Fe and PO_4^{-3} was minimized by incorporating a 0.7 s delay in reading the absorbance.[281] The separation of selenium from phosphate by protein precipitation with trichloroacetic acid allows Se determination in blood, serum, and seminal fluid by EAAS after thermal stabilization.[92] The addition of Pt as matrix modifier has a significant effect on both the absorbance/time profile of iron and the formation of gaseous phosphate decomposition products volatilized from a graphite surface.[253,276,277]

The use of Zeeman-effect background correction will largely eliminate this interference and allows the Se determination in all types of biological matrices.[164,199,239,241,257,258,263,278,285,288,290,295–299] This may be the future method of choice in which sample consumption and preparation are kept to a minimum.[162] Oxygen ashing in graphite tube and Zeeman effect background correction are two essential steps for an accurate direct determination of Se by EAAS in the presence of Cu/Mg matrix modifier.[241,247] Many authors[164,220,248,249,258,278,279,300,301] have proposed the use of the L'vov platform to avoid spectral interference and improve precision in the EAAS determination. EAAS with Zeeman background correction and a L'vov platform incorporated,[278] is an accurate method by comparing with the definitive isotope dilution-mass spectrometry (IDMS) method.[171] But, the IDMS method is twice as precise as the EAAS method.[278] The electrodeless discharge lamp gives a 3-fold increase in sensitivity compared to a hollow cathode lamp which is due to it being the more stable and more intense source of the two.[220]

Some authors[146,232,260] prefer to eliminate the organic matter with digestion pretreatment, followed by a separation with cation-exchange resin[230] or by complexation/extraction with DAN/cyclohexane,[244] 4-chloro-1,2-diaminobenzene/toluene,[146,151,232] DAN/toluene,[240,270] dithizone/CCl_4,[230,302] or APDC/MIBK.[65,222,260,303] When Cu(II) was replaced with the same amount of Ni(II), the Se extraction recovery was markedly lowered (98.4 and 82.1, respectively).[242] Although the reason for this is unknown, the advantage of Cu(II) over Ni(II) as a stabilizer is further supported by these results in addition to the fact that the Cu(II)–APDC complex is more stable than the Cu(II)–EDTA complex.[304] It is necessary to reduce the possible Se(VI) formed in the oxidation step to Se(IV). The reducing agents employed were HCl,[230,244] hydroxylamine,[232,302] or TiCl^3.[260,303] Selenium can be reduced and precipitated with ascorbic acid. Then Se is redissolved and injected into EAAS.[305]

Norheim et al.[244] have studied the thermal stabilization of selenium as Se-DAN complex by matrix modification with silver or nickel organometallic reagents, and the application of EAAS. This method agrees well with fluorimetry and no systematic error was observed.

2. HG-AAS

In the HG-AAS technique, it is necessary to digest the sample first (Table 4). The severe and systematic imprecisions reported for this technique[318,319] are almost exclusively due to the use of improper sample decomposition. After digestion, selenate must be

Table 4. Determination of Selenium by Hydride Generation Atomic Absorption Spectrometry

sample	sample treatment	detection limit (μg L^{-1})	RSD % between-run (within-run)	recovery %	ref
urine	$HNO_3/H_2SO_4/HClO_4$ digestion; HgO, $NaBH_4$	5 ng	≤20		267
blood	$HNO_3/HClO_4$ digestion; HCl reduction; $NaBH_4$	5.0 ng	4.3	100.5 ± 4.7	209
urine	$HNO_3/Mg(NO_3)_2$; $NaBH_4$		3		306
urine blood	$HNO_3/HClO_4$ digestion; HCl reduction; $NaBH_4$	20 ng	5	94	307
urine	$HNO_3/H_2SO_4/HClO_4$ digestion; HCl reduction; $NaBH_4$	4	5–8	101 (94–117)	308
blood	HNO_3 digestion; $NaBH_4$	2 ng g^{-1}	4.8		309
milk	oxygen combustion/silicic acid; dissolution in acid medium; $NaBH_4$	2 ng	1.6		67
serum	$HNO_3/H_2SO_4/HClO_4$ digestion; HCl reduction; antifoam; $NaBH_4$	10	5.6	108.7	225
blood	$HNO_3/HClO_4$ digestion at 125 °C; HCl reduction; $NaBH_4$		3.4	100	135
	$HNO_3/HClO_4$ digestion at 210 °C; HCl reduction; $NaBH_4$		1.4	108.5	135
plasma blood	HNO_3/H_2SO_4 digestion; HCl reduction; $NaBH_4$	13.5	4.04 4.54	101.1 (90–108)	83
serum	HNO_3 digestion; HCl reduction; $NaBH_4$	20	3.0	101 ± 5	164
plasma blood urine	$HNO_3/HClO_4$ digestion; HCl reduction; $NaBH_4$	1 ng	4–8		228
urine	HNO_3 digestion; antifoam; HCl, NH_2OH–HCl reduction	4			310
blood plasma	$HNO_3/HClO_3/HClO_4$ digestion; HCl reduction; $NaBH_4$	1 ng	19.4		311
serum plasma blood	$HNO_3/H_2SO_4/HClO_4$ digestion; HCl reduction; $NaBH_4$	5	2–6	102 (Se_T)	137
urine				97–104 (SeMet)	137
serum	combustion; HCl reduction; $NaBH_4$	600 ng	4.25	85	312
	$HNO_3/HClO_4$ digestion; HCl reduction; $NaBH_4$		2.35		
blood	$HNO_3/HClO_4$ digestion; HCl reduction; $NaBH_4$	0.3 (sensitivity)	30		229
blood serum urine	$HNO_3/Mg(NO_3)_2$/HCl digestion; HCl reduction; $NaBH_4$	0.15	1–2.6	100–101 Se(IV) Se(VI)	313
	$HNO_3/H_2SO_4/HClO_4$ digestion; HCl reduction; $NaBH_4$		0.9–4.5	104–105 Se(IV) 103 Se(VI)	313
urine blood	$HNO_3/HClO_4$ digestion; HCl reduction; KBH_4; quinolin-8-ol, thiourea, or phenanthroline (masking agents)	0.21		95.5–100.8	314
urine	HNO_3/H_2SO_4 digestion; HCl reduction		– (2.4)		160
	$HNO_3/H_2SO_4/HClO_4$ digestion; HCl reduction		– (3.5)		160
blood urine	$HNO_3/HClO_4$/formaldehyde digestion; HCl reduction; $NaBH_4$		2	96–105	315
serum	$HNO_3/HClO_4$ digestion; $NaBH_4$	0.46	2.5	94.9–106	316
blood	$HNO_3/H_2SO_4/HClO_4$ digestion; $NaBH_4$	2 ng g^{-1}			317
plasma serum	$HNO_3/H_2SO_4/HClO_4$ digestion; HCl reduction; $NaBH_4$[a]	1.2	3.4–4.8 (4.2–5.8)	95–109	259
serum milk	$HNO_3/H_2SO_4/HClO_4$ digestion; HCl reduction; $NaBH_4$[a]	0.31	8–11		142

[a] Flux injection system is utilized.

reduced to selenite because the hydride generation is almost exclusively done with Se(IV). This step can be carried out boiling with HCl[83,137,142,162,210,229,314,320–323] or hydroxylamine hydrochloride.[232] The formation of selenides is then obtained with a stronger reductant agent such as sodium borohydride. The optimal concentrations of reagents for hydride generation were 1.0% $NaBH_4$ and 10 M HCl.[181] The main interferences of this method occur in these reduction steps.[226] Interfering ions like Co^{+3}, Fe^{+3}, Te^{+4}, and Cu^{+2} were masked by the addition of 1,10-phenanthroline, quinidin-8-ol, or thiourea.[314] The microcolumn with cation exchange resin (Dowex 50w) manifold for the selective retention of the Cu interferent was coupled with hydride generation manifold through a flow injection sample injection valve.[324] The limiting factor for the HG-AAS is foam formation after tetraborate addition which reduces the applicable volume of sample to 20 μL.[164,227] Complete mineralization is one way to avoid excessive foam formation. However, this makes sample preparation more complex and increases the risk of losses of Se.[164] SeH_2 is carried by an Ar stream to the heated silica cell of the AAS instrument and atomized at 780 °C; absorbance is measured at 196 nm.[321,325] HG-AAS gave a good agreement with the results obtained by fluorimetry and NAA.[323]

The automation of a flow-injection system for the hydride generation of Se and its subsequent determination by AAS has been described.[142,324,326] These methods permit accurate determination using a minimal amount of analytical reagent and sample within a short time. Interferences were found to be typically less in the FI system due to the lower

Table 5. Determination of Selenium by Emission Spectrometric Methods

method	sample	treatment	species	detection limit (μg L^{-1})	RSD %	recovery %	ref
AFS	blood	Triton X-100 dilution		50			327
HG-AFS	plasma blood	$HNO_3/HClO_4$ digestion; HCl reduction; $NaBH_4$		1.4 ng/25 mL sample	4−7		228
HG-AFS	urine	HBr/Br_2 digestion; BH_4^-	SeMet SeCys selenopurine	0.5		~100	328
	serum		Se(IV) Se(VI)	1.0			328
HG-AFS	serum	glass atomizer; BH_4^- electrically heated; silica tube atomizer; BH_4^-		0.24 0.14	6−8 4.3−6.8		329
ICP-AES	urine	HCl; polydithiocarbamate resin	Se_T	0.3	9.1−14.9		330
ICP	urine	HNO_3 digestion		1	0.5−0.7		331
ICP-AES	urine	HNO_3 digestion; yttrium as internal standard		47			332
ETV-ICP-AES	urine	HCl; polydithiocarbamate resin; HNO_3; Ni modifier	Se(IV) Se(VI)	area: 3 ng weight: 0.990 ng	2.5	84	333
HG-ICP-AES	urine	HCl; polydithiocarbamate resin; HNO_3/H_2O_2 digestion; HCl reduction; $NaBH_4$	Se(VI) Se(IV)	0.04	18−25		334
HG-ICP	serum blood urine	$HNO_3/HClO_4$ digestion; HCl reduction; $NaBH_4$		0.60	6.6		335
MIP-AES	serum	HNO_3 digestion; $HCCl_3$/APDC/DDTC extraction		120	1.6	101	185
HG-MIP-AES	blood	$HNO_3/H_2SO_4/HClO_4$ digestion; chromatographic column; $NaBH_4$		1.25 ng g^{-1}	5.5	99−105	336

tetrahydroborate concentrations used and the better kinetic discrimination.[326]

C. Fluorescence and Atomic Emission Spectroscopy

Table 5 shows the main papers about determination of Se in body fluids using these techniques. Atomic emission or fluorescence atomic spectroscopy provide multielement analysis capabilities with the accuracy and precision required for the determination of many trace elements in biological materials such as Se in body fluids. Direct nebulization of diluted blood samples in fluorescence atomic spectroscopy (FAS), produces a very low sensitivity.[327] However, the detection limit is improved when the hydride generation technique is introduced.[228,329] But, in this case, acid digestion and hydride generation steps are required, as described previously for HG-AAS.[228] No observed statistically significant (paired-*t*-test, $P = 0.73$) differences between the results obtained for the determination of Se in blood sera by HG-AAS and HG-AFS have been observed.[329] However, the first is preferred because it gives improved precision, detection limit, and extended linear calibration range. The use of the hydride generation technique coupled with nondispersive atomic fluorescence spectrometry has proved to be a sensitive analytical tool for the determination of the elements forming volatile hydrides such as Se. The atomizer consists of a simple electrothermally heated quartz cell to which the gases evolved during the tetrahydroborate reduction are transported.[328,337] The use of sensitive laser-excited atomic fluorescence spectrometry for trace element determination has been discussed.[338] The detection limit for Se determination in blood was 80 fg for 10 μL, using $Pd(NO_3)_2$ as a matrix modifier.[338]

Atomic emission spectrometric (AES) methods that are suitable for determination of Se include the use of hollow-cathode discharge (HCD) and the inductively coupled plasma (ICP) as excitation source. The utilization of a hollow-cathode discharge tube makes the detection of elements with high ionization energies possible, even elements such as Se. When it was applied to the direct analysis of dried serum, no severe chemical interferences were encountered. The Na content of the serum was lower than the concentration which can cause a decrease in the spectral line intensity.[213]

A most promising analytical technique in recent years has been the development of the plasma source for emission spectrometry. In ICP technique, no physical interferences were found,[339] but the Se 196.026 nm lines have a significant baseline structure in an aqueous matrix, which is greatly reduced when the optical path is purged with N_2.[332] Although body fluids can be introduced directly or after simple dilution, for many samples, the solutions prepared from the original samples required 10- to 100-fold dilutions, which makes the detection of Se in the original sample impossible.[340] Internal standarization with yttrium compensated the differences between the aqueous calibration standards and the undiluted urine specimens.[332] So, this method[332] can be used as a rapid screening method for trace analysis in human urine.

The use of a more sensitive system of sample introduction in ICP as hydride generation[334−336] or rod electrothermal vaporization has been recommended. The former technique is more useful for analysis of complex samples than the more widely used HG-AAS.[185,336] In the latter technique, an aerosol formed externally by electrothermal vaporization is transported to the ICP-AES.[333] With this direct method about 300−400 analyses in a day can be performed.[333]

Table 6. Determination of Selenium by ICP Coupled to Mass Spectrometry

method	sample	treatment	species	detection limit ($\mu g\ L^{-1}$)	RSD %	recovery %	ref
ICP-MS	urine	ammonium reineckate; anion exchange column; ^{82}Se spike; $HNO_3/HClO_4$ digestion; HCl reduction; APDC precipitation; HNO_3 redissolution	($TMSe^+$)	0.04 μg		76.8−87.0	42
pneumatic nebulization-ICP-MS	plasma urine		Se^{74}, Se^{77}, Se^{82}	20−60 ng	1		341
HG-ICP-MS	plasma urine	$NaBH_4$	Se^{74}, Se^{77}, Se^{82}	0.6−1.8 ng	1		341
HG-ICP-MS	plasma	HNO_3/H_2O_2 digestion; HCl reduction; $NaBH_4$	Se^{74}	0.9 ng (50 mL)	1		342
			Se^{77}	0.3 ng (50 mL)			
	urine	$HNO_3/HClO_4$ digestion; HCl reduction; $NaBH_4$	Se^{82}	0.2 ng (50 mL)			

But most authors prefer previous treatment and preparation of samples such as digestion,[185,331,332,336] extraction with APDC and DDC/chloroform,[185] and/or ion exchange chromatography.[330,332,334-336] Poly(dithiocarbamate) resin is capable of concentrating and separating Se from the urine matrix[330,333-335] prior to ICP determination, because the major elements (Na, K, Ca, and Mg) present in urine do not complex with this resin. The recovery of Se(IV) and Se(VI) is different with respect to pH, which suggests the possibility of differentiating between the these two oxidation states. Se(IV) recovery does not depend on the pH range studied (pH 1−10), however Se(VI) was only recovered at pH 2.0.[333,334] After chelation, the resin was digested with HNO_3,[333] or HNO_3/H_2O_2[334,335] and injected into the ICP system. This resin did not mask the Cu interference completely.[335] Adding Te(VI) to form copper telluride is simple, but the detection limit was degraded. In order to suppress the Cu interference on the SeH_2 evolution, a poly(acrylamidoxime) resin was added to the sample in the reaction vessel.[335] A condensation tube and Chromosorb 102 column were used to separate the analyte species from hydrogen evolved during the course of the generation reaction, and to separate the analytes from the condensed contaminants that cause spectral interferences.[336]

D. Mass Spectroscopy

Another alternative to improve sensitivity is the use of mass spectrometry[42,341,342] coupled to an ICP system (Table 6). The plasma is used as an excitation source to ionize a high proportion of the Se, and these ions are introduced directly into the mass spectrometer to provide the analytical signal in ion counts/time. A comparative investigation between pneumatic nebulization and continuous hydride generation as sample introduction methods for ICP-MS was carried out for isotopic analysis of Se. The signal to background ratios were 30−50 times greater for the hydride system than pneumatic nebulization. Measurements of the three stable isotopes ^{74}Se, ^{77}Se, and ^{82}Se, can be carried out on a routine basis in blood, plasma, and urine.[341]

In order to increase the sensitivity, several preconcentration methods have been applied in ICP-MS. An ICP-MS method to premit the isolation and measurement of $TMSe^+$ from 1 L of human urine was developed.[42] The method was based on precipitation of $TMSe^+$ with ammonium reineckate, preseparation with anion-exchange resin, and final acid ($HNO_3/HClO_4$) decomposition. Ratios of the isotopes ^{74}Se or ^{77}Se and ^{82}Se were used for quantification. The reliability of the method was tested against an HPLC procedure.[42] Lyons et al.[343] have developed a chromatographic separation to eliminate chloride from the serum samples which can interfere in the mass detector. Other methods involve wet oxidation, reduction to selenite with HCl followed by measurements of isotope ratios in the gas stream (H_2Se) generated from on-line reduction of selenite with $NaBH_4$.[335] Twenty samples or 100 analyte solutions can be readily processed per 8 h.[342]

E. X-ray Spectrometric Analysis

The use of nuclear techniques (XRF and PIXE) in the study of the role of trace elements in biology and medicine has been described.[3,344] A substantial number of papers have recently been published on the topic of multielement X-ray spectrometric analysis of body fluids, using both XRF, photon-induced X-ray fluorescence and PIXE, the proton-induced technique[88,345] (Table 7). The major advantages of using the EDXRF system for the determination of trace elements in biological tissues are the relatively small sample size, easy sample preparation, and the ability to perform rapid multielement analysis in a single measurement without destroying the prepared sample.[350,351,358] Sampling, storing, sample pretreatment, and experimental conditions for Se determination in human serum, plasma, and whole blood, by X-ray emission spectrometric methods are described.[359]

Direct XRF has limited sensitivity, and therefore a preconcentration step is necessary in order to bring the trace elements to detectable levels. Most treatments involved ashing[346] or freeze-drying[360] (Table 7). But when volatile elements such as bromine or selenium are analyzed, simply drying the serum aliquot on a thin carrier and ambient temperature[88] or simple freeze-drying[361] were chosen in order to eliminate losses of these elements in the sample pretreatment. Simple physical evaporation of blood plasma and serum leads to detection limits around 50 ng g^{-1} of Se[85,362] while freeze-drying of tissues followed by grinding and pelleting yields a detection limit of 60 ng g^{-1} of Se.[363] Of course, ashing for 400 °C for 5 h,[346] could reduce these detection limits but then losses of Se become slightly greater. Vos et al.[351] have studied the possibilities of using a chelating filter of 2,2′-diaminodiethylamine (DEN)[364,365] for

Table 7. Determination of Selenium by X-ray Fluorescence Spectrometry

sample	treatment	instrumental conditions	detection limit ($\mu g\ L^{-1}$)	RSD %	recovery %	ref
		(1) Photon Induced				
blood	ashing (400 °C/5 h) and pelleting	20 mCi ^{109}Cd source Si(Li) detector	80			346
biological materials	$HNO_3/HClO_4$ (215 °C/30 min); reduction to Se^0 with HCl and ascorbic acid; filtration on carbon	target XRF (2 cm^2) counting time 1000 s	20 ng 5 ng g^{-1} (9.6 cm^2) 10 ng g^{-1} (2.0 cm^2)	26 (25 ng mL^{-1}) 14 (130 ng mL^{-1})	97 ± 8	347
serum	drying and dissolving HNO_3		50 ng g^{-1}	3		348
serum	ashing (O_2); H_2SO_4/HNO_3 digestion; ion-exchange separation	target XRF		15–25	97–100	349
blood	$H_2SO_4/HNO_3/HClO_4$ digestion; $Na_2SO_3/SnCl_2$ reduction to Se^0; coprecipitation with Te			4.2	94.6	155
blood	$H_2SO_4/HNO_3/HClO_4$ digestion; $Na_2SO_3/SnCl_2$ reduction to Se^0; coprecipitation with Te	low powder transmission	140 ng g^{-1}			156
		Ag target, X-ray tube 27.3 KeV, 140 μA	26 ng g^{-1}			
		Mo target, X-ray tube 29 KeV, 150 μA	17 ng g^{-1}			
serum	drying	Si(Li) detector 33 mm^2 detector area	4	13.9 ± 0.5	102.4	350
serum	drying in ambient temperature	irradiation 40Kv-40mA 3000 s	100	10		90
urine	gradual heating from 130 to 460 °C/9 h				10 H_2SeO_4 30 $TMSe^+$	351
		(2) Proton Induced (PIXE)				
serum	freeze-dried	Y as internal standard protons and beams (5–35 nA, 3 MeV)	21 (sum spec) 75 (single spec)	69		352
serum	dry-ashed (60 °C/1 h)	$PdCl_2$ as internal standard 1.8 MeV/100 min 4 MeV/30 min Si(Li) detector	<10 μg g^{-1}	6		353
serum	dried to 30 °C under reduced pressure	Cl_3Y as internal standard 0.3–0.5 μA/10 h 100–200 μC/5–10 min Van der Graaff accelerator Ge(Li) detector	10	8–12		354
serum	H_2O_2/H_2SO_4 digestion; HCl/hydrazine reduction	Te as internal standard Si(Li) detector	3 ng	10		355
bile	drying in ambient temperature under reduced pressure	Ru as internal standard		47–54		356
serum plasma blood	H_2O_2/H_2SO_4 digestion; HCl/SO_2/hydrazine reduction; Te coprecipitation	Y as internal standard 3.2 MeV from 4 MV Van der Graaff accelerator Ge(Li) detector	2–3 ng g^{-1}	<6		357

preconcentrating the trace elements in urine, but amino functions formed stronger complexes with the trace elements than did the DEN filters. For multielement analysis of human urine, 25 mL samples doped with yttrium as internal standard were evaporated gently and then ashed up to 460 °C overnight.[351] Other authors[346] have recommended a heating of up to 400 °C during 5 h in a silica crucible. However, the recoveries of Se, added as H_2SeO_3, were only 10%.[351] Acid addition (HNO_3) increases the recovery of H_2SeO_3 to 30%, being only 10% the recovery for trimethylselenonium chloride.[351] Selenium precipitation from the digestion liquids used by Robberecht and Van Grieken[347] was based on the reduction of selenite by 4 M HCl and ascorbic acid to colloidal Se and on the subsequent absorption on activated carbon. The absolute detection limit of 20 ng of Se is not as low as that of the commonly used hydride generation AAS technique, but it is possible to take larger samples and the relative XRF sensitivity is then improved.[347] Also the detection limit is about 10 time less than that of an XRF procedure published by Raptis.[366] A selective reduction of selenium compounds with a mixture of $SnCl_2$ and hydroxylamine and coprecipitation with tellurium was used.[155,156,367] Other authors[367,368] have utilized APDC and Fe as a coprecipitant. After total precipitation (25 min) at pH between 3.1–4.6, the resulting deposit was filtered, dried, protected with a thin Formvar foil and irradiated with photons for 1000 s period. In another procedure,[369] plasma is diluted in a polyethylene glycol-20000 solution, containing Y and V as internal standards, and a portion is evaporated on a polypropylene film at room temperature. The residue is analyzed by EDXRF, with a molybdenum anode, a molybdenum filter, and a Si(Li) detector.

A system for routine trace elemental analysis by X-ray tube, consists of a Si(Li) detector with an associated pulse processing system and a minicomputer.[350,361,370] Using the proposed method, Se and Rb concentrations are significantly higher than reference values.[361]

Holynska and Markowicz[156] obtained a better detection limit when they used a Mo target (tube 29 KeV, 15 μA) as primary radiation relative to excitation by 238Pu 100m Ci or Ag target (tube 27.3 KeV, 140 μA) excitation.

PIXE. The main advantage of PIXE is that one can measure automatically many elements of biological and medical interest in a quick single run, with little sample preparation.[354,371,372] The measurements can be carried out with or without sample treatment. Plasma or serum were treated with a preconcentration technique reducing Se(IV) or Se(VI) to Se(0) with HCl reflux or hydrazine dihydrochloride using tellurium (600 μg mL^{-1}) as coprecipitant and internal standard.[355,357,372] Sometimes YCl_3[352,354,357] or $PbCl_2$[353] have been employed as internal standard.

When no sample treatment is done, neither Se nor Pd losses take place during storage, dry-ashing (120 °C/30 min) and photon irradiation.[353] Good measurement precision requires a compromise between counting statistics, limitation of beam current, and reasonable data collection time.[353] Low-temperature ashing (LTA) procedure using oxygen plasma provokes losses of about 30–35% for different biological materials.[373] The analysis of 20 to 30 samples for the proposed method requires one day of chemical preparation and one day of instrumental analysis.

Once the sample was prepared it interacted for approximately 100 min with a beam of protons obtained by a Van der Graff accelerator (ca. 1.8 MeV) and the X-ray emission was recorded by a Si-(Li)[353,355,372,374] or Ge(Li) detector[354,357,375] connected to a multichannel spectra analyzer.

The only new trace elements which could be detected on the composite spectrum were Se and Sr. A systematic analysis of the Se peak in each single spectrum yields a standard deviation of 69% which means an absolute error of the order of 100%. This is not surprising since the Se peak is hardly visible above the background in all single spectra.[352] A combination of high-energy photon activation and low-energy photon detection provides the useful complementary method in trace element analytical chemistry.[375] However, detection using X-ray seems to be preferable for some elements such as Se than detection using low energy.[375]

Methods such as X-ray fluorescence, neutron activation analysis where decay time is often difficult to match for convenient elemental profiling, and secondary-ion mass spectrometry are also multielemental but currently do not seem to be as favored as PIXE, at least for trace elements.[354] Both the PIXE and XRF methods have been shown to be reliable techniques for Se determination provided an appropriate preconcentration method for each one is used. However, single values differ in some cases by more than 25%. These discrepancies are probably due to the fact that the targets, are too thin to be suitable for the determination of the total weights of Se and Te by the XRF techniques.[372]

F. Neutron Activation Analysis

In general, the determination of Se and other trace elements by neutron activation analysis has many desirable features including high sensitivity, reduced sample manipulation, multielement capability, and the flexibility to allow either short-lived (^{77m}Se) or long-lived (^{75}Se) isotopes to be utilized for the determination of elements. NAA started as a single-element technique when only Geiger-Muller counters and NaI(Tl) detectors were available. However, with the availability of multichannel analyzers and high-resolution Ge(Li) detectors, it has become a true multielement technique. Main literature data on Se determination by NAA in body fluids are presented in Table 8.

The long-lived radionuclide ^{75}Se has been used more often because its half-life allows chemical separation, but its activity can be measured only after long irradiation, long delay, and long counting time. This makes the measurement too expensive and limits the number of possible samples.[418] These inconveniences can be avoided by the use of the short-lived radionuclide ^{77m}Se. The determination of ^{77m}Se allowed the time of analysis to decrease significantly from 3 months for ^{75}Se to approximately 2 days.[400,408,419] Other alternative nuclides such as ^{76m}Se [125] and ^{81m}Se [339] have been employed. Thus, during a normal working day, the number of Se analyses that can be run approaches 100[407] or 150[397] samples. A method based on radiochemical neutron activation analysis is described which allows accurate measurement of stable isotopes, ^{74}Se, ^{76}Se, and ^{80}Se in body fluids.[420]

Instrumental NAA (INAA) does not involve any chemical separation (Table 8), and irradiated samples are simply counted over a period of time to get information about the desired elements. Different instrumental nuclear techniques, namely INAA, IPAA (photon activation analysis) and PGAA (prompt γ-ray activation analysis) were compared for Se determination in whole blood and plasma samples. Selenium could not be determined by PGAA because of low activities.[406] The use of reactor epithermal neutrons via long-lived isotopes provides accuracy, reliability, and detection limits similar to conventional thermal NAA. But the time required by the former is shortened by a factor of 3–4, because the waiting time used in the thermal procedure is too long and leads to poor precision because of low count rates.[373] Also, a substantial reduction of ^{32}P background activity is observed in epithermal NAA.[373] The advantages of the irradiation containers and their impurities have been discussed.[44,46,382] Quartz or silica ampules have been used;[72,94,125,373,376,377,379,381–383,389,390,392–395,410,412,414,415,421,422] polyethylene capsules,[53,54,268,379,380,387,394,410,411] and other polythene containers,[391,395,401–403] polystyrene,[53,54,405] and others[114,406] have also been used. Polyethylene capsules were lined with pure aluminum, because they were damaged during irradiation;[411,416] also quartz vials can be capped with aluminum foil.[72]

Direct Se determination in body fluids by INAA implies the presence of large amounts of Na, Cl, and P, whose radionuclides can contribute to the activity under the ^{77m}Se photopeak.[397,418] Interferences due

Table 8. Determination of Selenium by Neutron Activation Analysis

		instrumental conditions								
sample	treatment	flux ($n\ cm^{-2}\ s^{-1}$)	irradiation time	decay time	counting time	nuclide	detector	detection limit ($\mu g\ L^{-1}$)	RSD %	ref(s)
			(1) Instrumental Neutron Activation Analysis							
serum	dry or ash; $HNO_3/H_2SO_4/HClO_4$ digestion	7×10^{13}	10 d	3 month			Ge(Li)		4.8–5.7	107
urine	lyophilization	1.8×10^{12}	14 h	15 d		^{75}Se	Ge(Li)			46
urine blood		2.6×10^{12}	7–14 min	25–30 d		$^{75}Se_T$	Ge(Li)	0.001		376
urine blood		2.6×10^{12}	7–14 h			^{75}Se	Ge(Li)	7 ng g^{-1} wet weight		377
blood	freeze	1.4×10^{12}	4–6 h	1 wk			Ge(Li)		10	378
serum	lyophilization	1×10^{12}	2 min	1 min to 6 h	30–60 min		Ge(Li)		1.6–10.1	379
		2×10^{12}	8 h	2, 6, 15, 30 d	15 min to 5 h					
blood		4×10^{12}	7 h	1 wk		^{75}Se	Ge(Li)			380
serum	lyophilization; $HNO_3/HClO_4$ digestion; post-irradiation	1×10^{13}	12 d	20 d	6–15 h	^{75}Se	Ge(Li)		15.4	381
serum plasma	dried at 50 °C	5×10^{13}	10 d	3 month		^{75}Se	Ge(Li)		1.9	94, 382, 383
milk	dried at 70 °C, 24 h and at 100 °C, 8 h	5×10^{13}	48 h	60–90 d		^{75}Se	Ge(Li)		10	26, 384
serum										385
blood									5	
serum blood sperm	$H_2SO_4/HClO_4/HBr$ digestion; benzene/phenol extraction or HBr/HCl distillation	2.3×10^{12}	100 h			^{75}Se		2 ng		386
serum	lyophilization	$(5-10) \times 10^{11}$	4–6 h			^{75}Se	Ge(Li)			387
serum	lyophilization	6.5×10^{13}	100 h	2 wk			Ge(Li)			388
blood	lyophilization	5×10^{12} [a]	18 h	7 d	3000 s	^{75}Se	Ge(Li)	2 ng g^{-1}	10	389
		5×10^{12}						8 ng g^{-1}	21	
plasma		8×10^{13}	5 d	6–15 wk		^{75}Se	Ge(Li)			390
milk		2.6×10^{12}	10–14 h				Ge(Li)	1 ng g^{-1}	6.7	111
serum	lyophilization; HNO_3 digestion; gel permeation chromatography	5×10^{12}	12 h	1–3 w		^{75}Se	Ge(Li)			391
blood	lyophilization	2×10^{13}	30 h	2 w	50 min	^{75}Se	Ge(Li)			392
serum	dried at 50 °C, 24 h	5×10^{13}	72 h	6–8 w	4–8 h		Ge(Li)			72
plasma	dried at 50 °C	3×10^{13} [a]	24 h	21 d	7200 s	^{75}Se	Ge(Li)	100 ng g^{-1}	10–18	373
urine				30 d				700 ng g^{-1}	2.2–14	
plasma		5×10^{21}	5 d	7 months				20 ng g^{-1}	13–15	
urine								50 ng g^{-1}	0.34–6.9	
blood	lyophilization	7×10^{13}	24 h	4 w			Ge(Li)			393
serum		1×10^{13}	15 h	several weeks	3000 s	^{75}Se	Ge(Li)		2.4	394
blood	lyophilization; HNO_3 digestion	5×10^{12}	7–9 d	40 d	4 h	^{75}Se	HPGe			395
serum	lyophilization	2.2×10^{13}	265.5 h	1 month			^{75}Se	Ge(Li)		396
serum	lyophilization	2.2×10^{13}	30 h	1 month	1 h	^{76}Se	Ge(Li)			125
serum urine	dialysis; lyophilization	3×10^{11}	20 s	20 s		^{77m}Se	NaI(Tl)	30	10	103, 397–399
blood	lyophilization	2.3×10^{12}	20 s	3 s	19 s	^{77m}Se	Ge(Li)	50–65 ng g^{-1} dry weight	10.6	400, 401

Table 8 (Continued)

		instrumental conditions								
sample	treatment	flux ($n\ cm^{-2}\ s^{-1}$)	irradiation time	decay time	counting time	nuclide	detector	detection limit ($\mu g\ L^{-1}$)	RSD %	ref(s)
blood		1×10^{14}	5 s	15 s	20 s	^{77m}Se	Ge(Li)	300	8.9	402
serum								70	16	
	lyophilization							50	9.8	
	dialysis; lyophilization							5	2.6	
serum	HNO_3 digestion; infrared drying	5×10^{11}	10 s	10 s	600 s	^{77m}Se		42	4.2	268
blood										
serum		1×10^{14}	5 s	15 s	25 s	^{77m}Se			8.5	102
biological fluids	$HNO_3/H_2SO_4/H_2O_2$ digestion; HCl reduction; HBr; phenol/benzene extraction; chromatographic separation	1×10^{14}	5 s	15 s	20 s	^{77m}Se	Ge(Li)	2 ng		403
plasma	dialysis; lyophilization	1×10^{14}	5 s	15 s	25 s	^{77m}Se	Ge(Li)	5 ng	14–58	404
semen										
urine	formaldehyde	3.1×10^{11}	20 s	20 s	20 s	^{77m}Se	Ge(Li)	10 ng g^{-1}		53, 54, 405
serum										
urine	formaldehyde; anion-exchange column (Cl^-)	3.1×10^{11}	20 s	20 s	20 s	$TM^{77m}Se^+$ $^{77m}SeO_3^{2-}$	Ge(Li)	10		53, 54
serum										
urine	LiOH; o-phthalaldehyde (OPA)/2-mercaptoethanol derivatizing; anion-exchange column (NO_3^-)	3.1×10^{11}	20 s	20 s	20 s	^{77m}Se (total selenoamino acids)	Ge(Li)	40		54[b]
	LiOH; $Ba(NO_3)_2/(NH_4)_2SO_4$ column precipitation/ coprecipitation; OPA/2-mercaptoethanol derivatizing; anion-exchange column (NO_3^-)					$^{77m}SeO_3^{2-}$ ^{77m}Se total selenoamino acids				
plasma	lyophilization	$(1-5) \times 10^{13}$	15 s	18–20 s	20 s	^{77m}Se	Ge(Li)		18.2–19	406
blood										
milk	lyophilization	5×10^{13}	2 s	5 s	10 s	^{77m}Se ^{75}Se	Ge(Li)	2 ng	15	407
serum										
serum	$HNO_3/H_2SO_4/H_2O_2$ digestion; HCl reduction; $Pb(NO_3)_2$ coprecipitation; APDC complexation	1×10^{12}	30 s		60 s	^{77m}Se	Ge(Li)	17	3.2	394
serum	ultrafiltration; residue Se-protein	2×10^{13}	15 s	20 s	20 s	^{77m}Se	Ge(Li)	0.08		408
plasma	dried in vacuum	5×10^{13}	15 s	20 s	20 s	^{77m}Se	HPGe			395
blood	dried	1.7×10^{13}	15 s	20 s	20 s	^{77m}Se	HPGe			409
(2) Radiochemical neutron activation analysis										
blood	lyophilization; H_2SO_4/H_2O_2 digestion; HBr distillation; Dowex column (HCl)	1×10^{13}	48 h				NaI(Tl)		7.4	410
blood	HNO_3/H_2SO_4 digestion; H_2SO_4/HBr distillation; HCl/H_2SO_3 reduction	1×10^{13}	20 h	2 d		^{75}Se	Ge(Li)	8 ng g^{-1}	35	411

urine	evaporation; HNO_3/H_2SO_4 digestion; ascorbic acid precipitation	5 × 10^{13}	1 h	5 d		^{75}Se	NaI(Tl)	0.6	10	412
milk	lyophilization; oxygen flask combustion HCl/H_2SO_4; DDTC/toluene extraction	2 × 10^{12}	20–40 h			^{75}Se	NaI(Tl)			114
plasma urine	$HNO_3/H_2SO_4/H_2O_2$ digestion; HCl reduction; $Fe(NO_3)_3$, APDC precipitation; HNO_3 redissolution	5 × 10^{12}	3 d	1 wk	1000–2000 s	^{75}Se	Ge(Li)		12.2 16.4	339
serum	NH_2OH precipitation	2 × 10^{13}	7 d	1–2 wk	80 min	^{75}Se	Ge(Li)	0.2 ng		413
blood	lyophilization; HNO_3; HCl reduction; $HCCl_3$ extraction of Se-2-mercaptobenzothiazole; desiccation	1 × 10^{13}	7 d	27 h		^{75}Se	NaI(Tl)	4.8 ng	6.9	414
milk	dried at 65 °C; ethyl α-(isonitrosoaceto)acetate precipitation; $HNO_3/HClO_4$ redissolution	1 × 10^{13}	5–7 d			^{75}Se	NaI(Tl)		20	415
blood	$HNO_3/HClO_4/MgCl_2$ digestion; HCl reduction; NH_2OH/EDTA; Se-*o*-phenylenediamine, toluene extraction	2 × 10^{13}	20 h	23 d	3000–6000 s	^{75}Se	NaI(Tl)	0.5 ng	10	416
biological fluids	HNO_3/H_2SO_4 digestion; APDC/$HCCl_3$ extraction; electrophoretic separation	5 × 10^{13}	14 d			^{75}Se				417
plasma	$HNO_3/H_2SO_4/H_2O_2$ digestion; HCl reduction; $Fe(NO_3)_3$; APDC precipitation; HNO_3 redissolution	7 × 10^{12}	30 s	20 s	30 s	^{77m}Se			8.2	339
urine									14.9	
plasma	$HNO_3/H_2SO_4/H_2O_2$ digestion; HCl reduction; $Fe(NO_3)_3$; APDC precipitation, HNO_3 redissolution dithizone/$HCCl_3$ extraction; $SnCl_2$	5 × 10^{13}	10 min		1000 s	^{81m}Se	Ge(Li)		13.2	
urine									13.8	

[a] Epithermal neutrons. [b] Molecular-INAA.

to ^{23}Ne, ^{24}Na, ^{18}O, and ^{38}Cl were minimized by dialysis and lyophilization of the sample, and by using a NaI(Tl) detector.[103,397–399,402] The lyophilization step removes the water and greatly reduces the ^{19}O observed in the irradiated sample, and significantly enhances the measurement precision.[402] A decay time of a total of 3 months was sufficient to reduce the intensit of the γ-rays of the ^{24}Na and ^{82}Br and the from the ^{32}P β-rays.[94,382] The decontamination studies using ^{63}Cu, ^{51}Sr, ^{203}Hg, ^{59}Fe, ^{115}Cd, ^{65}Zn, ^{45}Ca, ^{60}Co, ^{24}Na, ^{42}K, ^{65}Mn, ^{99}Mo, ^{125}Sb, ^{75}S, and ^{32}P were carried out and interferents were not found.[415]

In order to reduce interferences and increase precision, several methods of radiochemical (RNAA) separation of Se have been described. Wet destruction of the sample with 12 M HNO_3 removes the interference from ^{38}Cl.[268] It is possible to use all the aromatic orthodiamines, which form selenodiazoles extractable to organic solvents.[416,423] The optimum conditions were 2×10^7 ng (*o*-phenylenediamine), 5×10^2 to 5×10^5 ng of Se mL^{-1}, pH 0.65–1.0, reaction time 2-h at 20 °C approximately, and toluene as an extraction efficiency solvent; the extraction using cyclohexane, heptane, or benzin was lower (<80%) than it was using the latter solvent.[416] Oxygen flask combustion combined with carbamate extraction into toluene has been proposed.[114] Also, another extraction system, 2-mercaptobenzothiazole into chloroform from HCl solution, has been developed.[414] After acid digestion, there are methods that involve distillation in HBr/HCl followed by further purification.[386,411] Precipitation of Se° with ethyl α-isonitrosoacetoacetate,[415] or APDC[339,394] can be used.

Speciation studies of Se have been carried out using previous separation of Se species and final measurement by NAA. Thus, Woittiez[391] separated six protein fractions of human serum by gel permeation chromatography (GPC). A method has been developed for the simultaneous determination of several Se species in serum[54] and urine[53,54,405] by anion exchange chromatography and molecular-NAA. The pH was adjusted to 10–11 in order to dissociate seleno amino acids from possible protein-binding sites. The elution of $TMSe^+$, SeO_3^{2-}, SeO_4^{2-}, selenomethionine, selenocystine, and selenocysteine has a recovery of ~100–101%, but the elution of SeMet occurs with a broad peak and with a significant fraction remaining on the resin. This vitiates any quantitative measurements of the various seleno amino acids.[53] In another paper, Blotcky et al.[54] have performed an optimized method that also allows the determination of total seleno amino acids in urine and blood serum. Because the selenite ion was found to interfere with the analysis, two separate procedures were developed for the determination of total seleno amino acids. For samples with nondetectable SeO_3^{2-}, a precolumn derivatization of amino acids with *o*-phthalaldehyde and 2-mercaptoethanol followed by anion- exchange chromatography was carried out. When detectable SeO_3^{2-} exists, a previous precipitation/coprecipitation step of SeO_3^{2-} by $Ba(NO_3)_2$ must be incorporated in order to eliminate the interferent SeO_3^{2-}.

NAA with isotope dilution techniques,[424] could be considered a definitive method. Of particular interest is the use of NAA to monitor stable isotopes in human metabolic studies. Being nonradioactive, stable isotopes can be used as metabolic tracers in all subjects, including high-risk groups such as pregnant women and infants where the use of radioisotopes is contraindicated.[418,425] A method combining radiotracer techniques with paper electrophoresis has shown that Se can be almost completely released from the biological matrix into ionic form as selenate after a simple pressure digestion.[417]

G. Chromatographic Methods

As can be seen in Table 9, the chromatographic techniques for Se analysis in body fluids have been divided in gas chromatography and high-performance liquid chromatography using column (HPLC) or thin layer (HPTLC) as stationary phase.

1. Gas Chromatography

At present, methods for determining Se by GC are based mainly on the quantification of a piazselenole formed in the reaction of Se (IV) with a chosen *o*-diamine in an acidic solution, using the sensitive electron capture detector (ECD).[429,441] The *o*-diamines more usually used include 2,3-diaminonaphthalene and 4-chloro, 4,5-dichloro, 4-nitro, and 3,5-dibromo derivatives of 1,2-diaminobenzene.[429,441] The best reagent found so far for Se(IV) determination by gas chromatography with ECD is 1,2-diamino-3,5-dibromobenzene, while 1,2-diamino-4-nitrobenzene is the more effective of the commercially available reagents.[426] The introduction of a second electrophore, such as the chloro or nitro group into the molecule, considerably improves the sensitivity, allowing the detection of amounts of Se in the order of picograms.[426,427] Also, this second electrophore may react quantitatively with Se(IV) in a wider range of pH.[442] Three different derivatizing reagents, 4-nitro-*o*-phenylenediamine (NPD), 3,5-dibromo-*o*-phenylenediamine (DBPD), and 4-(trifluoromethyl)-*o*-phenylenediamine (TFMPD) were investigated.[163] All three reagents performed equally well in terms of precision and accuracy. But, TFMPD was the best from the point of view of GC behavior and memory effect in the GC-MS system.[163] However, it is necessary to remove these compounds before the extraction of piazselenol, because they may produce unknown peaks in the chromatograms.[152] A procedure based on the reaction between Se(IV) and acetophenone[443] has been reported. Simultaneous dimethyl selenide and diselenide have been determined by gas chromatography using a multichannel nondispersive atomic fluorescence spectrometric detector and a miniature flame ($Ar-H_2$) as the atomizer.[444]

An indirect method for SeMet determination has been developed.[430] In the presence of $SnCl_2$, SeMet reacts with CNBr to form CH_3SeCN and, after extraction with Cl_3CH, is acid-digested to form Se(IV). Then, Se(IV) is derivatized with 4-nitro-*o*-phenylenediamine and determined by GC-ECD.[430] Also, an isotope dilution GC–MS methods for Se determination in body fluids have been described.[163,171,172,445] Several hydrolysis methods have been compared for determining selenoproteins.[446] After hydrolysis, SeMet was determined by reaction with CNBr and GC-FID (flame ionization detector) as described by Wu et al.[447] A known amount of an enriched Se isotope (^{82}Se) is added as internal standard. ^{75}Se or ^{76}Se was used as internal standard and the isotopic ratio of ^{80}Se to ^{82}Se was measured by dual ion monitoring making it possible to determine Se at the μg L^{-1} level.[163,171,172,445]

When total Se is determined, higher and lower oxidation states of Se must be converted to quadrivalent form.[154,429] In order to reduce selenate to selenite, acid digests from body fluids are boiled with HCl.[152,154,163,171,336,427–429] Concentrated HCl in reflux should be avoided since Se can be lost as volatile chloride adducts.[153,427] In general, from pH 2.0 to pH 0, where full protonation of the reagent begins, the height of the piazselenole peak is a constant maximum height.[426,429,441] The formation of piazselenole beginning from 1,2-diamino-3,5-dibromobenzene was quantitative after heating for 2 min at 60 °C.[152] In order to form the corresponding piazselenoles, some authors allow to stand Se and phenylenediamines at room temperature for 4 h (1,2-diamino-4-nitrobenzene)[427] or for 30 min (TFMPD).[163] Most reaction times for formation of piazselenoles are found between 60–90 min.[441] So, although 20 min seems to be enough in the formation of 5-(trifluoromethyl)-piazselenole at a range of temperature of 20–60 °C, a reaction time of 1 h was established for analytical purposes to allow for the variation in Se levels of the samples.[429] Although the piazselenoles can be extracted quantitatively at any pH, a low pH (usually below 1) is preferred, to avoid coextracting the excess of reagent.[426] The piazselenoles formed are then extracted by shaking with toluene,[153,171,178,426–428], benzene,[154] isooctane,[177] or dichloromethane.[349] Shaking time in the extraction process depends on the piazselenol and ranged between 20 s[427] and 5 min[153] and the phases are separated for 10 min.[427] In general, final organic extracts are quite stable when stored in the dark. No decomposition of 5-nitropiazselenole was noted for storage periods of up 3 weeks.[154] Piazselenole formed with TFMPD was not stable for more than 1 day on the desktop; but when frozen at −70 °C the samples are stable for at least 1 week.[163]

Interferences in the GC method for determining Se are minimal because of the selective nature of the reaction. However, two principal forms of interference are possible; the first of these sources is the result of the interaction of the diamine reagent with foreign ions present. But this interfering effect can be minimized by the judicious use of a masking reagent (such as EDTA).[57,427,429] Molybdenum employed as a catalyst in the digestion mixture did interfere in the formation of the Se-DAN complex, but could be conveniently masked with EDTA.[57] The second is the effect of acid remaining in the digestion residue which can cause spurious peaks. Electrophilic groups such as nitro or halogens can affect the sensitive ECD. Lanthanum hydroxide coprecipitation is a simple and rapid procedure for removing these interferences.[152] One peak appearing in the chromatograms under the GC conditions used, was particularly troublesome because of the closeness of its retention time to that of the 5-(trifluoromethyl)-piazselenole.[429] There are two alternatives to resolving this problem: improvement of column resolution or the use of a clean-up procedure. Florisilmagnesium sulfate treatment[154,427,429] and washing with HCl or $HClO_4$[429] have been employed in order to completely eliminate the interfering peak. Also, the addition of hydroxylamine sulfate and EDTA in combination with urea was found to eliminate the two interfering peaks occurring during the analysis of Se.[178,427]

The GC method can be a suitable method for a routine determination of Se in terms of labor and precision. One individual can analyze without difficulty at least 60 samples, including GC and calculations in ~12 h.[178] Other methods allow complete analysis of 18 samples daily for Se(IV) and total Se, or 36 samples for only total Se,[154] 30 determinations in 8 h,[152] or less than 3 h for a single sample including digestion and 2 h for formation of the complex.[57]

2. High-Performance Liquid Chromatography

One of the major advantages of HPLC with respect to GC is the higher versatility of their detectors. So, spectrophotometric,[131,448] fluorimetric,[175,433,449] atomic absorption spectrometry,[437] amperometric,[434] or radiochemical[435] detectors have been employed in the determination of Se by HPLC.

The procedure prior to injection in HPLC changes widely according to the detector used. In the first HPLC method that was described,[448] the Se–DAN complex was monitored using UV absorption, but the eluant caused complete quenching of the fluorescence. Shibata et al.[450] partly resolved this problem by using a reversed-phase system with acetonitrile as the eluant. Due to better sensitivity, currently, fluorimetric detectors are preferred. Formation of fluorescent Se–DAN complex[175,433,450] is normally used when the fluorimetric detector is coupled to the HPLC system. Also, fluorescent complex formed by the selective reaction between selenocysteine and *N*-[2-[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonic acid[449] has been utilized to determine SeCys in blood samples by HPLC. SeMet is determined in urine by ion-exchange HPLC procedure, after reaction between BrCN and SeMet.[128]

Determination of Se in serum by high-performance thin-layer chromatography (HPTLC) with fluorimetric detection has been proposed.[438–440] These methods are based in the fluorescence of the Se-DAN complex emitted from a thin layer. The chromatographic separation is carried out on HPTLC–silica gel plate with chloroform[438,439] or toluene/ethyl acetate (4:1)[440] as mobile phase. If the wet HPTLC is dipped into a solution consisting of paraffin oil/*n*-hexane or Triton X-100 dissolved in chloroform, the fluorescence intensity was enhanced by a factor of 25 and 90, respectively, compared with the untreated plate.[438,439] Oxidizing cations such as Fe^{+3} and Cu^{+2} can interfere with the formation of 2,1,3-naphthoselenodiazole, and oxalate ion can disturb the fluorimetric measurement. The adverse influence of these ions can be eliminated by the addition of EDTA, NaF and formic acid as masking reagents.[438,439] The excellent sensitivity of this procedure is proved by the detection limit of 250 fg of Se per spot.[439]

Table 9. Determination of Selenium by Chromatography

sample	treatment (detector)	species determined	detection limit ($\mu g\ L^{-1}$)	RSD %	recovery %	ref(s)
	(1) Gas Chromatography − Electron Capture Detector					
urine serum plasma	$HNO_3/Na_2MoO_4/H_2SO_4/HClO_4$ digestion; EDTA, DAN, hexane extraction	Se(IV)	0.5 ng			57
milk blood plasma	HNO_3 digestion; urea; HCl reduction; toluene extraction; 1,2-diamino-4-nitrobenzene or 1,2-diamino-3,5-dibromobenzene; toluene extraction	Se_T Se(−II, 0) Se(IV)	5 ng	1.7	94−106	153, 426
blood urine	$HNO_3/Mg(NO_3)_2$ digestion; HCl reduction; urea; 1,2-diamino-4-nitrobenzene dihydrochloride; toluene extraction		0.001 ng	10−40		427
milk	$HNO_3/Mg(NO_3)_2$ digestion; HCl reduction; urea; 1,2-diamino-4-nitrobenzene; toluene extraction		5 ng g^{-1}			177
plasma blood urine milk	HNO_3 digestion; urea; HCl reduction; 4-nitro-*o*-phenylenediamine; benzene extraction; $MgSO_4$/Florisil cleanup	Se_T Se(IV, VI)	0.19 ng g^{-1}	3.4	75−90	154
plasma blood	EDTA; HNO_3 digestion; urea; HCl reduction; 1,2-diamino-3,5-dibromobenzene; toluene extraction	Se_T Se(IV) + organo-Se		2.2	87−106	428
plasma blood	$HNO_3/Mg(NO_3)_2$ digestion; HCl reduction; hydroxylamine sulfate, EDTA, urea	Se_T	10	3	95−105	178
serum blood	$HNO_3/HClO_4$ digestion; HCl reduction; urea/$La(NO_3)_3/H_3N$; 1,2-diamino-3,5-dibromobenzene; toluene extraction	Se_T		3.9	87−108	152
milk saliva	$HNO_3/HClO_4$ digestion; HCl reduction; 4-(trifluoromethyl)-*o*-phenylenediamine; toluene extraction	Se_T	5			429
plasma				1.2	101.8	
blood				2.7	100.8	
urine				2.7		
blood urine	BrCN/$SnCl_2$;$HCCl_3$ extraction; $H_2SO_4/HClO_4$/molybdic acid digestion; 4-nitro-*o*-phenylenediamine; toluene extraction	SeMet	6 ng of SeMet g^{-1}	12.6−25.5		430
blood urine	$H_2SO_4/HClO_4$ digestion; toluene extraction				87−108	431
blood	$Na_2MoO_4/HClO_4/H_2SO_4$ digestion; 3,5-dibromo-*o*-phenylenediamine; toluene extraction		0.002 ng		92.6−107	432
blood urine	$HNO_3/H_3PO_4/H_2O_2$ digestion; HCl reduction; 4-nitro-*o*-phenylenediamine; toluene extraction (isotope dilution mass spectrometry)	Se_T	0.050 pg	1.4	≥95	171, 172
	(2) High-Performance Liquid Chromatography					
urine	evaporation of ethanolic extract; pellets dissolved in 50% aqueous ethanol	$TMSe^+$			85	419
urine	$HClO_4$ digestion; cation-exchange column	$TM^{75}Se^+$			90−95	131
serum	$HNO_3/HClO_4$ digestion; HCl reduction; EDTA/bromocresol purple/NH_4OH; DAN; cyclohexane extraction (spectrofluorimetric detection)	Se_T	0.050 ng	3.8	99.8−108.4	433
seminal plasma				1.7	99.1−108.6	
blood	$HNO_3/HClO_4/H_2SO_4$ digestion; HCl reduction; DAN; cyclohexane extraction (spectrofluorimetric detection)	Se_T	0.15 ng			175
serum	$HNO_3/HClO_4/H_2SO_4/H_2O_2$ digestion; EDTA; 4-nitro-*o*-phenylenediamine; *n*-hexane extraction (amperometric detection)	Se(IV)	0.040 ng	3.2−13.6		434

Table 9. (Continued)

sample	treatment (detector)	species determined	detection limit (μg L^{-1})	RSD %	recovery %	ref(s)
	(2) High-Performance Liquid Chromatography					
urine	^{75}Se-labeled sodium selenite; ultrafiltration (on line radioactivity detector)	Se(IV), TMSe$^+$	0.049	2.2	95 ± 5	435
plasma						
serum			25	4.5	96	436
urine	ethanol desalted; ion-exchange column; phenol–diethyl ether extraction; methanol (atomic absorption spectrometric detector)	selenoiocholine	31.3 ng	3.1	77 ± 4	437
		TMSe$^+$	43.9 ng	5.8	85 ± 0.4	
	(3) High-Performance Thin-Layer Chromatography					
serum	$HClO_4/H_2O_2/H_2SO_4$ digestion; HCl reduction; formic acid/EDTA/NaF; DAN; cyclohexane elution on column; $HCCl_3$ redissolution	Se$_T$	20 pg μL^{-1}		97	438
serum	HNO_3/H_2O_2 digestion; HCl reduction; 2,1,3-naphthoselenodiazole; cyclohexane elution on column; $HCCl_3$ redissolution	Se$_T$	250×10^{-6} ng		100.2	439
serum	HCl reduction; DAN[a]	Se$_T$	0.1 ng		100	440

[a] Thin-layer chromatography.

Prior to the formation of the fluorescent complex, human fluids must be acid digested with the methods already described in this work. Nitrite ion is accumulated during the digestion procedure and forms a fluorescent derivative with the DAN reagent that can interfere in final determination. Thus, an ammonium oxalate step was employed to eliminate nitrite from the digest.[175] Prior to the formation of Se–DAN complex, Se(IV) present in the digest must be reduced to Se(IV) with HCl.[175,433] Several conditions, 65 °C/40 min[175] or 40 °C/30 min, have been applied in the synthesis of Se–DAN. Afterward, this complex is extracted into cyclohexane and replaced in 1:1 methanol/2-propanol for injection in HPLC system; tetraphenylnaphthalene was used as internal standard.[175]

A HPLC-AAS interface based on thermochemical hydride generation was characterized for the determination of Se compounds in urine, such as selenocholine and TMSe iodide. Methanolic solutions of analytes were nebulized by a thermospray effect, pyrolyzed in a methanol–oxygen kinetic flame in the presence of H_2, and atomized in a microdiffusion flame maintained at the entrance to an untreated quartz T-tube. Both SeO_2 and TMSeI are converted into a H_2Se but only in the presence of H_2. Description of the apparatus and optimization of the method are given.[437]

With a radioactivity detector, no destructive sample pretreatment is required, and the chemical structures of the Se compounds to be analyzed remain intact.[435] This detector is independent of the chemical structure and valency state of the Se compounds as well as the matrix, in contrast with other detectors such as fluorimetric or AAS; a simple filtration step prior to injection is needed.[435] With a reversed-phase ion-pair HPLC method, a good resolution of radiolabeled Se complexes can be achieved.[435]

The electroactivity of the 5-nitropiazselenole permits the use of an amperometric detector (glassy carbon working electrode polarized at −0.45 V), coupled to the HPLC system. The method is sensitive (40 pg of Se), whereas in the same experiment with a spectrophotometric detector, the detection limit is 40 ng of Se.[434,451]

H. Electrochemical Methods

There are few electrochemical methods that can be applied for Se determination in body fluids (Table 10). The main problems with the electrochemical methods for Se determination are the interferences, organic or inorganic. So, considering the matrix effects, the method of standard addition is preferred.[455] With most electrochemical techniques the adsorption of the incompletely digested organic matrix may inhibit the electrode process and distort the response.[168,456–458] Complete mineralization makes sample preparation more complex and increases the risk of losses of Se. Different techniques of mineralization such as open[66,168,455] or closed[184] digestion procedure, ashing and digestion[64] have been proposed. Other authors prefer the separation of Se by volatilization of Se dioxide, ashing to 1150 °C.[67] Also, benzyltrimethylammonium methoxide has been used as a digesting solvent.[459]

In order to convert Se(VI) present in the digest to Se(IV) (the electroactive form), iodate/sulfite[157–159,453] or hydrochloric acid[168,184,452,455] has been utilized. Sometimes direct polarography has been carried out in order to only determine Se(IV).[452] According to many authors[455] by a relation curve of Se obtained versus the duration of heating in 95 °C water bath, 20 min was enough. They have developed adsorptive voltammetry for Se(IV) using ligands such as 2,3-diaminonaphthalene (DAN)[460] or 2,5-dimercapto-1,3,4-thiadiazole (DMTD).[459] These methods have sensitivities similar to that of cathodic stripping voltammetry (CSV) but suffer from less interferences.

Table 10. Determination of Selenium by Electrochemical Methods

method	sample	treatment	electrochemical conditions	detection limit (ng g^{-1})	RSD %	recovery %	ref
polarography	urine blood milk	$H_2O_2/HNO_3/HClO_4$ digestion; HCl reduction	Na_2SO_3/KIO_3 supporting electrolyte	(ng–pg) mL^{-1}			452
DPP-catalysis	blood	ashing; $HClO_4/H_2SO_4/KMnO_4$ digestion; Mandelic acid (sulfite)	$E_p = -0.15/-0.25$ V (vs SCE)		4.6		64
catalytic polarography	blood	$HNO_3/HClO_4$ digestion; $(NH_4)_2MoO_4$	Na_2SO_3/KIO_3/gelatin; NH_3/NH_4^+, EDTA, supporting electrolyte		7.73	98.6	157
catalytic polarography	serum	$H_2SO_4/HClO_4$ digestion; $(NH_4)_2MoO_4$	Na_2SO_3/KIO_3; NH_3/NH_4^+ supporting electrolyte	ng g^{-1} level	<10		159
catalytic polarography	blood	$HNO_3/HClO_4$ digestion;	Na_2SO_3/KIO_4 supporting electrolyte	5			453
catalytic polarography	serum	$H_2SO_4/HClO_4$ digestion; $(NH_4)_2MoO_4$			<5	99.7	454
oscillopolarography	plasma blood	$HNO_3/HClO_4$ digestion	Na_2SO_3/KIO_3; NH_3/NH_4^+, EDTA, supporting electrolyte				158
flow constant-current stripping voltammetry	blood milk	HNO_3 digestion; HCl reduction; Hg^{2+}	carbon fiber electrode $E_p = -0.45$ V (vs SCE); HCl supporting electrolyte		6–16	95–105	184
ASV-flow system	biological samples	dry under vacuum $Mg(ClO_4)_2$ dessicator; $HNO_3/HClO_4$ digestion; cation exchange resin	gold electrode $E_p = 1.0$ V (vs SCE)	4	11	99–101	66
DPCSV	milk	oxygen ashing with silicic acid	hanging mercury drop electrode $E_p = -0.40$ V (vs SCE) HNO_3 or HCl 0.15 M; $(NH_4)_2SO_4/EDTA/Cu^{2+}$	1 ng			67
DPCSV	urine	H_2SO_4/HNO_3 digestion; HCl reduction	hanging mercury drop electrode $E_p = -0.45$ V (vs Ag/AgCl) acid medium 1M supporting electrolyte			89–103	168
DPCSV	serum blood	$HNO_3/HClO_4$ digestion; HCl reduction	hanging mercury drop electrode $E_p = -0.50$ V (vs Ag/AgCl)	0.1	3.97	99.3 (91–108)	455

In order to correct the recovery, the method of standard additions was employed.[66,67,158,168]

The selenium peak may be shifted to a more negative potential in the presence of other metal ions such as Cu, Pb, and Cd.[461] The serious interference due to Cu can be corrected by determination of the diffusion current constants.[462] However such problems can be overcome by use of a separation method[66,141] which enables more specific determination of the element at similar potential for all sample materials. After the digestion procedure, Se was separated by liquid chromatography with IRA-200 strong cation-exchange resin. Detection of Se(IV) in the chromatographic effluent was made by anodic stripping voltammetry (ASV) at a tubular Au electrode.[66] The recovery of Se(IV) and the absence of interference was excellent with exception of Bi(III). Analytical results were excellent except when SiO_2 was present.[66] Some difficulties were encountered in differential pulse cathodic stripping voltammetry (DPCSV) on samples with a low Se content because high HCl concentration (>0.15 M) interfered with electrolysis.[67] Perchloric acid from 0.15 to 0.24 N does not affect the peak current much. Therefore, the solution acidity is selected within range for the procedure as indicated by Huang et al.[455] Thus, Se concentrations below 15 ng 0.5 g^{-1} can no longer be detected, although the absolute detection limit of DPCSV for Se lies at about 1 ng.[67]

The reduction of As(III), Cu(II), Fe(III), Zn(II), and Pb(II) does not interfere with the reduction of mercury(II) selenide, as a consequence of the constant-current stripping technique which yields lower values than those obtained by other techniques.[184]

A method for the determination of Se with differential pulse polarography (DPP) and catalysis has been developed. The Se(IV) and mandelic acid are adsorbed on the mercury drop, and a sharp polarographic peak is obtained.[64] Testing for interfering substances, the following limits of interference were established: Iron $< 0.22\ \mu g\ mL^{-1}$; V $< 1.38\ \mu g\ mL^{-1}$; Mn $< 0.10\ \mu g\ mL^{-1}$. The interference of more than 0.22 $\mu g\ mL^{-1}$ of iron can be eliminated by adding KSCN.[64]

Recently, new sensitive (ng mL^{-1}) catalytic polarographic methods for Se determination in body fluids[157-159,452,453,463] have been carried out. The residue obtained after the digestion process was analyzed in a supporting electrolyte containing Na_2SO_3 and KIO_4, and buffer solution H_3N/H_4N^+, pH 10. Of possible coexisting species tested, only Te[157] and H_2O_2[158] interfered significantly.

III. Quality Control and Reference Materials

Presently, there is a serious worry that quality control ensures reliable analytical measurements. Thus, the interlaboratory collaborative studies are of great interest, for demonstrating accurate values for Se obtained from different methods of determination. Thus, significant differences of experimental values from the certified values can be used to identify the analytical difficulties of the method of determination.[464]

An interlaboratory study of blood Se determinations was carried out.[84] The methods used were fluorimetry (61%), hydride generation AAS (23%), graphite furnace AAS (4%), gas chromatography (6%), neutron activation analysis (4%), and X-ray fluorimetry (2%). The intralaboratory and interlaboratory coefficients of variation ranged from 3.6 to 15.9% and 8.3 to 55%, respectively.[84] An interlaboratory test[215] in the fluorimetric determination of Se, has shown a within laboratory coefficient of variation (repeatibility) of 4.8% and between laboratories (reproducibility) of 6.0%. Moreover, it has been validated by interlaboratory studies and neutron activation analysis[465,466] and another seven different analytical methods.[318] The within batch variation of the improved method was about 2%, while the between batch variation over a period of two years was less than 10%.[93] Three methods for determination of Se in biological fluid samples have been compared using certified reference materials: acid decomposition fluorimetry, HG-AAS, and EAAS.[467] HG-AAS gave an unacceptably high coefficient of variation of 35% ($n = 5$).[467] Also, there was little difference ($P < 0.05$) in Se results obtained by fluorimetric and hydride generation methods. However, other studies[291,468] show that results obtained by HG-AAS for human body fluids are in agreement with the those found with independent analytical techniques. Accurate results can be obtained when a proper sample decomposition technique is used.[291] There was no difference between the means of any of the methods.[162] The benefit of a common set of standards, preferably those of matrix and Se concentrations similar to those the samples, in reducing interlaboratory CV's has been demonstrated.[469]

IV. Concluding Remarks

1. Sampling and storage depend on the type of fluid that is going to be analyzed. In general, collection must be made using plastic vials perfectly washed. If the analysis is going to be carried out within few days, samples may be stored refrigerated, but for longer periods, congelation or lyophilization processes are recommended.
2. Although there are alternative methods such as dry ashing, acid digestion is the method of choice for most authors to minimize losses by volatilization. A simple preparation of the samples, such as desiccation, lyophilization, or simple dilution can be only carried out for graphite furnace atomic absorption spectrometry, and some nuclear or atomic emission techniques. Acid digestion methods must be optimized according to the instrumental method used and the Se species present in the body fluid. Nitric–perchloric or nitric–perchloric–sulfuric acid mixtures can be used for the total destruction of organic matter. Procedures for the acid digestion using a nitric–phosphoric–peroxide mixture have been used in order to eliminate the perchloric acid.
3. Spectrofluorimetric technique is considered a definitive method because its high sensitivity and precision. This technique utilizes the fluorescent complex (Se–DAN) formed by the reaction between 2,3-diaminonaphthalene and selenium in the tetravalent oxidation state. After the digestion step, the Se(VI) must be reduced to Se(IV) by heating with HCl. The optimum pH for the piazselenole formation

is between 1–2, at 50–60 °C for 30 min. The inclusion of cyclodextrins with surfactants can produce a significant synergistic enhancement effect on the fluorescence intensity. $TMSe^+$ ion and other Se compounds present in urine have been separated and determined by cation exchange chromatography and fluorimetry.

4. Atomic absorption spectrometry with graphite furnace technique can be used for sensitive, rapid, and direct determination of Se in large batches of routine samples. The main problems of this determination are poor precision, spectral and matrix interferences, and losses due to volatility. Spectral interferences from iron and/or phosphate present in body fluids can be compensated by deuterium arc, or Zeeman background correction, with or without a L'vov platform. The use of matrix modifiers, such as salts of Ni, Cu, Pb, Ag, Pt, and/or Mg, helps the thermal stabilization of Se and allows ashing temperatures of up to 1200 °C.

5. When the hydride generation technique is used in methods such as atomic absorption spectrometry, it is necessary to digest the sample. Then the selenate ion present in the digested sample must be reduced to selenite ion, and the formation of the selenides is produced with a stronger reductant as sodium borohydride. The H_2Se is carried by an Ar stream to the heated silica cell, and selenium is atomized at 780 °C. The main problem of this technique is its poor precision. Complete mineralization is a decisive step to avoid excessive foam formation. A flow-injection system for hydride generation permits accurate determination of Se using a minimal amount of reagent and sample within a short time.

6. Direct nebulization of diluted body fluids in atomic emission spectroscopy produces very low sensitivity. Several methods of preconcentration, such as digestion, extraction, and/or ion exchange chromatography have been employed to increase the sensitivity. The detection limit can also be improved when the hydride generation technique is introduced, or with the use of mass spectrometry. The development of the plasma source in emission spectrometry using yttrium as internal standard allows Se determination in undiluted body fluids.

7. The main advantage of nuclear techniques (XRF and PIXE) is that many elements can be measure automatically in a quick single run with little sample preparation. However, preconcentration steps are recommended for both techniques for Se determination in body fluids, because of the poor sensitivity and precision. Simple drying or freeze-drying, as well as digestion, reduction, and/or coprecipitation steps can be used. In XRF, a Mo or a Ag target has been employed as primary radiation, and Si(Li) detector associated at the pulse processing system. In the PIXE technique, the prepared sample must interact for a time with a beam of protons obtained from a Van der Graff accelerator, and the X-ray emission is recorded by a Si(Li) or Ge(Li) detector.

8. Instrumental activation analysis (INAA) has many desirable features such as high sensitivity and specificity, reduced sample manipulation, and multielemental capability. Short-lived (^{77m}Se) or long-lived (^{75}Se) isotopes can be utilized. The time required by the former isotope is lower than that for the long-lived isotope. Lyophilization or dialysis of the sample are used for minimizing interferences, such as ^{23}Ne, ^{24}Na, ^{18}O, or ^{38}Cl.

Also, methods of radiochemical separation (RNAA) have been described in order to improve the precision and sensitivity, and to reduce interferences. These methods can involve acid digestion, extraction with organic solvents, and precipitation. Other methods of separation, such as anion exchange chromatography, have been employed for speciation studies. The use of NAA with isotope dilution techniques is of particular interest in human metabolic studies. Stable nonradioactive isotopes can be used as metabolic tracers in all subjects, including at-risk groups such as pregnant females and infants, where the use of radioisotopes is contraindicated.

9. Determination of Se by GC using electron capture detection is based on the quantification of the piazselenole formed in the reaction of Se(IV) with an *o*-diamine. Therefore, pretreatment of sample is the same as for spectrofluorimetric technique: Acid digestion, reduction, formation, and extraction of the Se-DAN complex, are necessary steps for GC determination. The introduction of a second electrophore, such as chloro or nitro groups, considerably improves sensitivity with the electron capture detector. Interference effects due to foreign ions can be minimized by the use of a masking agent as EDTA. Coprecipitation or adsorption chromatography have been used for eliminating troublesome peaks in the chromatogram. Isotope dilution GC–MS methods, using the ^{75}Se or ^{76}Se isotopes as internal standard, have been described. Isotopic ratio of ^{80}Se to ^{82}Se is utilized for Se determination at the $\mu g\ L^{-1}$ level.

10. Methods by high-performance liquid chromatography (HPLC) are being developed. The advantage with regard to gas chromatographic methods is the versatility of their detectors. Fluorimetric, amperometric, radiochemical, or atomic absorption spectrometric detectors can be employed. The sample pretreatment depends on the type of detector utilized. The fluorimetric detector is often used and utilizes the fluorescence of the Se–DAN complex for the determination. Also, this detector has been employed in HPTLC determination.

11. Acid digestion and reduction to Se(IV) are necessary steps for the Se determination by electrochemical methods. The adsorption of the incompletely digested organic matrix may inhibit the electrode process and distort the response. Complete mineralization could increase the risk of losses of Se. Liquid chromatography with cation-exchange resin has been used to eliminate the presence of other interferent metal ions.

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References

(1) Iyengar, G. V. *Sci. Total Environ.* **1991**, *100*, 1.
(2) Morrison, G. H. *CRC Crit. Rev. Anal. Chem.* **1979**, *11*, 287.
(3) Valkovic, V. *Phys. Med.* **1991**, *VII*, 51.

(4) Schroeder, H. A.; Frost, D. V.; Balassa, J. J. *J. Chronic Dis.* **1970**, *23*, 227.
(5) Levander, O. A. *Annu. Rev. Nutr.* **1987**, *7*, 227.
(6) Wilber, C. *Clin. Toxicol.* **1980**, *17*, 171.
(7) Yang, G.; Zhou, R.; Yin, S.; Gu, L.; Yan, B.; Liu, Y.; Liu, Y.; Li, X. *J. Trace Elem. Electrolytes Health Dis.* **1989**, *3*, 77.
(8) Yang, G.; Yin, S.; Zhou, R.; Gu, L.; Yan, B.; Liu, Y.; Liu, Y. *J. Trace Elem. Electrolytes Health Dis.* **1989**, *3*, 123.
(9) Keshan Disease Research Group. *Chin. Med. J.* **1979**, *92*, 471.
(10) Keshan Disease Research Group. *Chin. Med. J.* **1979**, *92*, 477.
(11) Salonen, J. T.; Alfthan, G.; Huttunen, J. K.; Puska, P. *Am. J. Epidemiol.* **1984**, *124*, 342.
(12) Clark, L. C. *Fed. Proc.* **1985**, *44*, 2584.
(13) Combs, G. F., Jr.; Clark, L. C. *Nutr. Rev.* **1985**, *43*, 325.
(14) Sando, K. *Geka to Taisha* **1989**, *23*, 225; Chem. Abstr. **1990**, *112*, 34729r.
(15) Huttunen, J. K. *Acta Pharmacol. Toxicol.* **1986**, *59*, 311.
(16) Oster, O.; Prellwitz, W. *Biol. Trace Elem. Res.* **1990**, *24*, 91.
(17) Välimäki, M. J.; Harju, K. J.; Ylikahri, R. H. *Clin. Chim. Acta* **1983**, *130*, 291.
(18) Bjoerneboe, G.-E. Aa.; Johnsen, J.; Bjoerneboe, A.; Bache-Wiig, J. E.; Moerland, J.; Drevon, C. A. *Ann. Nutr. Metab.* **1988**, *32*, 56.
(19) Olmsted, L.; Schrauzer, G. N.; Flores-Arce, M.; Dowd, J. *Biol. Trace Elem. Res.* **1989**, *20*, 59.
(20) *Recommended Dietary Allowances*, 10th ed.; National Academy Press: Washington, DC, 1989; p 217.
(21) Robberecht, H. J.; Deelstra, H. A. *Talanta* **1984**, *31*, 497.
(22) Sanz Alaejos, M.; Díaz Romero, C. *Clin. Chem. (Winston-Salem, N.C.)* **1993**, *39*, 2040.
(23) Xia, Y.; Hill, K. E.; Burk, R. F. *J. Nutr.* **1989**, *119*, 1318.
(24) Ip, C.; Ganther, H. E. *Cancer Res.* **1990**, *50*, 1206.
(25) Lane, H. W.; Lotspeich, C. A.; Moore, C. E.; Ballard, J.; Dudrick, S. J.; Warren, D. C. *J. Parenter. Enteral Nutr.* **1987**, *11*, 177.
(26) Lombeck, I.; Kasperek, K.; Bachmann, D.; Feinendegen, L. E.; Bremer, H. J. *Eur. J. Pediatr.* **1980**, *134*, 65.
(27) Levander, O. A. *Fed. Proc.* **1985**, *44*, 2579.
(28) Lockitch, G. *Crit. Rev. Clin. Lab. Sci.* **1989**, *27*, 483.
(29) Fishbein, L. *Int. J. Environ. Anal. Chem.* **1984**, *17*, 113.
(30) Magos, L.; Berg, G. G. In *Biological Monitoring of Toxic Metals*; Clarkson, T. W., Friberg, L., Nordberg, G. F., Sager, P. R., Eds.; Plenum Press: New York, 1988; p 383.
(31) Van Dael, P.; Deelstra, H. *Proc. Bioavail.* **1989**, *72*, 112.
(32) Akesson, B.; Borglund, M. The Proceedings of the Fourth International Symposium on Uses of Selenium and Tellurium, Banff, Alberta, Canada, 1989; p 405; *Chem. Abstr.* **1991**, *114*, 141865v.
(33) Shamberger, R. J. *Clin. Physiol. Biochem.* **1986**, *4*, 42.
(34) Zachara, B. A.; Wasowicz, W.; Gromadzinska, J.; Sklodowska, M.; Krasomski, G. *Biol. Trace Elem. Res.* **1986**, *10*, 175.
(35) Beilstein, M. A.; Tripp, M. J.; Whanger, P. D. *J. Inorg. Biochem.* **1981**, *15*, 339.
(36) Shamberger, R. J. *Biochemistry of Selenium*; Plenum Press: New York, 1983.
(37) Hawkes, W. C.; Wilhelmsen, E. C.; Tappel, A. L. *J. Inorg. Biochem.* **1985**, *23*, 77.
(38) Nahapetian, A. T.; Young, V. R.; Janghorbani, M. *Anal. Biochem.* **1984**, *140*, 56.
(39) Nahapetian, A. T.; Janghorbani, M.; Young, V. R. *J. Nutr.* **1983**, *113*, 401.
(40) Foster, S. J.; Ganther, H. E. *Anal. Biochem.* **1984**, *137*, 205.
(41) Nahapetian, A. T.; Young, V. R.; Janghorbani, M. *Anal. Biochem.* **1984**, *140*, 56.
(42) Sun, X. F.; Ting, B. T. G.; Janghorbani, M. *Anal. Biochem.* **1987**, *167*, 304.
(43) Nève, J.; Vertongen, F.; Capel, P. *Am. J. Clin. Nutr.* **1988**, *48*, 139.
(44) Blotcky, A. J.; Rack, E. P. *J. Res. Natl. Bur. Stand. (U.S.)* **1986**, *91*, 93.
(45) Cornelis, R. *Mikrochim. Acta* **1991**, *III*, 37.
(46) Cornelis, R.; Speecke, A.; Hoste, J. *Anal. Chim. Acta* **1975**, *78*, 317.
(47) Bratakos, M. S.; Kanaki, H. C.; Vasiliou-Waite, A.; Ioannou, P. V. *Sci. Total Environ.* **1990**, *91*, 161.
(48) Shendrikar, A. D.; West, P. W. *Anal. Chim. Acta* **1975**, *74*, 189.
(49) Bem, E. M. *Environ. Health Perspect.* **1981**, *37*, 183.
(50) Howe, M. *Arch. Environ. Health* **1979**, *34*, 444.
(51) Palmer, I. S.; Olson, O. E.; Ketterling, L. M.; Shank, C. E. *J. Am. Diet. Assoc.* **1983**, *82*, 511.
(52) Hasunuma, R.; Tsuda, M.; Ogawa, T.; Kawanishi, Y. *Bull. Environ. Contam. Toxicol.* **1990**, *44*, 501.
(53) Blotcky, A. J.; Hansen, G. T.; Borkar, N.; Ebrahim, A.; Rack, E. P. *Anal. Chem.* **1987**, *59*, 2063.
(54) Blotcky, A. J.; Ebrahim, A.; Rack, E. P. *Anal. Chem.* **1988**, *60*, 2734.
(55) Jaffé, W. G.; Ruphael, M. D.; de Mondragón, M. C.; Cuevas, M. A. *Arch. Latinoam. Nutr.* **1972**, *22*, 595.
(56) Van der Torre, H. W.; Van Dókkum, W.; Schaafsma, G.; Wedel, M.; Ockhuizen, T. *Br. J. Nutr.* **1991**, *65*, 69.
(57) Young, J. N.; Christian, G. D. *Anal. Chim. Acta* **1973**, *65*, 127.
(58) Valentine, J. L.; Faraji, B.; Kang, H. K. *Environ. Res.* **1988**, *45*, 16.
(59) Bunker, V. W.; Lawson, M. S.; Stansfield, M. E.; Clayton, M. F. *Br. J. Nutr.* **1988**, *59*, 171.
(60) Chen, X.; Yang, G.; Chen, J.; Chen, X.; Wen, Z.; Ge, G. *Biol. Trace Elem. Res.* **1980**, *2*, 91.
(61) Iyengar, G. V.; Kasperek, K.; Feinendegen, L. E. *Sci. Total Environ.* **1978**, *10*, 1.
(62) Grant, A. B. *New Zealand*, J. *Sci.* **1981**, *24*, 65.
(63) Fourie, H. O.; Peisach, M. *Analyst* **1977**, *102*, 193.
(64) Wang, D.; Pan, J. *Anal. Proc. (London)* **1987**, *24*, 329.
(65) Leitner, S. S.; Savory, J. *Anal. Chim. Acta* **1975**, *74*, 133.
(66) Andrews, R. W.; Johnson, D. C. *Anal. Chem.* **1976**, *48*, 1056.
(67) Han, H.-B.; Kaiser, G.; Tölg, G. *Anal. Chim. Acta* **1981**, *128*, 9.
(68) Versieck, J. *J. Res. Natl. Bur. Stand. (U.S.)* **1986**, *91*, 87.
(69) Kant, A. K.; Moser-Veillon, P. B.; Reynolds, R. D. *Nutr. Res. (N.Y.)* **1989**, *9*, 717.
(70) Morisi, G.; Patriarca, M.; Marano, G.; Giampaoli, S.; Taggi, F. *Ann. Ist. Super. Sanita* **1989**, *25*, 393. *Chem. Abstr.* **1990**, *112*, 113820s.
(71) Baptista, R. J.; Bistrian, B. R.; Blackburn, G. L.; Miller, D. G.; Champagne, C. D.; Buchanan, L. *Am. J. Clin. Nutr.* **1984**, *39*, 816.
(72) Friel, J. K.; Gibson, R. S.; Peliowski, A.; Watts, J. *J. Pediatr. (St. Louis)* **1984**, *104*, 763.
(73) Versieck, J. *Crit. Rev. Clin. Lab. Sci.* **1985**, *22*, 97.
(74) Behne, D.; Jürgensen, H. *Lancet* **1977**, *I*, 148.
(75) Ellis, N.; Lloyd, B.; Lloyd, R. S.; Clayton, B. E. *J. Clin. Pathol.* **1984**, *37*, 200.
(76) Suzuki, T.; Hongo, T.; Ohba, T.; Kobayashi, K.; Imai, H.; Ishida, H.; Suzuki, H. *Nutr. Res. (N.Y.)* **1989**, *9*, 839.
(77) Thorling, E. B.; Overvad, K.; Heerfordt, A.; Foldspang, A. *Biol. Trace Elem. Res.* **1985**, *8*, 65.
(78) Thorling, E. B.; Overvad, K.; Geboers, J. *Ann. Clin. Res.* **1986**, *18*, 3.
(79) Hansson, L.; Pettersson, J.; Eriksson, L.; Olin, A. *Clin. Chem. (Winston-Salem, N.C.)* **1989**, *35*, 537.
(80) Rudolph, N.; Shing, S. L. *Pediatr. Res.* **1978**, *12*, 789.
(81) Robinson, M. F.; Godfrey, P. J.; Thomson, C. D.; Rea, H. M.; Van Rij, A. M. *Am. J. Clin. Nutr.* **1979**, *32*, 1477.
(82) Gromadzinska, J.; Wasowicz, W.; Sklodowska, M.; Strozynski, H. *J. Clin. Chem. Clin. Biochem.* **1988**, *36*, 255.
(83) Lloyd, B.; Holt, P.; Delves, H. T. *Analyst* **1982**, *107*, 927.
(84) Koh, T.-S. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 664.
(85) Tsukamoto, Y.; Iwanami, S.; Marumo, F. *Nephron* **1980**, *26*, 174.
(86) Akesson, B.; Mortensen, B. *J. Inorg. Biochem.* **1988**, *33*, 257.
(87) Kasperek, K.; Iyengar, G. V.; Kiem, J.; Borberg, H.; Feinendegen, L. E. *Clin. Chem. (Winston-Salem, N.C.)* **1979**, *25*, 711.
(88) Robberecht, H.; Van Grieken, R.; Shani, J.; Barak, S. *Anal. Chim. Acta* **1982**, *136*, 285.
(89) Imai, H.; Suzuki, T.; Kashiwazaki, H.; Takemoto, T.; Izumi, T. *Nutr. Res.* **1990**, *10*, 1205.
(90) Lombeck, I.; Kasperek, K.; Feinendegen, L. E.; Bremer, H. J. *Clin. Chim. Acta* **1975**, *64*, 57.
(91) Ericson, S. P.; McHalsky, M. L.; Rabinow, B. E.; Kronholm, K. G.; Arceo, C. S.; Weltzer, J. A.; Ayd, S. W. *Clin. Chem. (Winston-Salem, N.C.)* **1986**, *32*, 1350.
(92) Saeed, K.; Thomassen, Y. *Anal. Chim. Acta* **1982**, *143*, 223.
(93) Koh, T.-S.; Benson, T. H. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 918.
(94) Juergensen, H.; Behne, D. *J. Radioanal. Chem.* **1977**, *37*, 375.
(95) Sullivan, J. F.; Blotcky, A. J.; Jetton, M. J.; Hahn, M. K. J.; Burch, R. E. *J. Nutr.* **1979**, *109*, 1432.
(96) Salonen, J. T.; Alfthan, G.; Huttunen, J. K.; Pikkarainen, J.; Puska, P. *Lancet* **1982**, *II*, 175.
(97) Cocho, J. A.; Parrado, C.; Cervilla, J. R.; Alonso-Fernández, J. R.; Fraga, J. M. *Quím. Clín.* **1984**, *3*, 19.
(98) Virtamo, J.; Valkeila, E.; Alfthan, G.; Punsar, S.; Huttunen, J. K.; Karvonen, H. J. *Am. J. Epidemiol.* **1985**, *122*, 276.
(99) Aro, A.; Alfthan, G.; Soimakallio, S.; Voutilainen, E. *Clin. Chem. (Winston-Salem, N.C.)* **1986**, *32*, 911.
(100) Corrocher, R.; Cassaril, M.; Bellisola, G.; Gabrielli, G.; Hulpe, M.; Garofoli, E.; Nicoli, N. *Tumori* **1986**, *72*, 617; *Chem. Abstr.* **1987**, *106*, 136218s.
(101) Westermarck, T.; Raunu, P.; Kirjarinta, M.; Lappalainen, L. *Acta Pharmacol. Toxicol.* **1977**, *40*, 465.
(102) Willett, W. C.; Morris, J. S.; Pressel, S.; Taylor, J. O.; Polk, B. F.; Stampfer, M. J.; Rosner, B.; Schneider, K.; Hames, C. G. *Lancet* **1983**, *II*, 130.
(103) McConnell, K. P.; Broghamer, W. L., Jr.; Blotcky, A. J.; Hurt, O. J. *J. Nutr.* **1975**, *105*, 1026.
(104) Chen, S. Y.; Collipp, P. J.; Boasi, L. H.; Isenschmid, D. S.; Verolla, R. J.; San Roman, G. A.; Yeh, J. K. *Ann. Nutr. Metabol.* **1982**, *26*, 186.
(105) Bukkens, S. G. F.; De Vos, N.; Kok, F. J.; Schouten, E. G.; De Bruijn, A. M.; Hofman, A. *J. Am. Coll. Nutr.* **1990**, *9*, 128.
(106) Rossi, L. C.; Clemente, G. F.; Santaroni, G. *Arch. Environ. Health* **1976**, *31*, 160.
(107) Behne, D.; Matamba, P. A. *Fresenius' Z. Anal. Chem.* **1975**, *274*, 195.

(108) Parr, R. M. *Protocol for Washing Breast and Use of the Milk Collection Vessels*; IAEA/WHO Joint Research Program on Trace Elements in Human Milk, 1978.
(109) Litov, R. E.; Sickles, V. S.; Chan, G. M.; Hargett, I. R.; Cordano, A. *Nutr. Res.* **1989**, *9*, 585.
(110) Smith, A. M.; Picciano, M. F.; Milner, J. A. *Am. J. Clin. Nutr.* **1982**, *35*, 521.
(111) Clemente, G. F.; Ingrao, G.; Santaroni, G. P. *Sci. Total Environ.* **1982**, *24*, 255.
(112) Binnerts, W. T. *Neth. Milk Dairy J.* **1979**, *33*, 24.
(113) Varo, P.; Koivistoinen, P. *Int. J. Vitam. Nutr. Res.* **1981**, *51*, 79.
(114) Polkowska-Motrenko, H.; Dermelj, M.; Byrne, A. R.; Fajgelj, A.; Stegnar, P.; Kosta, L. *Radiochem. Radioanal. Lett.* **1982**, *53*, 319.
(115) Maus, R. W.; Martz, F. A.; Belyea, R. L.; Weiss, M. F. *J. Dairy Sci.* **1980**, *63*, 532.
(116) Debski, B.; Picciano, M. F.; Milner, J. A. *J. Nutr.* **1987**, *117*, 1091.
(117) Levander, O. A.; Moser, P. B.; Morris, V. C. *Am. J. Clin. Nutr.* **1987**, *46*, 694.
(118) Mannan, S.; Picciano, M. F. *Am. J. Clin. Nutr.* **1987**, *46*, 95.
(119) Shearer, T. R.; Hadjimarkos, D. M. *Arch. Environ. Health* **1975**, *30*, 230.
(120) Karlsen, J. T.; Norheim, G.; Froslie, A. *Acta Agric. Scand.* **1981**, *31*, 165.
(121) Kumpulainen, J.; Vuori, E.; Kuitunen, P.; Mäkinen, S.; Kara, R. *Int. J. Vitam. Nutr. Res.* **1983**, *53*, 420.
(122) Varo, P.; Nuurtamo, M.; Koivistoinen, P. *J. Dairy Sci.* **1984**, *67*, 2071.
(123) Smith, A. M.; Picciano, M. F. *J. Nutr.* **1986**, *116*, 1068.
(124) Pleban, P. A.; Mei, D.-S. *Clin. Chim. Acta* **1983**, *133*, 43.
(125) Antoniou, K.; Vassilaki-Grimani, M.; Lolis, D.; Grimanis, A. P. *J. Radioanal. Chem.* **1982**, *70*, 77.
(126) Robberecht, H. J.; Deelstra, H. A. *Clin. Chim. Acta.* **1984**, *136*, 107.
(127) Deagen, J. T.; Butler, J. A.; Zachara, B. A.; Whanger, P. D. *Anal. Biochem.* **1993**, *208*, 176.
(128) Wolf, W. R.; LaCroix, D. E.; Slagt, M. E. *Anal. Lett.* **1992**, *25*, 2165.
(129) Oyamada, N.; Ishizaki, M. *Bunseki Kagaku* **1982**, *31*, 17.
(130) Oyamada, N. *Ibaraki−ken Eisei Kenkyusho Nempo* **1983**. *21*, 17; *Chem. Abstr.* **1984**, *100*, 135289e.
(131) Hoffman, J. L.; McConnell, K. P. *Arch. Biochem. Biophys.* **1987**, *254*, 534.
(132) Drabek, K.; Kalouskova, J. *J. Radioanal. Nucl. Chem.* **1987**, *119*, 119.
(133) Robberecht, H. J.; Van Grieken, R. E.; van den Bosch, P. A.; Deelstra, H.; Vanden Berghe, D. *Talanta* **1982**, *29*, 1025.
(134) Tamari, Y.; Ohmori, S.; Hiraki, K. *Clin. Chem. (Winston-Salem, N.C.)* **1986**, *32*, 1464.
(135) Verlinden, M. *Talanta* **1982**, *29*, 875.
(136) Yang, R.; Huang, J.; Feng, G.; Mei, Z. *Med. Lab. Sci.* **1986**, *43*, 331.
(137) Welz, B.; Melcher, M.; Nève, J. *Anal. Chim. Acta* **1984**, *165*, 131.
(138) Welz, B. *Instrum.-Res.* **1986**, *2*, 46.
(139) Liu, J.; Xu, B.; Xiao, H.; Chen, F.; Zhou, Q. *Gongye Weisheng Yu Zhiyebing* **1991**, *17*, 230; *Chem. Abstr.* **1992**, *116*, 147240a.
(140) Geahchan, A.; Chambon, P. *Clin. Chem. (Winston-Salem, N.C.)* **1980**, *26*, 1272.
(141) Adeloju, S. B.; Bond, A. M.; Briggs, M. H.; Hughes, H. C. *Anal. Chem.* **1983**, *55*, 2076.
(142) Negretti de Brätter, V. E.; Brätter, P.; Tomiak, A. *J. Trace Elem. Electrolytes Health Dis.* **1990**, *4*, 41.
(143) Henze, G.; Monks, P.; Tölg, G. *Fresenius' Z. Anal. Chem.* **1979**, *295*, 1.
(144) Raptis, S.; Knapp, G.; Mayer, A.; Tölg, G. *Fresenius' Z. Anal. Chem.* **1980**, *300*, 18.
(145) Schreiber, B.; Linder, H. R. *Fresenius' Z. Anal. Chem.* **1979**, *298*, 404.
(146) Nève, J.; Hanocq, M.; Molle, L. *Mikrochim. Acta* **1980**, *I*, 259.
(147) Ihnat, M. *Anal. Chim. Acta* **1976**, *82*, 293.
(148) Analytical Methods Committee. *Analyst* **1979**, *104*, 778.
(149) Janghorbani, M.; Ting, B. T. G.; Nahapetian, A.; Young, V. R. *Anal. Chem.* **1982**, *54*, 1188.
(150) Olson, O. E.; Palmer, I. S.; Cary, E. E. *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 117.
(151) Nève, J.; Hanocq, M.; Molle, L.; Lefebvre, G. *Analyst* **1982**, *107*, 934.
(152) Uchida, H.; Shimoishi, Y.; Tôei, K. *Analyst* **1981**, *106*, 757.
(153) Shimoishi, Y. *Analyst* **1976**, *101*, 298.
(154) Cappon, C. J.; Smith, J. C. *J. Anal. Toxicol.* **1978**, *2*, 114.
(155) Holynska, B.; Lipinska-Kalita, K. *Radiochem. Radioanal. Lett.* **1977**, *30*, 241.
(156) Holynska, B.; Markowicz, A. *Radiochem. Radioanal. Lett.* **1977**, *31*, 165.
(157) Cai, Y.; Zhu, L.; Lin, J. *Lihua Jianyan, Huaxue Fence* **1987**, *23*, 360; *Anal. Abstr.* **1988**, *50*, 6D43.
(158) Lin, S. *Lihua Jianyan, Huaxue Fence* **1987**, *23*, 91; *Anal. Abstr.* **1988**, *50*, 1D47.
(159) Liu, X.; Tu, Y.; Cheng, W.; Wu, Z. *Shengwu Huaxue Yu Shengwu Wuli Jinzhan* **1987**, *6*, 52; *Chem. Abstr.* **1988**, *108*, 218519w.
(160) Bunker, V. W.; Delves, H. T. *Anal. Chim. Acta* **1987**, *201*, 331.
(161) Reamer, D. C.; Veillon, C. *Anal. Chem.* **1983**, *55*, 1605.
(162) Macpherson, A. K.; Sampson, B.; Diplock, A. T. *Analyst* **1988**, *113*, 281.
(163) Aggarwal, S. K.; Kinter, M.; Herold, D. A. *Anal. Biochem.* **1992**, *202*, 367.
(164) Welz, B.; Melcher, M.; Schlemmer, G. *Fresenius' Z. Anal. Chem.* **1983**, *316*, 271.
(165) Ting, B. T. G.; Nahapetian, A.; Young, V. R.; Janghorbani, M. *Analyst* **1982**, *107*, 1495.
(166) Levesque, M.; Vendette, E. D. *Can. J. Soil Sci.* **1971**, *51*, 85.
(167) Nygaard, D. D.; Lowry, J. H. *Anal. Chem.* **1982**, *54*, 803.
(168) Adeloju, S. B.; Bond, A. M.; Briggs, M. H. *Anal. Chem.* **1984**, *56*, 2397.
(169) Watkinson, J. H. *Anal. Chim. Acta* **1979**, *105*, 319.
(170) Lalonde, L.; Jean, Y.; Roberts, K. D.; Chapdelaine, A.; Bleau, G. *Clin. Chem. (Winston-Salem, N.C.)* **1982**, *28*, 172.
(171) Reamer, D. C.; Veillon, C. *Anal. Chem.* **1981**, *53*, 2166.
(172) Reamer, D. C.; Veillon, C. *J. Nutr.* **1983**, *113*, 786.
(173) Raptis, S. E.; Kaiser, G.; Tölg, G. *Fresenius' Z. Anal. Chem.* **1983**, *316*, 105.
(174) Alfthan, G. *Anal. Chim. Acta* **1984**, *165*, 187.
(175) Handelman, G. J.; Kosted, P.; Short, S.; Dratz, E. A. *Anal. Chem.* **1989**, *61*, 2244.
(176) Holak, W. *J. Assoc. Off. Anal. Chem.* **1976**, *59*, 650.
(177) Stijve, T.; Philippossian, G. *Trav. Chim. Aliment. Hyg.* **1978**, *69*, 74.
(178) McCarthy, T. P.; Brodie, B.; Milner, J. A.; Bevill, R. F. *J. Chromatogr. Biomed. Appl.* **1981**, *14*, 9.
(179) Pettersson, J.; Hansson, L.; Örnemark, U.; Olin, A. *Clin. Chem. (Winston-Salem, N.C.)* **1988**, *34*, 1908.
(180) Brumbaugh, W. G.; Walther, M. S. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 570.
(181) Mattos, S. V. M.; Mansur Netto, E.; Fenelon, A. S. *Rev. Bras. Patol. Clin.* **1992**, *28*, 11; *Chem. Abstr.* **1993**, *118*, 208779y.
(182) Lamleung, S. Y.; Cheng, V. K. W.; Lam, Y. W. *Analyst* **1991**, *116*, 957.
(183) Matusiewicz, H.; Sturgeon, R. E.; Berman, S. S. *J. Anal. At. Spectrom.* **1991**, *6*, 283.
(184) Hua, C.; Jagner, D.; Renman, L. *Anal. Chim. Acta* **1987**, *197*, 257.
(185) Fricke, F. L.; Rose, O., Jr.; Caruso, J. A. *Talanta* **1976**, *23*, 317.
(186) Fleming, C. R.; Lie, J. T.; McCall, J. T.; O'Brien, J. F.; Baillie, E. E.; Thistle, J. L. *Gastroenterology* **1982**, *83*, 689.
(187) Stoeppler, M.; Backhaus, F. *Fresenius' Z. Anal. Chem.* **1978**, *291*, 116.
(188) Scheubeck, E.; Gehring, J.; Pickel, M. *Fresenius' Z. Anal. Chem.* **1979**, *297*, 113.
(189) Watkinson, J. H. *Anal. Chem.* **1966**, *38*, 92.
(190) Haddad, P. R.; Smythe, L. E. *Talanta* **1974**, *21*, 859.
(191) Grzebula, S.; Witkowski, P. *Pol. Arch. Weter.* **1977**, *20*, 125.
(192) Whetter, P. A.; Ullrey, D. E. *J. Assoc. Off. Anal. Chem.* **1978**, *61*, 927.
(193) Gomanova, M. I.; Shemyakin, F. M.; Kudrin, A. N.; Nikolaev, S. M.; Chernysheva, L. F. *Farmatsiya (Moscow)* **1977**, *26*, 56.
(194) Lane, H. W.; Dudrick, S.; Warren, D. C. *Proc. Soc. Exp. Biol. Med.* **1981**, *167*, 383.
(195) Moreno Domínguez, T.; García Moreno, C.; Mariné Font, A. *Analyst* **1983**, *108*, 505.
(196) Peters, H. J.; Koehler, H. *Zentralbl. Pharm., Pharmakother. Laboratoriumsdiagn.* **1982**, *121*, 127.
(197) Bayfield, R. F.; Romalis, L. F. *Anal. Biochem.* **1985**, *144*, 569.
(198) Vaessen, H. A. M. G.; van Ooik, A.; Zydendorp, J. *Z. Lebensm. Unters. Forsch.* **1985**, *181*, 189.
(199) Koops, J.; Klomp, H.; Westerbeek, D. *Neth. Milk Dairy J.* **1989**, *43*, 185.
(200) Sheehan, T. M. T.; Gao, M. *Clin. Chem. (Winston-Salem, N.C.)* **1990**, *36*, 2124.
(201) Kawamoto, H.; Tanaka, T. *Rinsho Kagaku (Nippon Rinsho Kagakkai)* **1992**, *21*, 179; *Chem. Abstr.* **1993**, *118*, 18689w.
(202) Bem, E. M. *Chem. Anal. (Warsaw)* **1979**, *24*, 155.
(203) Niu, J.; Wang, B. *Huanjing Kexue* **1989**, *10*, 45; *Chem. Abstr.* **1989**, *111*, 190767m.
(204) Chan, C. C. Y. *Anal. Chim. Acta* **1976**, *82*, 213.
(205) Spallholz, J. E.; Collins, G. F.; Schwarz, K. *Bioinorg. Chem.* **1978**, *9*, 453.
(206) Michie, N. D.; Dixon, E. J.; Bunton, N. G. *J. Assoc. Off. Anal. Chem.* **1978**, *61*, 48.
(207) Pettersson, J.; Olin, A. *Talanta* **1991**, *38*, 413.
(208) Hall, R. J.; Gupta, P. L. *Analyst* **1969**, *94*, 292.
(209) Clinton, O. E. *Analyst* **1977**, *102*, 187.
(210) Bye, R.; Lund, W. *Fresenius' Z. Anal. Chem.* **1988**, *332*, 242.
(211) Vogel, A. I. *A Text-book of macro and semimicro qualitative inorganic analysis*, 4th ed.; Longmans: London, 1954; p 584.
(212) Brown, M. W.; Watkinson, J. H. *Anal. Chim. Acta* **1977**, *89*, 29.
(213) Szilvassy-Vamos, Z.; Lazar, J.; Horvath, M.; Kertai-Simon, A. *J. Anal. At. Spectrom.* **1990**, *5*, 705.
(214) Zheng, Y.-X.; Lu, D.-H. *Mikrochim. Acta* **1992**, *106*, 3.
(215) Vaessen, H. A. M. G.; van Ooik, A. *Z. Lebensm. Unters. Forsch.* **1987**, *185*, 468.

(216) Hasunuma, R.; Ogawa, T.; Kawaniski, Y. *Bull. Environ. Contam. Toxicol.* **1993**, *50*, 19.
(217) Herrador, M. A.; Jiménez, A. M.; Navas, M. J.; Troncoso, A. M.; Asuero, A. G. *Z. gesamte hyg. Ihre Grenzgeb.* **1986**, *32*, 285; *Chem. Abstr.* **1986**, *105*, 96092s.
(218) Afsar, H.; Apak, R.; Tor, I. *Analyst* **1989**, *114*, 1319.
(219) Lund, W.; Thomassen, Y.; Doevle, P. *Anal. Chim. Acta* **1977**, *93*, 53.
(220) Fuavao, V. A.; Porter, B. L.; Sneddon, J. *Anal. Lett.* **1989**, *22*, 71.
(221) Lin, W.; He, Y.; Huang, L.; Zhao, S.; Huang, G.; Wu, J.; Zhang, L. *Lihua Jianyan, Huaxue Fence* **1989**, *25*, 33; *Chem. Abstr.* **1990**, *112*, 51595m.
(222) Eurola, M.; Ekholm, P.; Ylinen, M.; Koivistoinen, P.; Varo, P. *Acta Agric. Scand.* **1989**, *39*, 345.
(223) Brooks, R. R.; Willis, J. A.; Liddle, J. R. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 130.
(224) Porter, B. L.; Fuavao, V. A.; Sneddon, J. *At. Spectrosc.* **1983**, *4*, 185.
(225) Oster, O.; Prellwitz, W. *Clin. Chim. Acta* **1982**, *124*, 277.
(226) Verlinden, M.; Deelstra, H.; Adriaenssens, E. *Talanta* **1981**, *28*, 637.
(227) Ringstad, J.; Thelle, D. *Acta Pharmacol. Toxicol.* **1986**, *Suppl. 59*, 336.
(228) Brown, A. A.; Ottaway, J. M.; Fell, G. S. *Anal. Proc. (London)* **1982**, *19*, 321.
(229) Norheim, G.; Haugen, A. *Acta Pharmacol. Toxicol.* **1986**, *59*, 610.
(230) Ishizaki, M. *Talanta* **1978**, *25*, 167.
(231) Ishizaki, M. *Bunseki Kagaku* **1977**, *26*, 206.
(232) Nève, J.; Hanocq, M. *Anal. Chim. Acta* **1977**, *93*, 85.
(233) Saeed, K.; Thomassen, Y.; Langmyhr, F. J. *Anal. Chim. Acta* **1979**, *110*, 285.
(234) Rail, C. D.; Kidd, D. E.; Hadley, M. W. *Int. J. Environ. Anal. Chem.* **1980**, *8*, 79.
(235) Tada, Y.; Yonemoto, T.; Iwasa, A.; Nakagawa, K. *Bunseki Kagaku* **1980**, *29*, 248.
(236) Alfthan, G.; Kumpulainen, J. *Anal. Chim. Acta* **1982**, *140*, 221.
(237) Hughet, C.; Chappius, P.; Peynet, J.; Thuillier, F.; Legrand, A.; Rousselet, F. *Ann. Biol. Clin.* **1983**, *41*, 277.
(238) Morita, K.; Mishima, M. *Bunseki Kagaku* **1981**, *30*, 170.
(239) Pleban, P. A.; Munyani, A.; Beachum, J. *Clin. Chem. (Winston-Salem, N.C.)* **1982**, *28*, 311.
(240) Tulley, R. T.; Lehmann, H. P. *Clin. Chem. (Winston-Salem, N.C.)* **1982**, *28*, 1448.
(241) Carnrick, G. R.; Manning, D. C.; Slavin, W. *Analyst* **1983**, *108*, 1297.
(242) Kumpulainen, J.; Raittila, A. M.; Lehto, J.; Koivistoinen, P. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 1129.
(243) Clavel, J. P.; Chalvignac, B.; Thuillier, A. *J. Pharm. Clin.* **1983**, *2*, 55.
(244) Norheim, G.; Saeed, K.; Thomassen, Y. *At. Spectrosc.* **1983**, *4*, 99.
(245) Prosbova, M.; Vrzgula, L.; Kralicekova, E. *Chem. Listy* **1984**, *78*, 861; *Chem. Abstr.* **1984**, *101*, 187283p.
(246) Paschal, D. C.; Kimberly, M. M. *At. Spectrosc.* **1986**, *7*, 75.
(247) Nève, J.; Molle, L. *Acta Pharmacol. Toxicol.* **1986**, *59*, 606.
(248) Morisi, G.; Patriarca, M.; Menotti, A. *Clin. Chem. (Winston-Salem, N.C.)* **1988**, *34*, 127.
(249) Nève, J.; Chamart, S.; Trigaux, P.; Vertongen, F. *At. Spectrosc.* **1987**, *8*, 167.
(250) Chen, Z.; Chen, Y. *Guangpuxue Yu Guangpu Fenxi* **1990**, *10*, 69; *Anal. Abstr.* **1991**, *53*, 6F27.
(251) Eckerlin, R. H.; Hoult, D. W.; Carnrick, G. R. *At. Spectrosc.* **1987**, *8*, 64.
(252) Moynier, I.; Bourret, E.; Fussellier, M.; Bardet, L. *Analusis* **1989**, *17*, 369.
(253) Saeed, K. *J. Anal. At. Spectrom.* **1987**, *2*, 151.
(254) Jacobson, B. E.; Locktch, G. *Clin. Chem. (Winston-Salem, N.C.)* **1988**, *34*, 709.
(255) Pohl, B.; Knowles, M.; Grund, A. *Fresenius' Z. Anal. Chem.* **1987**, *327*, 20.
(256) Charlot, C.; Rieu, D.; Touati, J. *Analusis* **1989**, *17*, 272.
(257) Van Cauwenbergh, R.; Robberecht, H.; Van Dael, P.; Deelstra, H. *J. Trace Elem. Electrolytes Health Dis.* **1990**, *4*, 127.
(258) McMaster, D.; Bell, N.; Anderson, P.; Love, A. H. G. *Clin. Chem. (Winston-Salem, N.C.)* **1990**, *36*, 211.
(259) McLaughlin, K.; Dadgar, D.; Smyth, M. R.; McMaster, D. *Analyst* **1990**, *115*, 275.
(260) Chi, X.; Shi, Y.; Wan, B. *Beijing Shifan Dauxue Xuebao, Ziran Kexueban* **1989**, *3*, 61; *Chem. Abstr.* **1990**, *113*, 144361t.
(261) Pohl, B.; Schneider, T. *Lab. Prakt.* **1990**, *14*, 244, 246.
(262) Forrer, R.; Gautschi, K.; Lutz, H. *J. Trace Elem. Electrolytes Health Dis.* **1991**, *5*, 101.
(263) Hoenig, M. *Analusis* **1991**, *19*, 41.
(264) Kimura, T.; Sugino, K.; Tamura, Y.; Fukunaga, A. *Yamaguchi Igaku* **1990**, *39*, 601. *Chem. Abstr.* **1991**, *115*, 131068u.
(265) Welz, B.; Schlemmer, G.; Voellkopf, U. *Spectrochim. Acta* **1984**, *39B*, 501.
(266) Alexander, J.; Saeed, K.; Thomassen, Y. *Anal. Chim. Acta* **1980**, *120*, 377.
(267) Kneip, T. J. *Health Lab. Sci.* **1977**, *14*, 53.
(268) Ward, N. I.; Ryan, D. E. *Anal. Chim. Acta* **1979**, *105*, 185.
(269) Aaseth, J.; Thomassen, Y.; Alexander, J.; Norheim, G. *New Engl. J. Med.* **1980**, *303*, 944.
(270) Nève, J.; Hanocq, M.; Molle, L. *J. Pharm. Belg.* **1980**, *35*, 345.
(271) Dillon, L. J.; Hilderbrand, D. C.; Groon, K. S. *At. Spectrosc.* **1982**, *3*, 5.
(272) Cramb, R.; Starkey, B. *Ann. Clin. Biochem.* **1987**, *24 (suppl. 1)*, S1−93.
(273) Logemann, E.; Kruetzfeldt, B.; Rokitzki, L. *Beitr. Gerichtl. Med.* **1989**, *47*, 97; *Chem. Abstr.* **1989**, *111*, 192190s.
(274) Blackmore, D. J.; Stanier, P. *At. Spectrosc.* **1984**, *5*, 215.
(275) Ni, Z.; Shan, X.; Jin, L.; Luan, S.; Zhang, L.; Subramanian, K. S. *ACS Symp. Ser.* **1991**, *445 (Biol. Trace Elem. Res)*, 206; *Chem. Abstr.* **1991**, *115*, 129318g.
(276) Saeed, K.; Thomassen, Y. *Anal. Chim. Acta* **1981**, *130*, 281.
(277) Bauslaugh, J.; Radziuk, B.; Saeed, K.; Thomassen, Y. *Anal. Chim. Acta* **1984**, *165*, 149.
(278) Lewis, S. A.; Hardison, N. W.; Veillon, C. *Anal. Chem.* **1986**, *58*, 1272.
(279) Schlemmer, G.; Welz, B. *Spectrochim. Acta* **1986**, *41B*, 1157.
(280) Janssen, E. *VDLU FA-Schriftenr* **1992**, *35 (Oekologische Aspekte Extensiver Landbewirtschaftung)*, 315; *Chem. Abstr.* **1993**, *118*, 164535j.
(281) Sampson, B. *J. Anal. At. Spectrom.* **1987**, *2*, 447.
(282) García-Olalla, C.; Robles, L. C.; Aller, A. L. *Anal. Sci.* **1991**, *7*, 611.
(283) Knowles, M. B.; Brodie, K. G. *J. Anal. At. Spectrom.* **1988**, *3*, 511.
(284) Itai, K.; Takikawa, S.; Tsunoda, H. *Biomed. Res. Trace Elem.* **1991**, *2*, 87.
(285) Pohl, B.; Knowles, M.; Grund, G. *GIT Lab. Med.* **1987**, *10*, 269.
(286) Knowles, M. B.; Brodie, K. G. *J. Anal. At. Spectrom.* **1989**, *4*, 305.
(287) Voth-Beach, L. M.; Shrader, D. E. *Spectroscopy (Springfield, OR)* **1986**, *1*, 49, 52, 55.
(288) Knowles, M.; Frary, B. D. *Int. Lab.* **1988**, *April*, 52.
(289) Mo, S.; Zheng, D.; Chen, R. *Fenxi Huaxue* **1989**, *17*, 814; Anal. *Abstr.* **1990**, 11D13.
(290) Manning, D. C. *At. Absorpt. Newsl.* **1978**, *17*, 107.
(291) Welz, B.; Verlinden, M. *Acta Pharmacol. Toxicol.* **1986**, *suppl. 59*, 577.
(292) Jowett, P. L. H.; Banton, M. I. *Anal. Lett.* **1986**, *19*, 1243.
(293) Saeed, K.; Thomassen, Y. *At. Spectrosc.* **1983**, *4*, 163.
(294) Hilderbrand, D. C. *At. Spectrosc.* **1983**, *4*, 164.
(295) Fernández, F. J.; Giddings, R. *At. Spectrosc.* **1982**, *3*, 61.
(296) Sotera, J. J.; Kahn, H. L. *Int. Lab.* **1983**, *13*, 24.
(297) Deschuytere, M. A.; Robberecht, H. J.; Deelstra, H. *Bull. Soc. Chim. Belg.* **1988**, *97*, 99.
(298) Jagdt, S.; Schlesing, H. *Lab. Prakt.* **1989**, *13*, 1036, 1038.
(299) Shimmura, T.; Nakazaki, M.; Shiroishi, K. *Toyama-ken Eisei Kenkyusho Nenpo* **1990**, *14*, 175; *Chem. Abstr.* **1992**, *116*, 145539f.
(300) Voellkopf, V.; Grobenski, Z. *At. Spectrosc.* **1984**, *5*, 115.
(301) Zhao, J.; Jiang, S.; Chen, S.; Jiang, D.; Chakraborti, D. *Fresenius' J. Anal. Chem.* **1990**, *337*, 877.
(302) Oyamada, N. *Jpn. J. Ind. Health* **1982**, *24*, 320.
(303) Chi, X.; Wan, B.; Shi, Y. *Guangpuxue Yu Guangpu Fenxi* **1988**, *8*, 40; *Chem. Abstr.* **1988**, *109*, 243187q.
(304) Kamada, T.; Yamamoto, Y. *Talanta* **1980**, *27*, 473.
(305) Ihnat, M.; Westerby, R. *Anal. Lett.* **1974**, *7*, 257.
(306) Welz, B.; Melcher, M. *Fresenius' Z. Anal. Chem.* **1978**, *290*, 106.
(307) Valentine, J. L.; Kang, H. K.; Dang, P.-M.; Schluchter, M. *J. Toxicol. Environ. Health* **1980**, *6*, 731.
(308) Cox, D. H.; Bibb, A. E. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 265.
(309) Meyer, A.; Hofer, Ch.; Knapp, G.; Tölg, G. *Fresenius' Z. Anal. Chem.* **1981**, *305*, 1.
(310) Schierling, P.; Oefele, C.; Schaller, K. H. *Aerztl. Lab.* **1982**, *28*, 21; *Chem. Abstr.* **1982**, *96*, 98879y.
(311) Irsch, B.; Schäfer, K. *Fresenius' Z. Anal. Chem.* **1985**, *320*, 37.
(312) Piwonka, J.; Kaiser, G.; Tölg, G. *Fresenius' Z. Anal. Chem.* **1985**, *321*, 225.
(313) Hansson, L.; Pettersson, J.; Olin, A. *Talanta* **1987**, *34*, 829.
(314) Long, S.; Yu, P. *Guangpuxue Yu Guangpu Fenxi* **1986**, *6*, 62; *Chem. Abstr.* **1987**, *106*, 98722s.
(315) Zhang, L.; Guo, J. *Lihua Jianyan, Huaxue Fence* **1987**, *23*, 171; 177; *Anal. Abstr.* **1988**, *50*, 1D48.
(316) Cui, X.; Jiang, H.; Han, G.; Zhang, Q.; Yang, Y. *Shandong Yike Daxue Xuebao* **1988**, *26*, 61; *Chem. Abstr.* **1988**, *109*, 207765t.
(317) Okamoto, S.; Kojima, M.; Matsukoa, Y.; Hatanaka, M. *Shiga-kenritsu Eisei Kankyo Senta Shoho* **1988**, *22*, 49; *Chem. Abstr.* **1988**, *109*, 3295e.
(318) Ihnat, M.; Thomassen, Y.; Wolynetz, M. S.; Veillon, C. *Acta Pharmacol. Toxicol.* **1986**, *59*, 566.
(319) Ihnat, M.; Wolynetz, M. S.; Thomassen, Y.; Verlinden, M. *Pure Appl. Chem.* **1986**, *58*, 1063.
(320) Florence, T. M. *Talanta*, **1982**, *29*, 345.
(321) Dawcynski, H.; Winnefeld, K.; Weiland, G.; Boewe, D. *Zentralbl. Pharm. Pharmakother. Laboratoriumsdiagn.* **1988**, *127*, 404.
(322) Bye, R. *Talanta* **1990**, *37*, 1029.

(323) Tamari, Y.; Yoshida, M.; Takagi, S.; Chayama, K.; Tsuji, H.; Kusaka, Y. *Bunseki Kagaku* **1992**, *41*, T77.

(324) Offeley, S. G.; Seare, N. J.; Tyson, J. F.; Kibble, H. A. B. *J. Anal. At. Spectrom.* **1991**, *6*, 133.

(325) Dawcynski, H.; Bach, W.; Peters, H.; Vorberg, B.; Yersin, A. *Zentralbl. Pharm. Pharmakother. Laboratoriumsdiagn.* **1989**, *128*, 481.

(326) Welz, B.; Schubert-Jacobs, M. *At. Spectrosc.* **1991**, *12*, 91.

(327) Thompson, K. C.; Godden, R. G. *Analyst* **1976**, *101*, 174.

(328) D'Ulivo, A.; Lampugnani, L.; Sfetsios, I.; Zamboni, R. *Spectrochim. Acta* **1993**, *48B*, 387.

(329) Brown, A. A.; Ottaway, J. M.; Fell, G. S. *Anal. Chim. Acta* **1985**, *172*, 329.

(330) Barnes, R. M.; Genna, J. S. *Anal. Chem.* **1979**, *51*, 1065.

(331) Koyama, E.; Katsura, S.; Fukumura, M.; Nagashima, N. *Bunseki Kagaku* **1980**, *29*, 211.

(332) Kimberly, M. M.; Paschal, D. C. *Anal. Chim. Acta* **1985**, *174*, 203.

(333) Barnes, R. M.; Fodor, P. *Spectrochim. Acta* **1983**, *38B*, 1191.

(334) Fodor, P.; Barnes, R. M. *Spectrochim. Acta* **1983**, *38B*, 229.

(335) Zhuang, M.; Barnes, R. M. *Appl. Spectrosc.* **1984**, *38*, 635.

(336) Robbins, W. B.; Caruso, J. A.; Fricke, F. L. *Analyst* **1979**, *104*, 35.

(337) D'Ulivo, A.; Lampugnani, L.; Zamboni, R. *J. Anal. At. Spectrom.* **1990**, *5*, 225.

(338) Heitmann, U.; Mueller, A.; Bucsener, H.; Hese, A.; Schoknecht, G. *Colloq. Atomspektrom. Spurenanal.* **1989**, *5*, p 11; *Chem. Abstr.* **1991**, *115*, 149242f.

(339) Ting, B. T. G.; Pagounes, J.; Janghorbani, M.; Young, V. R. *J. Radioanal. Chem.* **1982**, *70*, 133.

(340) Schramel, P. *Spectrochim. Acta* **1983**, *B38*, 199.

(341) Janghorbani, M.; Ting, B. T. G. *Anal. Chem.* **1989**, *61*, 701.

(342) Ting, B. T. G.; Mooers, C. S.; Janghorbani, M. *Analyst* **1989**, *114*, 667.

(343) Lyons, T. D. B.; Fell, G. S.; Hutton, R. C.; Eaton, A. N. *J. Anal. At. Spectrom.* **1988**, *3*, 601.

(344) Valkovic, V. *Nucl. Instrum. Methods Phys. Res.* **1989**, *B40–41*, 848.

(345) Colautti, P.; Enzi, G.; Moschini, G.; Muzzio, P. C.; Stievano, B. M. *Ric. Clin. Lab.* **1976**, *6*, 169.

(346) Paradellis, T. *Eur. J. Nucl. Med.* **1977**, *2*, 277.

(347) Robberecht, H.; Van Grieken, R. *Anal. Chim. Acta* **1983**, *147*, 113.

(348) Chaitchik, S.; Shenberg, C.; Nir-El, Y.; Mantel, M. *Biol. Trace Elem. Res.* **1988**, *15*, 205.

(349) Bethel, V.; Hamm, V.; Knöchel, A. *Fresenius' Z. Anal. Chem.* **1989**, *335*, 855.

(350) Sky-Peck, H. H.; Joseph, B. J. *Clin. Biochem. (Ottawa)* **1981**, *14*, 126.

(351) Vos, L.; Robberecht, H.; Van Dyck, P.; Van Grieken, R. *Anal. Chim. Acta* **1981**, *130*, 167.

(352) Barrette, M.; Lamoureux, G.; Lebel, E.; Lecomte, R.; Paradis, P.; Monaro, S. *Nucl. Instrum. Methods* **1976**, *134*, 189.

(353) Berti, M.; Buso, G.; Colautti, P.; Moschini, G.; Stievano, B. M.; Tregnaghi, C. *Anal. Chem.* **1977**, *49*, 1313.

(354) Hyvonen-Dabek, M.; Nikkinen-Vilkki, P.; Dabek, J. T. *Clin. Chem. (Winston-Salem, N.C.)* **1984**, *30*, 529.

(355) Buso, G. P.; Colautti, P.; Moschini, G.; Hu, X.; Stievano, B. M. *Nucl. Instrum. Methods Phys. Res.* **1984**, *B231*, 177.

(356) Boerma, D. O.; Smit, E. P.; Roosnek, N. *Nucl. Instrum. Methods Phys. Res.* **1989**, *B36*, 60.

(357) Simonoff, M.; Hamon, C.; Moretto, P.; Llabador, Y.; Simonoff, G. *Nucl. Instrum. Methods Phys. Res.* **1988**, *B31*, 442.

(358) Shenberg, C.; Mantel, M.; Izak-Biran, T.; Rachmiel, B. *Biol. Trace Elem. Res.* **1988**, *16*, 87.

(359) Robberecht, H.; Deelstra, H.; Van Grieken, R. *Biol. Trace Elem. Res.* **1990**, *25*, 149.

(360) Beitz, L.; Charbit, P.; Schuon, H. *Electromedica* **1975**, *1*, 13.

(361) Standzenieks, P.; Rindby, A.; Selin, E. *Nucl. Instrum. Methods* **1978**, *153*, 269.

(362) Robberecht, H.; Van Grieken, R. *Anal. Chim. Acta* **1982**, *136*, 285.

(363) Stump, I.; Kearney, J.; d'Auria, J.; Popham, J. *Mar. Pollut. Bull.* **1979**, *10*, 270.

(364) Smits, J. A.; Van Grieken, R. E. *Anal. Chem.* **1980**, *52*, 1479.

(365) Smits, J.; Van Grieken, R. *Int. J. Environ. Anal. Chem.* **1981**, *9*, 62.

(366) Raptis, S.; Wegscheider, W.; Knapp, G.; Tölg, G. *Anal. Chem.* **1980**, *52*, 1292.

(367) Holynska, B.; Markowicz, A. *Chem. Anal. (Warsaw)* **1982**, *27*, 263.

(368) Nagj, M.; Injuk, J.; Valkovic, V. *J. Radioanal. Nucl. Chem.* **1988**, *127*, 243.

(369) Sargentini-Maier, M. L.; Maier, E. A.; Ruch, C.; Dufour, P.; Oberling, F.; Leroy, M. J. F. *J. Trace Elem. Electrolytes Health Dis.* **1987**, *1*, 99.

(370) Pella, P. A.; Dobbyn, R. C. *Anal. Chem.* **1988**, *60*, 684.

(371) Weber, G.; Robaye, G.; Bartsch, P.; Collignon, A.; Beguin, Y.; Roelandts, I.; Delbrouck, J. M. *Nucl. Instrum. Methods Phys. Res.* **1984**, *B3*, 326.

(372) Liu, N. Q.; Galassini, S.; Moschini, G.; Nagj, M.; Injuk, J.; Valkovic, V. *J. Radioanal. Nucl. Chem.* **1989**, *130*, 39.

(373) Chisela, F.; Brätter, P. *Anal. Chim. Acta* **1986**, *188*, 85.

(374) Corrocher, R.; Ferrari, S.; de Gironcoli, M.; Bassi, A.; Olivieri, O.; Guarini, P.; Stanzial, A.; Barba, A. L.; Gregolini, L. *Clin. Chim. Acta* **1989**, *179*, 121.

(375) Kato, T.; Sato, T.; Mori, K. *Anal. Sci.* **1990**, *6*, 103.

(376) Barocas, A.; Chamard, P.; Clemente, G. F.; Giorcelli, F.; Mastinu, G. G.; Pompei, F.; Zucaro Labellarte, G. *J. Radioanal. Chem.* **1976**, *34*, 19.

(377) Clemente, G. F. *J. Radioanal. Chem.* **1976**, *32*, 25.

(378) Hankins, D. A.; Riella, M. C.; Scribner, B. H.; Babb, A. L. *Surgery* **1976**, *79*, 674.

(379) Nadkarni, R. A.; Morrison, G. H. *Radiochem. Radioanal. Lett.* **1976**, *24*, 103.

(380) Zdankiewicz, D. D.; Fasching, J. L. *Clin. Chem. (Winston-Salem, N.C.)* **1976**, *22*, 1361.

(381) Versieck, J.; Hoste, J.; Barbier, F.; Michels, H.; De Rudder, J. *Clin. Chem. (Winston-Salem, N.C.)* **1977**, *23*, 1301.

(382) Behne, D.; Juergensen, H. *J. Radioanal. Chem.* **1978**, *42*, 447.

(383) Behne, D.; Wolters, W. *J. Clin. Chem. Clin. Biochem.* **1979**, *17*, 133.

(384) Lombeck, I.; Kasperek, K.; Harbisch, H. D.; Feinendegen, L. E.; Bremer, H. J. *Eur. J. Pediatr.* **1977**, *125*, 81.

(385) Lombeck, I.; Kasperek, K.; Bonnermann, B.; Feinendegen, L. E.; Bremer, H. J. *Eur. J. Pediatr.* **1978**, *129*, 139.

(386) Zmijewska, W.; Semkow, T. *Chem. Anal. (Warsaw)* **1978**, *23*, 583.

(387) Nakahara, H.; Nagame, Y.; Yoshizawa, Y.; Oda, H.; Gotoh, S.; Murakami, Y. *J. Radioanal. Chem.* **1979**, *54*, 183.

(388) Zmijewska, W.; Wodkiewicz, L.; Masiak, M. *Chem. Anal. (Warsaw)* **1980**, *25*, 531.

(389) Kostadinov, K.; Dzhingova, R. *J. Radioanal. Chem.* **1981**, *63*, 5.

(390) Krivan, V.; Geiger, H.; Franz, H. E. *Fresenius' Z. Anal. Chem.* **1981**, *305*, 399.

(391) Woittiez, J. R. W.; Van Kamp, G. J.; Das, H. A. *J. Radioanal. Chem.* **1982**, *70*, 239.

(392) Lin, S.-M. *Radioisotopes* **1983**, *32*, 155.

(393) Teherani, D. K.; Nagj-Vezekenyi, K. *J. Radioanal. Nucl. Chem.* **1986**, *104*, 53.

(394) Lavi, N.; Mantel, M.; Alfassi, Z. B. *Analyst* **1988**, *113*, 1855.

(395) McOrist, G. D.; Fardy, J. J. *J. Radioanal. Nucl. Chem.* **1989**, *134*, 65.

(396) Yonezawa, Ch.; Imai, H.; Hongo, T.; Hoshi, M.; Tachikawa, E.; Kabuto, M.; Suzuki, T. *Bunseki Kagaku* **1992**, *41*, 581.

(397) Blotcky, A. J.; Arsenault, L. J.; Rack, E. P. *Anal. Chem.* **1973**, *45*, 1056.

(398) Broghamer, W. L., Jr.; McConnell, K. P.; Blotcky, A. L. *Cancer* **1976**, *37*, 1384.

(399) Miller, L.; Mills, B. J.; Blotcky, A. J.; Lindeman, R. D. *J. Am. Coll. Nutr.* **1983**, *2*, 331.

(400) Egan, A.; Kerr, S.; Minski, M. J. *Dev. Toxicol. Environ. Sci.* **1977**, *1*, 353.

(401) Egan, A.; Kerr, S. A.; Minski, M. J. *Radiochem. Radioanal. Lett.* **1977**, *28*, 369.

(402) McKown, D. M.; Morris, J. S. *J. Radioanal. Chem.* **1978**, *43*, 411.

(403) Morris, J. S.; Anderson, H.; Cordis, M. *Trans. Am. Nucl. Soc.* **1980**, *34*, 174.

(404) Morris, J. S.; Smith, M. F.; Morrow, R. E.; Heimann, E. D.; Hancock, J. C.; Gall, T. *J. Radioanal. Chem.* **1982**, *69*, 473.

(405) Blotcky, A. J.; Hansen, G. T.; Opelanio-Buencamino, L. R.; Rack, E. P. *Anal. Chem.* **1985**, *57*, 1937.

(406) Gökmen, I. G.; Gordon, G. E.; Aras, N. K. *J. Radioanal. Nucl. Chem.* **1987**, *113*, 453.

(407) Woittiez, J. R. W.; Nieuwendijk, B. J. T. *J. Radioanal. Nucl. Chem.* **1987**, *110*, 603.

(408) McOrist, G. D.; Fardy, J. J.; Florence, T. M. *J. Radioanal. Nucl. Chem.* **1987**, *119*, 449.

(409) McOrist, G. D.; Fardy, J. J.; Sleet, R. *J. Radioanal. Nucl. Chem.* **1992**, *158*, 293.

(410) Tjioe, P. S.; de Goeij, J. J. M.; Houtman, J. P. W. *J. Radioanal. Chem.* **1977**, *37*, 511.

(411) Rosenberg, R. J. *J. Radioanal. Chem.* **1979**, *50*, 109.

(412) Weingarten, R.; Shamai, Y.; Schlesinger, T. *Int. J. Appl. Radiat. Isot.* **1979**, *30*, 585.

(413) Gebre-Medhin, M.; Gustanov, K. H.; Gamstorp, L.; Plentin, L. O. *Acta Paediatr. Scand.* **1985**, *74*, 886.

(414) Itawi, R. K.; Turel, Z. R. *J. Radioanal. Nucl. Chem.* **1986**, *106*, 81.

(415) Singh, M.; Sawant, A. D. *J. Radioanal. Nucl. Chem.* **1987**, *114*, 83.

(416) Kalousková, J.; Drábek, K.; Pavlík, L.; Hodík, F. *J. Radioanal. Nucl. Chem.* **1989**, *129*, 59.

(417) Yang, J. Y.; Yang, M. H.; Lin, S. M. *Anal. Chem.* **1990**, *62*, 146.

(418) Gordon, G. E.; Gökmen, I. G.; Aras, N. K.; Henkin, R. I. *J. Radioanal. Chem.* **1982**, *70*, 145.

(419) Kraus, R. J.; Foster, S. J.; Ganther, H. E. *Anal. Biochem.* **1985**, *147*, 432.

(420) Janghorbani, M.; Ting, B. T. G.; Young, V. R. *Am. J. Clin. Nutr.* **1981**, *34*, 2816.

(421) Wester, P. O. *Acta Med. Scand.* **1974**, *196*, 489.

(422) Girardi, F.; Marafante, E.; Pietra, R.; Sabbioni, E.; Marchesini, A. *J. Radioanal. Chem.* **1977**, *37*, 427.
(423) Drábek, K.; Kalouskova, J.; Pavlik, L. Czech. CS 269.160 15 Mar 1991; *Chem. Abstr.* **1991**, *115*, 178867u.
(424) Veillon, C.; Alvarez, L. In *Metal Ions in Biological Systems*; Sigel, H., Ed.; Marcel Dekker: New York, 1983; Vol. 16, p 103.
(425) Janghorbani, M.; Christensen, M. J.; Nahapetian, A.; Young, V. R. *Am. J. Clin. Nutr.* **1982**, *35*, 647.
(426) Tôei, K.; Shimoishi, Y. *Talanta* **1981**, *28*, 967.
(427) Poole, C. F.; Evans, N. J.; Wibberley, D. G. *J. Chromatogr.* **1977**, *136*, 73.
(428) Kurahashi, K.; Inoue, S.; Yonekura, S.; Shimoishi, Y.; Tôei, K. *Analyst* **1980**, *105*, 690.
(429) Dilli, S.; Sutikno, I. *J. Chromatogr.* **1984**, *298*, 21.
(430) Zheng, O.; Wu, J.; Xie, L. *Anal. Biochem.* **1989**, *178*, 77.
(431) Xu, F.; Li, C.; Jin, X. *Huanjing Kexue* **1987**, *8*, 84; *Chem. Abstr.* **1987**, *107*, 55051a.
(432) Wang, Q.; Lu, Z.; Wu, B. *Zhonghua Loading Weisheng Zhibeyin Zazhi* **1989**, *7*, 230; *Chem. Abstr.* **1989**, *111*, 211279w.
(433) Vézina, D.; Bleau, G. *J. Chromatogr.* **1988**, *426*, 385.
(434) Aelvoet, C.; Hanocq, M. *Analusis* **1989**, *17*, 131.
(435) Baldew, G. S.; de Goeij, J. J. M.; Vermeulen, N. P. E. *J. Chromatogr.* **1989**, *496*, 111.
(436) Aoyama, E.; Nakagawa, T.; Suzuki, Y.; Tanaka, H. *ACS Sym. Ser.* **1990**, *445 (Biol. Trace Elem. Res.)*, 240.
(437) Blais, J.-S.; Huyghues-Despointes, A.; Momplaisir, G. M.; Marshall, W. D. *J. Anal. At. Spectrom.* **1991**, *6*, 225.
(438) Funk, W.; Kerler, R.; Schiller, J. T.; Dammann, V. *JHRC CC, J. High Resolut. Chromatogr., Chromatogr. Commun.* **1982**, *5*, 534.
(439) Funk, W.; Dammann, V.; Couturier, T.; Schiller, J.; Völker, L. *JHRC CC, J. High Resolut. Chromatogr., Chromatogr. Commun.* **1986**, *9*, 224.
(440) Stahr, H. M.; Kinder, J.; Nicholson, D.; Hyde, W. *J. Liq. Chromatogr.* **1982**, *5*, 1191.
(441) Dilli, S.; Sutikno, I. *J. Chromatogr.* **1984**, *300*, 265.
(442) Shimoishi, Y. *J. Chromatogr.* **1977**, *136*, 85.
(443) Jordanov, N.; Daskalova, K.; Rizov, N. *Talanta* **1974**, *21*, 1217.
(444) D'Ulivo, A.; Papoff, P. *J. Anal. At. Spectrom.* **1986**, *1*, 479.
(445) Ducros, V.; Favier, A. *J. Chromatogr., Biomed. Appl.* **1992**, *583*, 35.
(446) Xie, L.; Ouyang, Z. *Sepu.* **1990**, *8*, 250. *Anal. Abstr.* **1991**, *53*, 6F129.
(447) Wu, J.; Ouyang, Z. *Sepu.* **1987**, *5*, 236. *Anal. Abstr.* **1988**, *50*, 5D245.
(448) Wheeler, G. L.; Lott, P. F. *Microchem. J.* **1974**, *19*, 390.
(449) Hawkes, W. C.; Kutnink, M. A. *J. Chromatogr.* **1991**, *576*, 263.
(450) Shibata, Y.; Morita, M.; Fuwa, K. *Anal. Chem.* **1984**, *56*, 1527.
(451) Aelvoet, C.; Hanocq, M. *Analusis* **1986**, *10*, 523.
(452) Wu, D. Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1,031,134; *Chem. Abstr.* **1992**, *116*, 15067q.
(453) Wan, Y. *Fenxi Shiyanshi* **1988**, *7*, 32; *Chem. Abstr.* **1989**, *111*, 3617c.
(454) Liu, X.; Zhu, L.; Zhao, Y.; Zhu, Y.; Yao, L.; Han, Z. *Lihua Jianyan, Huaxue Fence* **1991**, *27*, 54, 56; *Chem. Abstr.* **1991**, *115*, 4531q.
(455) Huang, B.; Zhang, H.; Pu, G.; Yin, F.; Zheng, S.; Yang, H. *Anal. Lett.* **1985**, *18*, 279.
(456) *Princeton Applied Research Workshop Manual*; Princeton: Princeton, NJ, 1973.
(457) Nürnberg, H. W. *Electrochim. Acta* **1977**, *22*, 935.
(458) Valenta, P.; Rutzel, H.; Nürnberg, H. W.; Stoepler, M. *Fresenius' Z. Anal. Chem.* **1977**, *285*, 25.
(459) Chiang, L.; James, B. D.; Magee, R. J. *Mikrochim. Acta* **1989**, *II*, 149.
(460) Tanaka, S.; Sugawara, K.; Taga, M. *Anal. Sci.* **1990**, *6*, 475.
(461) Adeloju, B.; Bond, A. M.; Hughes, H. C. *Anal. Chim. Acta* **1983**, *148*, 59.
(462) Hasdemir, E.; Somer, G. *Analyst* **1990**, *115*, 297.
(463) Yang, S.; Lin, Y.; Liu, X.; Wu, J.; Hu, Y.; Chen, Y.; Tan, G.; Luo, S. *Zhonghua Yixue Jianyan Zazhi* **1991**, *14*, 336; *Chem. Abstr.* **1992**, *117*, 22732b.
(464) Versieck, J. *J. Micronutr. Anal.* **1989**, *6*, 261.
(465) Thomson, C. D.; Robinson, M. F. *Am. J. Clin. Nutr.* **1980**, *33*, 303.
(466) Robinson, M. F.; Thomson, C. D. *Nutr. Abst. Rev.* **1983**, *53*, 3.
(467) Kumpulainen, J.; Koivistoinen, P. *Kem.-Kemi* **1981**, *8*, 372. *Chem. Abstr.* **1981**, *95*, 113827e.
(468) IUPAC, Comission on Toxicology, Subcommittee on Toxicology of selenium. *Pure Appl. Chem.* **1987**, *59*, 927.
(469) Koh, T.-S. *Anal. Chem.* **1987**, *59*, 1597.

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