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SELENIUM ANALYSIS: A REVIEW

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Abstract. *Selenium is an essential microelement for the proper functioning of the human body. It is involved in many metabolic functions and processes having mainly an antioxidant role. it participates in the regulation of the immune system, nervous system, endocrinological system and reproductive system. However, excess selenium has toxic effects. Therefore a proper diet is required, which involves from one side knowing the concentration of selenium in food and from the other side quantifying selenium in biological samples in order to intervene in case of deficiency or intoxication. Over time, a number of analytical methods have been developed: spectrophotometry, spectrofluorimetry, atomic spectrometry, electroanalytical methods, neutron activation methods, x-ray methods, chromatographic methods and plasma spectroscopy methods.*
Keywords: selenium, selenium deficiency, selenium analysis, selenium quantification

1. Introduction

Because it is vital to healthy development, disease prevention and wellbeing, selenium is considered an essential element in human nutrition [1-3]. At least 30 selenoproteins (thioredoxin reductases, gultathione peroxidases and iodothyronine deionidases) are known, in which selenium is present in the form of selenocysteine (Se-Cys) and selenomethionine (Se-Met) [4-8]. The role of selenium as an antioxidant is mainly fulfilled by the glutathione peroxidases class, which act by neutralizing reactive oxygen species (ROS) [9]. In addition to the antioxidant role, it also acts as an antimutagenic, anticarcenogenic, antiviral, antibacterial, antifungal and antiparasitic agent [10, 11]. The anticarcinogenic role has been demonstrated in a variety of cellular models and even in some clinical studies [12].

Being a powerful antioxidant, involved in cellular defense against reactions with free radicals, the risk of deficiency seems to increase in proportion to age. There is evidence that most degenerative diseases originate in the harmful reactions of free radicals. These diseases include atherosclerosis, cancer, inflammatory joint, asthma, diabetes, senile dementia and degenerative eye disease [7, 8, 13].

It has been observed that selenium deficiency disrupts mitochondrial activity, and supplements with Se intensifies the activity of selenoenzymes,

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protecting the integrity of the membrane system and the structure of mitochondria. The activity of the hormone glutathione peroxidase can be used as a predictive marker of semen quality, being a suitable tool to evaluate the fertilization capacity of sperms. Selenium deficiency can cause reproductive disorders, in some cases leading to infertility. The low concentration of this element in the seminal material is characterized by decreased sperm motility and a low amount of adenosine-5'-triphosphate (ATP) enzymes, which is required for testicular development and spermatogenesis [2].

In addition, selenium inhibits viral cytotoxic effects through its antioxidant action [4, 10, 14]. Selenium deficiency promotes the mutation, replication and virulence of RNA viruses. In a selenium-rich region of China, recovery rates were observed to be three times higher than average compared to other regions where selenium levels are lower, regarding SARS-CoV-2 infection [15] . Given the influence of selenoproteins on viral pathogenicity through the cellular and viral mechanisms in which they are involved, a correlation between pathogenicity and increased mortality was observed in SARSCoV-2 infection [14, 16]. Patients with COVID-19 who have severe forms of the disease or pre-existing comorbidities may benefit from a selenium-rich diet or the administration of selenium-containing dietary supplements [16]. In addition to selenium deficiency which is directly associated with two syndromes [17] (Keshan's disease and Kasihn-Beck disease), excess selenium has toxic effects. Excess selenium can have as side effects nausea, vomiting, diarrhea, fatigue, skin lesions, muscle spasms, neurological disorders and even death [10, 18, 19].

The daily recommended dose in USA is 20 μg/day for children under 3 years of age and up to 55 μg/day for adults. These values can be increased to 60 μg/day for pregnant women and 70 μg/day for breastfeeding women [20]. The dose recommended for adults by the World Health Organization (WHO) is 50 μg/day for adults. At around 1500 μg/day toxic effects begin to appear [21]. The necessary selenium is obtained through a balanced diet, with an adequate selection of meat, vegetable and dairy products. Proper assimilation of selenium from these foods depends on the bioavailability, bioaccessibility and bioactivity of the compounds per se [22].

Some food matrices were analysed after digestion with concentrated nitric acid and hydrogen peroxide in a Milestone microwave digestion system. ^{78}Se isotope was quantified on an Agilent 7800 ICP-MS in helium collision mode by the use of an octopole based cell with ^{74}Ge as internal standard. The results obtained are presented in Table 1. In view of the above, quantification of selenium in biological materials may become clinically imperative in situations of excessive or insufficient intake. Also, the quantification of selenium in food matrices is an important topic in order to establish a proper diet.

Table 1. Selenium content from some food matrices

Food matrix	***Selenium content (mg/kg)***
Mixed nuts (peanuts, almonds, walnuts, cashew)	3.84
Shiitake mushrooms	0.39
Green salad	<0.02
Tomatoes	<0.02
Potatoes	<0.02
Apples	<0.02
Bananas	0.04
Pork meat	0.27
Cow meat	0.21
Chicken meat	0.17
Fish meat	0.14
Canned tuna	0.81
Eggs	1.07
Oat cereals	0.11
Hard goat cheese	0.06
Whole milk	0.03

2. Sample preparation

In general, sample preparation is one of the most important steps in determining trace elements. The selection of the appropriate digestion method is critical in the analysis of substrates containing organic selenium compounds. Some digestion methods may work for some, but not necessarily for all substrates, errors usually occur when determinations are made on organic matrix, the accuracy, precision and repeatability of analytical methods being negatively influenced by the digestion process, if it is not performed properly [23].

An interesting alternative is the traditional liquid-liquid extraction and, more recently, the extraction mediated by colloidal mycelium. The critical point is the temperature above which the aqueous solutions of nonionic surfactant are disturbed. The micelles of such nonionic surfactants known as Triton X-100 or X-114 have a non-polar core and an extended polar layer, in which both the extracted solvents and the extracted complexes can be solubilized. Above the critical point, the solution is separated into two phases, a rich phase containing a high concentration of surfactant in a small volume and a poor phase, with a surfactant concentration close to the critical micellar concentration (CMC). The hydrophobic species (hydrophobic organic compounds or metal ions after reaction with a suitable hydrophobic ligand) present in the sample are able to interact with the mycelium, thus being concentrated in the small volume of the surfactant-rich phase. This procedure is called "cloud point extraction" (CPE) [7].

Atomic absorption with hydride generation and atomic fluorescence spectrometry are techniques that possess the advantages of high sensitivity and versatility. Selenium is reduced from Se(VI) to Se(IV), usually by heating with

hydrochloric acid and then Se(IV) is converted to hydrogen selenide by reduction with sodium borohydride. The hydride is transported by a gas stream (usually argon) to a cell in the light beam of an atomic absorption spectrometer. The cell is heated either electrically or by a flame to oxidize selenium from hydride to elemental selenium. Temperatures of about 850° C are required. All samples, blood, plasma, urine and tissues can be treated equally well by this technique [19]. The mixture of nitric-perchloric acid or the mixture of nitric-perchloric-sulfuric acid has proven to be the most effective method of preparing samples for HG-AAS [24].

Research to improve extraction has led to the development of microextraction (ME) techniques, which involve simple pre-concentration with low costs. Of all the MEs, dispersive liquid-liquid microextraction is the most favorable for selenium. On the other hand, atomic absorption spectrometry was the most commonly used instrumentation. Selenium and ME have rarely been coupled to spectrophotometry and X-ray spectrophotometry methods. Another technique used was a double preconcentration process consisting of microextraction before preconcentration, followed by the determination of selenium using cathodic stripping voltammetry (ME-CSV) [25].

Several methods for selenium microextraction have been developed, such as dispersive liquid-liquid microextraction (DLLME), ultrasound assisted emulsification and dispersive liquid-liquid microextraction (USAE-DLLME), ionic liquid dispersive microextraction (ILDME), ultrasound assisted emulsification and ionic liquid dispersive liquid-liquid microextraction (USA-IL-DLLME), dispersive solid phase microextraction (DSPME) and electrochemically controlled in tube solid phase microextraction (EC-in-tube-SPME) [25]. Some studies have described the use of formic acid in sample preparation prior to analysis, including analytical methods using inductively coupled plasma mass spectrometry (ICP-MS), electrothermal atomic absorption spectroscopy (ET-AAS), inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry with electrothermal vaporization (ETV-ICP-MS) [2].

For the determination of selenium both by UV-VIS spectrophotometry and by ICP-MS, open system digestion techniques have been reported [10], [18], but the digestion of samples with nitric acid and hydrogen peroxide is most frequently used in closed microwave system [4]. Also, biological samples were analyzed on ICP-MS such as serum and breast milk without prior digestion, by dilution with nitric acid and TritonX-100, and blood samples by dilution with ammonium hydroxide solution, disodium EDTA and TritonX -100 [26].

For the analysis of different species of selenium by HPLC-ICP-MS, the extraction method is more complicated and involves boiling in water, the use of Tris-HCl buffer, acid hydrolysis and enzymes such as proteases, pronases, proteinases and protease-lipases [4].

3. Analytical methods

The methods used for the determination of selenium and its different species are: UV-VIS spectroscopy, neutron activation analysis (NAA), hydride generation atomic absorption spectroscopy (HG-AAS), inductively coupled plasma atomic emission spectroscopy (ICP-AES / ICP-OES), electrothermal atomic absorption spectroscopy (ET-AAS), inductively coupled plasma mass spectrometry (ICP-MS), molecular and atomic fluorescence spectrometry, high performance liquid chromatography (HPLC), voltammetry, atomic emission spectroscopy (AES) and spectrophotometric methods [7, 18, 27, 28].

The need to determine the selenium content and, in particular, the content of its species in food or food supplements has developed along with the variety of food supplements on the market. Although organic selenium species are dominant in selenium-enriched products, inorganic selenium species, especially Se (IV) and sometimes Se (VI), have also been found in food supplement products. It has been shown that the HPLC-HG-AAS system allows the determination of inorganic selenium species in samples of different types, however the method currently used for this purpose is HPLC-ICP-MS [29]. Gas chromatography (GC) can also be used to identify selenium compounds, mainly to determine volatile species. GC can also be applied to non-volatile species after derivatization (e.g. with ethyl chlorate, isopropyl chloroformate, diethylethoxymethylene malonate), but this method is time consuming [22].

Catalytic-kinetic methods that have been widely studied are an attractive alternative for quantification of selenium. However, they are time consuming, have a short linear concentration range and their accuracy is poor. In addition, most of these methods are not sensitive to the determination of selenium (IV) in the range of ppb. For this, several methods for the determination of Se (IV) after its preconcentration by solid phase extraction have been reported. But all these methods could not measure Se (IV) concentrations lower than 10 ng/mL and are time consuming methods due to the preconcentration and dissolution steps [30].

Because selenium is found in low concentrations and in complex matrices, selective and sensitive methods are needed to determine it. The most widely used are atomic absorption spectroscopy with hydride generation (HG-AAS), electrothermal atomic absorption spectroscopy (ET-AAS), instrumental and radiochemical neutron activation analysis (INAA and RNAA) and inductively coupled plasma mass spectrometry (ICP-MS). Of these, ICP-MS analysis is the most suitable technique for quantification of selenium [4].

3.1. Spectrophotometric methods

Spectrophotometric methods have been very popular, being used to determine metals, but recently they have become obsolete because they consume a lot of time and do not provide a sufficiently low detection limit. The detection limit of this methods can be reduced by special sample preparation, for example extraction, sorption, chelation, to concentrate the analyte that has to be analysed. Analyses of different selenium species are possible using different reaction conditions (e.g. different pH values) or a combination of different methods [31, 32].

Due to its importance, several analytical techniques have been developed for the determination of selenium. Several spectrophotometric methods have been reported with certain chromogenic reagents, such as 3,3-diaminobenzidine, dithiozone, 8-hydroxyquinoline, chromotropic acid, J acid, 1-naphthylamine-7-sulfonic acid and Leuco Crystal Violet [3, 33]. Common reagents in the spectrophotometric determination of selenium are dithizone, o-phenyldiamine and chromotropic acid. Methods based on the use of amines require a long reaction time (~ 2 hours), are not selective and are subject to different interferences. Another reagent used for the spectrophotometric determination of selenium is 6-amino-1-naphthol-3-sulfonic acid (acid J), which forms with Se (IV) at pH 1-2.5 a yellow complex having a maximum absorption at 392 nm . The method is practically free of interference and allows to obtain a detection limit of 80 ng/mL. With 2,3-diaminonaphthalene selenium reacts in the presence of bromide ions that act as a catalyst, forming a complex that can be extracted with cyclohexane in an acidic medium. The maximum absorption of the complex is 378.5 nm, and the detection limit of the method is 12 ng/ml [8, 31].

A classical method of determining selenium is based on the formation of a yellow complex between Se (IV) and 3,3'-diaminobenzidine (DAB) in acidic medium. The complex is extracted with toluene and has a maximum absorption at 420 nm. This reaction is specific for selenium, being recommended for the determination of selenium in surface waters [31]. Another proposed method is based on the reaction of Se with KI in acidic medium to release iodine, which reacts with starch to form a blue product which has a maximum absorption at 589 nm. The limit of quantification of the method was calculated at 0.012 mg/L with a recovery between 50 and 120% and an accuracy of plus-minus 15%. This method has been used to determine selenium in water, plant material, human hair and waste [1].

The study by Revanasiddappa et al. reported the development of a simple, sensitive and selective method for the determination of selenium with a new reagent, Variamine Blue (VB), based on the release of iodine in the reaction of selenium with potassium iodide in an acidic environment and the subsequent oxidation of VB by the iodine released to form a purple species. The developed method has been successfully used for the determination of selenium in water, soil, plant materials, human hair, cosmetics and pharmaceuticals [3].

An interesting reaction is the catalytic reduction of methylene blue by selenosulfides. The sensitivity of the method can be increased by applying a cationic surfactant such as cetyltrimethylammonium bromide (CTAB) [31]. In a paper published by Pathare and Sawant the operating conditions for the spectrophotometric determination of selenium (1V) with the sodium salt of hexamethylene imincarbodithioate (NaHMICdt) as a ligand by the liquid-liquid extraction technique are presented. Under acidic conditions, selenium forms a yellow complex with the ligand and can be extracted into toluene, having a maximum absorption at 335 nm. The absorbance of selenium samples (1V) increases linearly in the concentration range of 0.5-4.0 mg/L, at pH = 1 (4N HCl). It was found that the stoichiometry of the complex is 1: 4. The proposed method is extremely sensitive, reproducible and has been applied satisfactorily to determine the amount of selenium in polluted water, plant material, wheat flour and shampoo-drug [34].

An automatic injection spectrophotometric method has been developed for the rapid, simple, selective and sensitive determination of selenium (IV) in various multivitamin and mineral pharmaceutical formulations. The method is based on the oxidation of 4-aminoantipyrine (4-amino-1,2-dihydro-1,5-dimethyl-2-phenyl-3H-pyrazol-3-one; 4-AAP) by selenium in acid medium and coupling with N-(naphthalen-1-yl)-ethane-1,2-diamine dihydrochloride (NEDA) to give a purple derivative. A peristaltic pump was used to introduce dilute Se(IV), HCl, 4-AAP and NEDA solutions into the reaction coil through an automatic system. Lambert-Beer law was obeyed for the concentration range 0.05-5.0 mg/mL. This method has been found suitable for estimating the concentration of selenium (IV) in various multivitamin and mineral pharmaceutical formulations, such as tablets and capsules. Studies show that selenium (IV) can easily participate in oxidative coupling reactions, in acidic environment, with 4-AAP-NEDA [35].

3.2. Spectrofluorimetry

Spectrofluorimetry was used to determine selenium in hair and other biological substrates. Quantification is based on the reaction of selenium with an organic ligand such as 3,3'-diaminobenzidine (DAB) or 2,3-diaminonaphthalene (DAN) to form a fluorescent complex [36]. The resulting fluorophore (a piazselenol) is extracted from the aqueous acid solution in cyclohexane, in which its fluorescence can be measured (excitation: 366 nm, emission: 525 nm). In practice, the process is quite complicated: the organic materials must be completely digested, and the Se^{IV} thus produced must be reduced before the complexation process is possible. DAN is potentially photolabile and the efficiency of piazselenol formation depends on pH. The volumetric ratio of digestive acid to the sample must be optimized for a particular heating system, and the performance of the heating device (usually an electrically heated aluminum block) is critical; invariable change

requires re-optimization of the temperature program [19]. Also, interference from coextractants can often lead to reduced sensitivity [36].

A kinetic spectrofluorimetric methods was developed as well, in which Se(IV) acts as a catalyst for the reduction of resorufin by sulphide in the presence of cetylpyridinium chloride. The linearity of the method was studied in the range of 5 – 1000 μg/L, with a detection limit of 1 μg/L. Although the samples were prepared in concentrated hydrochloric acid to ensure the reduction of Se(VI) to Se(IV), it was shown that only Se(IV) was catalytically active [37].

Another method reported is based on a oxidation reaction of selenium with 2-(a-pyridyl)thioquinaldinamide (PTQA) a fluorescent compound was obtained. The determination was carried out by flow injection analysis (FIA), showing a linear range for Se(IV) between 10 ppb – 2.2 ppm [38].

3.3. Atomic spectroscopy

Sample introduction as a hydride, for atomic absorption spectroscopy (HG-AAS) and other atomic spectroscopic techniques, has a number of advantages in determining selenium and other volatile hydride-forming elements. Currently, the most common source of atomization for the generation of AAS hydrides is the heated quartz T tube. The T-tube can be heated either by a flame (usually an air-acetylene flame) or in an electric oven. Electric heating offers the possibility of choosing an optimal atomization temperature for each element. Some of the main problems associated with conventional processes is that the hydrides formed are diluted by co-evolved hydrogen and carrier gas, thus reducing the sensitivity of the determination. Also, many hydrides are not efficiently atomized at the maximum temperature reached in quartz tubes and therefore suffer from gaseous interference [39].

The acidic sample solutions are usually treated with sodium (III) tetrahydroborate as the reducing agent to generate the volatile covalent hydride of the element under analysis. The tetrahydroborate ion converts Se (IV) to hydride (H_2Se) and the reaction of $NaBH_4$ with HCl produces a constant stream of hydrogen. Hydrides and excess hydrogen are removed from the generating vessel using an argon stream in a chemically generated diffusion flame. The hydrides are atomized and the resulting atoms are detected by atomic fluorescence. The hydride generation process is subject to chemical interference. Transition metal ions (eg nickel, copper and cobalt) can cause signal suppression. One of the advantages of using atomic fluorescence is that the additional sensitivity allows the sample to be diluted to overcome these interference effects. Since only Se (IV) forms a volatile hydride, it is necessary to pre-treat the samples consisting in their disaggregation and reduction [40], [41]. Depending on the preconcentration procedure used, the detection limits reported were between 0.12 – 0.24 ng/mL [39].

Hydride generation can be performed in batches or alternatively, mechanized or even automated, using different sample introduction techniques. Continuous flow analysis using a multi-channel peristaltic pump has been used in several hydride generation accessories. The main advantage is simplicity, but it involves a high consumption of samples and reagents, which are fed continuously as long as the pump is turned on. Flow injection analysis (FIA) is a useful automation technique that can be used to generate hydrides. Several papers have been published on the use of FIA for the generation of selenium hydride in HG-AAS, HG-AFS and to a lesser extent in atomic emission spectroscopy (HG-ICP-OES) [20]. In the flow injection system for hydride-generating atomic absorption spectrometry, the sample solution is diluted with a carrier liquid (water) and the sensitivity is reduced. Capturing hydrides with liquid nitrogen and electrothermal release is a difficult process [42].

Although AFS combines the advantage of a wide, dynamic, linear range, typical for atomic emission techniques and the high selectivity of atomic absorption for some elements, the method has so far not found widespread use. The problems in the analysis of real samples are due to the interference effects of the matrix. Coupling a fluorescence technique with a vapor generation technique has the potential to overcome these problems [40, 41].

Atomic fluorescence is based on a principle similar to that of atomic absorption spectroscopy and thus the construction of analytical instruments is similar. Radiation from a strong source (lamp without EDL electrodes, laser) is absorbed by the plasma generated by the flame or by an electrothermal atomizer. The absorption determines the excitation of the atoms that emit radiation (fluorescence) when returning to the ground level. The wavelength of the emitted fluorescence can be the same as that of the excitation radiation (resonant fluorescence), longer (passing through an intermediate state) or shorter (thermoluminescence). The intensity of fluorescent radiation is proportional to the concentration of atoms in the plasma, which allows quantitative analysis (for example by a comparison with a calibration curve). The intensity of fluorescent radiation is measured by the detector, which is placed at 90° with respect to the excitation radiation coming from the source. This eliminates the effect of excitation radiation on the intensity of fluorescence. However, the result may still be affected by the scattering of radiation by the sample [31, 33, 43]. The hydride generation atomic fluorescence spectrometry (HG-AFS) method was used for selenium determination in water soluble protein and peptide fractions with a detection limit of 0.2 ng/g [40].

Determination of total selenium at trace and ultra-trace levels is sometimes performed by electrothermal atomic absorption spectroscopy (ET-AAS) [20]. The technique shows similar sensitivity to the hydride generation technique, having the advantage of a more simple sample preparation, but the disadvantage of spectral

and vapor phase interferences. The detection limit reported for biological sample was as low as 12 ng/mL [44].

3.4. Electroanalytical methods

One of the electroanalytical methods reported for the determination of selenium is cathodic stripping voltammetry (CSV) with a hanging mercury drop electrode (HMDE), where selenium in reduced and deposit in the form of HgSe or as an intermetallic compound, if Rh or Cu are added in the sample [45].

Other electroanalytical techniques described for the determination of total selenium content are differential pulse cathode stripping voltammetry (DPCSV), with a detection limit of 121 ng/mL, as well as the differential pulse polarography (DPP) method. The latter was used to determine selenium Se (IV) in a nitric acid medium, reaching a high diffusion current intensity. When using a strong basic electrolyte (pH> 9), the limit of quantification was 0.08 ng/mL. Another method applied for the determination of Se (IV) was the catalytic polarography method, providing the detection limit of 0.04 ng/mL. [31], and adsorption stripping voltammetry (AdSV) by using a Bi/Hg film coated electrode at the detection limit of 0.07 ng/mL and the quantification limit of 0.25 ng/mL [46].

3.5. Neutron activation analysis

The methods of analyzing selenium by neutron activation are based on the phenomenon of artificial radioactivity. In this phenomenon, called activation, radioactive nuclei are formed as a result of the bombardment of a certain substance with neutrons, ions or photons. The sample is irradiated by a cold neutron beam. Nuclear neutron capture elements release characteristic gamma rays, prompt to de-excitation. The measurement of gamma rays allows the determination of the elements in the sample. Thus, the isotopes formed can be identified based on measurements of radiation energy and half-life. Quantitative analysis is based on the dependence of the radioactive activity of a given isotope and its concentration [47].

The use of neutron activation assay for selenium quantification has been successfully applied to biological samples such as hair, nails and urine. The results obtained were in the range of 0.3 mg/kg – 1.6 mg/kg [48]. The advantage of this technique is that the sample is not destroyed and can be used multiple times [31]. Another study on selenium analysis from human urine by NNA reported a detection limit of 0.6 ng/mL [49].

3.6. X-ray methods

One method for quantifying selenium is X-ray fluorescence analysis, excited by synchrotron radiation. The method allows a high sensitivity when the determined quantities are very small. In parallel, selenium species can be

investigated with X-ray absorption spectrometry techniques, such as XANES (X-ray absorption near edge structure) and EXAFS (Extended X-Ray Absorption Fine Structure) [50]. In a study conducted in USA the micro X ray absorption spectroscopy techniques (XANES and EXAFS) were used to determine the species of selenium release into the environment following mining activities [51]. The x ray absorption near edge structure spectroscopy was also use to investigate selenium species in selenium enriched button mushrooms and selenized yeasts [52].

X-ray fluorescence (XRF) and particle-induced X-ray emission (PIXE) are two analytical methods that were used for selenium analysis. In the XRF method, the sample is excited by X-rays and then emits secondary X-rays (so-called X-ray fluorescence). Each element emits a characteristic spectrum of radiation (qualitative analysis) and the intensities of the spectral line are proportional to the content of that element in the sample (quantitative analysis). In the PIXE method, atoms are excited by a rapid proton bombardment, and the sensitivity of the method is 100 times higher than that of XRF [31]. Total reflection X-ray fluorescence method (TXRF) was used in quantification of Se(IV) in water, after reduction with ascorbic acid. A detection limit of 0.8 ng/mL was reported [53]. In a recent study following the causal relationship between COVID and selenium levels, TXRF was also used to quantify selenium in serum [16]. In a recent study following the causal relationship between COVID and selenium levels, total reflection X-ray fluorescence (TXRF) was used to quantify selenium in serum [16].

3.7. Chromatographic methods

Chromatographic methods, in particular gas chromatography (GC) and high performance liquid chromatography (HPLC), allow the quantification of selenium at different oxidation states and the identification of specific compounds, both organic and inorganic. Unfortunately, detectors used in chromatography, such as capture electron detectors (CEDs), thermal conductivity detectors (TCDs), flame ionization detectors (FIDs), photoionization detectors (PIDs), UV-Vis spectrophotometers , IR, UV) or fluorimeters (RF), have a relatively low sensitivity and do not have sufficiently low detection limits. The situation is improved by the use of atomic absorption spectrometers, atomic fluorescence spectrometers or plasma spectrometers with emission or mass detection and with different excitation sources [54].

A method for the determination of selenium was developed on a GC equipment with an electron capture detector based on the reaction of Selenium(IV) with 3-bromo-5-trifluoromethyl-1,2-diaminobenzene to form 4-bromo-6-trifluoromethylpiazselenol. The methods detection limit was calculated at 2.2 ng/mL [55].

3.8. Plasma spectroscopy methods

The sample to be studied is transformed into aerosols by a nebulizer or a spark ablation system in which a fragment of the sample is evaporated from its surface and introduced into the neutral gas stream (e.g. argon) to which a high frequency signal is applied) by inductive coupling (without electrode). The signal energy heats the gas (argon + sample) to 10000 ° C - the plasma state, in which most atoms are ionized. Excited atoms emit radiation and based on this emission spectrum can determine the chemical composition (quantitative and qualitative). The method based on plasma excitation and emission spectrum analysis is known as ICP-AES or ICP-OES (inductively coupled plasma atomic emission spectroscopy) [56]. ICP-MS (Inductively Coupled Plasma Mass Spectrometry) measure the ions created in the plasma, that are separated according to their mass ratios, so as to allow the identification and quantification of the present analytes. ICP-MS provides a higher sensitivity (i.e. low detection limits) for a wide range of elements [10].

In recent years, inductively coupled plasma mass spectrometry (ICP-MS) has generated much interest as a more viable instrumental method for determining a wide range of elements. In addition to the ability to provide trace detection limits, the method has the advantage of a simultaneous and rapid multi-element determination procedure [36]. Selenium isotopes that can be analysed through ICP-MS are ^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se, and ^{82}Se. From these the most abundant isotope is ^{80}Se, but the spectral interference with $^{40}Ar_2^+$ makes it unsuitable for analysis [45]. The reported detection limits are as low as 0.01 mg/kg [2].

In a study conducted by Prioteasa et al on biological sample analysis (breast milk and blood) the reported detection limit and quantification limit for selenium were 0.07 μg/L and 0.14 μg/L, respectively [26]. Inductively coupled plasma mass spectrometry is possibly the most widely used technique for the quantification of selenium in recent years, as it allows the simultaneous analysis of a large number of elements, is linear in several orders of magnitude, reaches very low limits of quantification and very short time of analysis [2], [4], [10], [23], [26]. The reported detection limits for the ICP-MS technique in multiple studies are summarized in Table 2. ICP-MS in tandem with high pressure liquid chromatography (HPLC) is often used to identify different species of selenium [4, 40]. The detection limits for the analytical methods discussed above are presented in Table 3.

Table 2. ICP-MS detection limits

Elements analyzed	***Limits of detection***	***References***
Co, Cr, Mo, Nb, Zr, Mn, Si	< 0.05 ppb	[57]
Co, Cr, Mo, Ti, Fe, Al, V, Mn	< 0.001 ppb	[58]
Hg	0.01 μg/g	[59]
Mn, As, Pb, Co, Ni, Cu, Zn, Se, Ca, Cr, Cd	0.001 ppb – 1.104 ppb	[26]

Table 3. Detection limits of the analytical methods used for the determination of Selenium

Technique		***Detection limits / Range of detection limits***
Spectrophotometry		12 ppb – 5 ppm
Spectrofluorimetry		1 ppb – 10 ppb
Atomic spectroscopy	HG-AAS	0.12 ppb – 0.24 ppb
	HG-AFS	0.2 ppb
	ET-AAS	12 ppb
Electroanalytical methods	DPCSV	121 ppb
	DPP	0.08 ppb
	Catalytic polarography	0.04 ppb
	AdSV	0.25 ppb
Neutron activation analysis		0.6 ppb
X ray method	TXRF	0.8 ppb
Chromatographic methods (GC)	GC	2.2 ppb
Plasma spectroscopy methods	ICP-MS	0.07 ppb

Conclusions

Over time, a number of analytical methods have been developed to quantify total selenium and its species. A positive evolution of methods performances is observed, such as lower quantification limits, improved repeatability, better precision and accuracy. It's important to mention that the detection limits are dependent on a number of factors such as sample pretreatment, sample preparation and instrument configuration, therefore the values may vary and are not absolute.

The choice of an appropriate technique should be made together with a suitable method of sample preparation considering the matrices to be tested and the expected selenium concentration. Among the techniques mentioned, inductively coupled plasma mass spectrometry (ICP-MS) appears to be the most widely used technique nowadays for the determination of total selenium.

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