ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
30 (2002) 1425–1429

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Short communication

Differential pulse cathodic stripping voltammetric determination of selenium in pharmaceutical products

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Received 22 April 2002; received in revised form 23 July 2002; accepted 30 July 2002

Abstract

The aim of this paper is to determine selenium from pharmaceutical products by differential pulse cathodic stripping voltammetry. Firstly, were established the optimum parameters for voltammetric determination of selenium (electrolyte, deposition time, pulse duration, pulse amplitude, etc.) and secondly, the content of selenium was determined in five pharmaceutical products. The drug samples were treated with a mixture of 6 ml HNO_3 and 1 ml H_2O_2 in the microwave oven. Due to the matrix effects the method of addition is preferred. The peak potential is −0.545 V vs. Ag/AgCl, and the calibration curve is linear up to 0.125 ng/ml, but selenium was determined from pharmaceutical products used the calibration curve in the range 8–64 ng/ml, due to the concentration of selenium in these tablets.

Keywords: Selenium; DPCSV; Pharmaceutical products

1. Introduction

Selenium is an essential element for plants, animals and human body, but at high concentration it can become toxic. The range between the concentration in which selenium is essential and toxic is very narrow.

The toxicity of selenium depends to its chemical species, inorganic and organic forms in natural compounds. The most frequently inorganic species are selenite and selenate; organoselenium compounds in biological samples with direct Se–C bonds are methylated compounds, selenoamino acids, selenoproteins and their derivatives [1].

The compounds of selenium protect the cell membranes from oxidative damage; catalyze the reactions of the intermediate metabolism; inhibit the toxicity of some heavy metals (As, Cd, Pb, Tl, Hg and Sn) [2].

A selenium deficiency can produce a necrotic degeneration of liver, pancreas, heart and kidney and sometimes can increase risk of cancer. But

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0731-7085/02/$ - see front matter
PII: S0731-7085(02)00454-5

high selenium concentration can produce for animals: inflammation of the feet, softening and loss of hoofs and horns; and for humans: losing hair and nails, irritation of skin and eyes.

The selenium-responsive disease are necrotic liver degeneration in rats, mice, rabbits, and pigs; muscular dystrophies (myopathies) in lambs, calves, pigs, horses, and turkeys; infertility in ewes; unthriftiness in cattle and sheep; poor hair and feather development in pigs, horses, and chickens; exudative diathesis in chicks; and atrophy and fibrosis of the pancreas in chicks [3].

Selenium act as an anticarcinogen, but it is also a potent toxin, the problem is whether anticarcinogenic effects can be mediated by cytotoxicity. One of the toxic effects of selenium is DNA fragmentation and DNA fragmentation induced by other toxic compounds can lead to an activation of the nuclear enzyme poly(ADP–ribose)polymerase. This enzyme utilizes NAD as a substrate and extensive DNA fragmentation may lead to cell death via NAD depletion. It is possible that selenite can interact with other DNA-damaging compounds so that is selectively kills DNA-damaged cells [4].

Selenium deficiency can increase the risk of ischemic heart disease, accelerate the progression of liver disease in chronic alcoholism, acquired immunodeficiency syndrome [5].

Selenium is a component of some enzymes and proteins with an antioxidation role; it can influence the thyroid hormone production and has an important action in E vitamin absorption. The most important quantity of selenium is in liver, kidney, heart and spleen. The concentration of plasmatic selenium depends to the age, sex, geographical region, season, and to the alimentation [6].

The selenium concentration in foods depends on where the plants are grown. Thus, most fruits and vegetables contain less than 0.01 μg/g selenium, while mushrooms, garlic and radish contain 0.13, 0.25 and 0.04 μg/g, respectively.

In environment, the concentration of selenium can be: in waters 0.09 ng/ml for sea and ocean, but in the fresh water it is much lower; in the atmosphere 1 ng/ml and in the soils, up to 80 μg/g.

The concentration of selenium necessary per day for human body is 0.050–0.200 mg and the maximum concentration accepted in drinking water is 10 μg/l [7].

Selenium can be determinate by voltammetric methods from various environmental [8], biological [7,9], pharmaceutical and food samples.

The novelty of this paper consists in a rapid and reliable technique for sampling of selenium from a lot of drugs using microwave (MW) digestion and differential pulse cathodic stripping voltammetry (DPCSV) determination. In these conditions, it is possible to obtain analytical information on a lot of samples in due time.

2. Experimental

2.1. Apparatus

For cathodic stripping voltammetry (CSV) determinations an electrochemical system polarographic and voltammetric ensemble Trace Master 5 and POL 150 Polarographic Analyzer (Radiometer, Copenhagen) was used. The electrochemical cell contained a working electrode: hanging mercury drop electrode, a reference electrode: Ag/AgCl and as auxiliary electrode: platinum wire.

The solutions were deaerated with analytical grade argon (99.999%) at the start of each experiment and a flow of argon was maintained over the solution during the experiment to prevent oxygen interference. All experiments were performed at a constant temperature of 25 °C.

Reductions and digestion were carried out by a Milestone model MLS-1200 Mega (Milestone Laboratory Systems, Italy) MW oven (1000 W of maximum power) equipped with six high pressure (up to 100 bar) Teflon® containers.

2.2. Reagents

Containers for sample digestion must be cleaned with hydrochloric acid and washed with ultrapure water (Milli-Q) before utilization. In the case of stripping analysis, the reagents used for preparation of supporting electrolyte and standards must be of high purity, because the reagents can contain

heavy metals, which may influence the determination of analytes at the nanogram per milliliter.

The reagents used were analytical grade (Merck) and all the solutions were prepared in Milli-Q water.

Stock standard selenite solution was prepared by dissolving 0.1406 g SeO_2 in 100 ml Milli-Q water. Dilute solutions were prepared before every use.

2.3. Digestion and reduction of Se (VI)

To determine selenium from pharmaceutical products the sampling process has a great influence on the results of analysis. The homogenization, digestion and in some cases the separation steps have an important role to assure an accurate determination.

For determination of selenium from drug samples firstly it was necessary digestion of the samples as following: ten tablets were taken and homogenized. Then a number of aliquot samples were digested.

Sample digestion and Se (VI) reduction were made in a MW oven in concordance with experimental conditions. This procedure has some advantages on the classic digestion techniques such as the reduction of the digestion time, because it is possible to obtain the digestion of six samples simultaneously. In the same time rapidity and the reproducibility were assured and the contamination of the samples and the risk of losses of selenium are decreasing.

The digestion procedure varied in function of the matrix and it is necessary to have in mind the analytical technique used for selenium determination.

For DPCSV determination of selenium, the samples ($\sim$100 mg) were treated with a mixture of 6 ml HNO_3 and 1 ml H_2O_2 in the MW oven with the following program: 2 min at 250 W, 2 min at 0 W, 6 min at 250 W, 5 min at 400 W, 5 min at 650 W and finally 5 min ventilation.

After digestion, for reduction the Se (VI) to Se (IV) the samples are treated with 0.5 ml HCl 37% by heating.

3. Results and discussion

The low concentrations of the element in different kind of samples require a highly sensitive and selective method for their accurate determination.

CSV was used as a sensitive and selective voltammetric method for determination of trace amounts of selenium [10–12]. The most widely used stripping mode is differential pulse voltammetry designed to compensate the charging background current.

The advantages of voltammetric methods are that they can determine simultaneously four to six analytes in the presence of each other, it is possible to make speciation (e.g. selenium [2,13,14], manganese, chromium, etc.).

In this paper different physico-chemical parameters were studied in the view to establish the best conditions to determine selenium by DPCSV.

The form in which selenium is adsorbed onto a mercury drop electrode is dependent upon the chosen supporting electrolyte. The composition of the supporting electrolyte may affect the selectivity and the sensitivity of the measurement.

The voltammetric behavior of selenium was studied in different types of electrolytes namely HNO_3 (0.1 M), $HClO_4$ (0.1 M), HCl (0.1 M) and KCl (0.1 M). It was observed that among acids utilized, HNO_3 assure the best sensitivity.

The possible mechanism of reaction (for concentration step) is the following:

$$SeO_3^{2-} + 8H^+ + 6e^- = H_2Se + 3H_2O \quad (1)$$

$$H_2Se + Hg = HgSe + 2H^+ + 2e^- \quad (2)$$

And for the stripping step the reaction is:

$$HgSe + 2H^+ + 2e^- = H_2Se + Hg \quad (3)$$

The deposition potential imposed on the working electrode is chosen according to the species to be determined and is maintained for a deposition period depending on their concentrations.

The deposition potential is applied to the working electrode to cause the metal of interest to deposit onto or into its surface.

The amount of metal plated is proportional to the deposition time.

Table 1
The optimum parameters established for cathodic stripping voltammetric determination of selenium

Purge time	300 s
Deposition time	600 s
Waiting time	30 s
Initial potential	−350 mV
Final potential	−750 mV
Step amplitude	2 mV
Pulse duration	20 ms
Pulse amplitude	50 mV
Scan rate	25 mV/s
Stirring speed	300 rpm

Therefore the influence of deposition time on the determination of selenium by DPCSV was studied. It was observed that a good sensitivity was achieved for 600 s, but if the deposition time is increasing on obtain a second peak.

Also, the influence of: pulse duration (20, 40 and 60 ms), pulse amplitude and scan rate onto sensitivity of the determination of selenium was studied. Finally, the optimum parameters for DPCSV determination of selenium were selected and presented in the Table 1.

Under the optimum parameters obtained, standard calibration curves for selenium were constructed by plotting slope, against concentration.

DPCSV determination of selenium can be applied up to 0.125 ng/ml and the equation for the calibration curve is $y = 0.3557x - 0.5071$ ($R^2 = 0.9985$) in the range 0.125–1 ng/ml.

For the determination of selenium from pharmaceutical products was used the equation for the calibration curve is $y = 0.5082x - 0.0128$ ($R^2 = 0.9999$) in the range of 8–64 ng/ml, due to the concentration of selenium in these tablets. Also, the linear regression equation are presented: $y = 0.3557x - 0.5071$ (in the range 8–64 ng/ml) and $y = 0.5082x + 0.0128$ (in the range 0.125–1 ng/ml). The correlation coefficients were 0.9992–0.9999 indicating good linearity.

The results obtained for five pharmaceutical products are presented in Table 2.

There are not observed interferences in the determination of selenium in the presence of the common excipients and additives of the tablets, such as talc, magnesium stearate, starch, lactose, glucose and dextrose.

Two of the drugs analyzed (Optima Forte and Eurovita) contain together with selenium another microelements such as: zinc, molybdenium, chromium, nickel, vanadium, tin and some vitamins (A, E, C, B_1, B_2, B_6, B_{12}, D, K). The other three pharmaceutical products (Antioxidant, Selenium-Walmark and Orgasel) contains only selenium as active principle and common excipients.

The standard addition method was applied by adding selenite solution to the previously analysed tablets, to check the validity of the proposed methods.

The precision of the proposed method was evaluated by a replicate analysis of samples containing selenium in different concentrations. The low values of the RSD in different levels reflect the high precision of the proposed method.

The results of the analysis of commercial tablets and the recovery study (standard addition method) of the drug, suggested that there is no interference

Table 2
Determination of selenium from different pharmaceutical products

Sample	Se content (μg/g)	Se found (μg/g)[a]	Recovery (%)	RSD ($n = 5$) (%)
(1) Optima forte	16.73	15.51 ± 1.20	92.71	7.73
(2) Antioxidant	59.31	49.00 ± 4.35	82.62	8.87
(3) Seleniu Walmark	151.78	145.90 ± 7.05	96.13	4.83
(4) Eurovita	13.42	13.56 ± 0.84	101.04	6.19
(5) Orgasel	116.60	99.60 ± 5.23	85.42	5.25

[a] Average of more distinct analysis. (1) Stanley Pharmaceuticals Ltd, Vancouver, Canada; (2) TISHCON Corp, USA; (3) WALMARK Co., Ltd, Trinec-Czech Republic; (4) LARGO-FLORIDA, USA; (5) Human and Veterinary Therapeutics Society, 'Hipocrate', Romania.

from any excipients, which may be present in tablets.

The samples were stable in time for more than 1 month and the results of the selenium determination were reproducible. These results were cross-checked by the reported method.

For the determination of the repeatability of the method, selenium was analyzed five times in the same sample.

4. Conclusions

For environmental and biological samples it is very important to know the real concentration of selenium for understanding its biochemical cycle, mobility and toxicity.

In comparison with another techniques, DPCSV has a great advantage of reduces analysis time and also the interferences.

The results obtained in this paper enable us to consider that DPCSV corresponds as technique for the determination of selenium in different pharmaceutical products.

The results obtained by us are included in the limits of the determination of DPCSV.

Due to the fact that this technique is very sensitive and has a good selectivity, it can be recommended for the determination of selenium in various pharmaceutical products.

The analysis of commercial tablets and the recovery study (standard addition method) of the drug, suggested that there is no interference from any excipients, which may be present in tablets.

The results of selenium determination by electrometric techniques in various pharmaceutical products enable to conclude that this method is good and more rapid than hydride generation atomic absorption spectrometry, the only technique with similar performances.

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