Technical Paper

DETERMINATION OF MERCURY IN PHARMACEUTICALS BY GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY WITH CHEMICAL MODIFIER

Viorica Alexiu,[a] Elena Chirtop,[b] Luminita Vladescu,[c] and Mihai Simion[a]

[a] *Magistra C&C, 9th Aviator Belghiru Street, Constanta, Romania, Tel/Fax: 0040241634742, E-mail: baumtech@rdslink.ro, baumtech@yahoo.com.*
[b] *Pharmaplant-Biogalenica, 313th, Splaiul Unirii Street, Bucharest, Romania*
[c] *University of Bucharest, 13th B, Regina Elisabeta Street, Bucharest, Romania*

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Abstract

The mercury concentration in pharmaceuticals is usually very low. Therefore, to achieve a precise and accurate analysis it is necessary to have a proficient technique. The determination of mercury by graphite furnace atomic absorption spectrometry often requires the use of a chemical modifier. The chemical modifier is used to stabilize the analyte or volatilise the bulk of sample matrix. In this work, we have studied the effects of $HCl+H_2O_2$ and $PdCl_2$ on the sensitivity of mercury determination in graphite furnace atomic absorption spectrometry. The best results were achieved using 10% $PdCl_2$ and 4% HCl + 4% H_2O_2 concentration. The detection limits (3σ) of the method with 4% HCl + 4%H_2O_2 and $PdCl_2$ 10% as matrix modifiers, were 0.1 μg/mL and 0.02 μg/mL, respectively. After this work, a method with $PdCl_2$ 10% has been proposed for determination of mercury in pharmaceutical samples. The ashing and atomizing temperatures were 250 °C and 1800 °C, respectively. The method was successfully applied to the determination of mercury in pharmaceutical samples, with a recovery range of 96-102%.

Key words: GF-AAS, mercury, chemical modifier, thimerosal

Introduction

Mercury is considered a global pollutant as the element and many of its compounds are highly toxic and readily released into the environment because of their high volatility and mobility.[1,2]

Mercury can be present in numerous forms, including two ionic states: Hg(I) and Hg(II); where Hg(I) represents mercurous compounds that are not very common in the environment and Hg(II) which represents mercuric salts that are commonly found in the environment.

Elemental mercury (Hg^0) is highly volatile, but is only slightly soluble in water. The problem with this form is that it can be easily transported in the atmosphere, and can be oxidized to Hg(II) and deposited in soil, vegetation and/or water bodies.

Inorganic Hg(II), can be methylated to organic forms, primarily to monomethyl mercury which is the most toxic species and also the form in which mercury strongly bioaccumulates in biological food chains, posing potential hazards to humans and wildlife.[3]

Organic mercury is far more dangerous than inorganic mercury,[4] while methyl mercury is identified as a well-known neurotoxic agent and is often correlated with inorganic mercury activity.

Exposure to organomercurials such as methyl mercury produces predominantly central nervous system (CNS) effects that are commonly severe and include coma and death. Organomercurials are typically encountered in the food chain as a result of methylation of inorganic mercury salts by bacteria.

There have been reports of various clinical presentations where a pharmaceutical organomercurial has been applied to surgical wounds and membranes as desiccants and antiseptics.[5,6]

The strongly toxic compounds of mercury have been exploited for bactericides, fungicides and insecticides and its brilliant hues have lead to mercury use in paints. It is also an excellent preservative and disinfectant, accounting for its presence in many chemical reagents and other applications in forms such as mercurochrome and thimerosal.

Thimerosal is a mercury-containing organic compound (ethylmercury thiosalicylate). Thimerosal has been added to some vaccines and other products because it is an antibacterial agent. When thimerosal is degraded or metabolised, it is transformed into ethyl mercury, another organic derivate of mercury. Thimerosal should be reduced or eliminated in vaccines as a precautionary measure and to reduce general exposure to mercury. When thimerosal is used as preservative in vaccines, it is present in concentrations up to 0.01% (50 μg thimerosal/0.5 mL)

Some vaccines may be preservative-free but may contain traces of thimerosal (less than 0.5 μg/0.5 mL).[7,8,9]

Many researches have attempted to determine mercury levels in the blood, urine, tissues, pharmaceutical samples and hair of humans and animals. Several methods have used atomic absorption spectrometry (AAS),[10,11] atomic fluorescence spectroscopy,[12,13] or inductively coupled plasma mass spectrometry (ICP-MS).[14] However, among the available methods, cold vapour (CV) AAS is the most widely used.[15,16,17,18]

The direct determination of mercury by graphite furnace atomic absorption spectrometry (GF-AAS) is difficult because of excessive background signals and potential losses in the pyrolysis stage.[19] A matrix modifier (or chemical modifier), is added in large excess to guarantee that the analyte element is converted into the compound of highest thermal stability.[20,21,22,23] Chemical modifier permits the use of a higher temperature in ashing and atomization steps, without losses of the analyte.

In this work the optimal conditions for determination of mercury by graphite furnace atomic absorption spectrometry, (drying temperature and time, ashing temperature and time, atomization temperature and time) in pharmaceutical products (thimerosal and products with thimerosal) were investigated. A method has been proposed for the determination of mercury in pharmaceutical samples with palladium chloride as chemical modifier.

This is an alternative method to the official method for indirect determination of thimerosal by could vapour atomic absorption spectrometry,[24] which requires large consumption of reagents and long time of analysis. The method was applied to indirect determination of thimerosal in gamaglobulinic vaccine.

Experimental

Apparatus

All measurements were carried out with Perkin-Elmer 3300 atomic absorption spectrophotometer, equipped with a deuterium lamp for background correction, a Perkin-Elmer HGA-600 graphite furnace and Perkin-Elmer AS-60 autosampler. Argon with a purity of 99.996% was used as the carrier gas for the mercury vapour and as the purge and protective gas for the graphite atomizer. A mercury electrode-less discharge lamp was used as a light source. Samples for slurry preparation were weighted using an MT-5 micro analytical balance (METTLER) with a resolution of 0.001 mg. The instrumental parameters are given in Table 1.

Table 1. Instrumental parameters for determination of mercury by GF-AAS with chemical modifier.

PARAMETERS	
Wavelength, nm	253.7
Slit, nm	0.8
Lamp current, mA	10.0
Calibration mode	Absorbance, peak height
Background correction	Deuterium lamp

Reagents

All reagents used were analytical grade: USP-thimerosal reference standard (99.98% certified value), hydrogen peroxide 30% (Fluka), hydrochloric acid 37% (Merck), palladium chloride (Merck), twice deionised water (Membrapure Water System) was employed throughout.

Solutions

Stock organic mercury standard solution, 1000 μg/mL was prepared by dissolving 2.0185 g of thimerosal reference standard in 500 mL deionised water, and adjusting the volume to 1000 mL with deionised water.

Working organic mercury standard solutions were prepared by diluting stock organic mercury solution in deionised water to give final concentrations of 0.10 μg/mL-1.50 μg/mL.

Hydrogen peroxide 4% and hydrochloric acid 4% solution (chemical modifier).

Palladium chloride: 10% solution (chemical modifier).

The samples and calibrating standards were transferred into the furnace by injections of 40 μL, respectively, followed by 10 μL of chemical-modifier solution.

Sample preparation

The thimerosal samples were obtained in batches supplied from producers. Sample solution, 100 μg/mL thimerosal, was prepared from 50 mg of thimerosal which was weighted with analytical balance ($\pm 2\times10^{-4}$ g accuracy), dissolved in approximately 100 mL deionised water in a 500 mL volumetric flask, making up to the mark with deionised water and mixed well.

Working sample solution of 1 μg/mL thimerosal, was prepared by dilution of the 100 μg/mL sample solution.

Gamaglobulinic vaccine samples were delivered to the drugs control laboratory from drugs manufacturers and there were checked regarding the packaging and labelling procedures. For each vaccine batch a mean sample was prepared from 10 vials of 0.5 mL each. 2.5 mL of the mean sample were diluted with deionised water in a 5 mL volumetric flask and made up to the mark with deionised water.

Results and discussion

The influence of ashing and atomization temperature on the determination of mercury in pharmaceutical samples, by GF-AAS with H_2O_2 4%+HCl 4% and with $PdCl_2$ 10% as chemical modifiers was investigated. A standard solution containing 0.5 μg/mL organic mercury was used for optimization of parameters.

Mercury determination method by GF-AAS with H_2O_2 4%+HCl 4%

Ashing temperature between 200 °C – 500 °C was investigated. The optimum ashing temperature was found to be 250 °C when the atomizing temperature at 1100 °C was used (Figure 1).

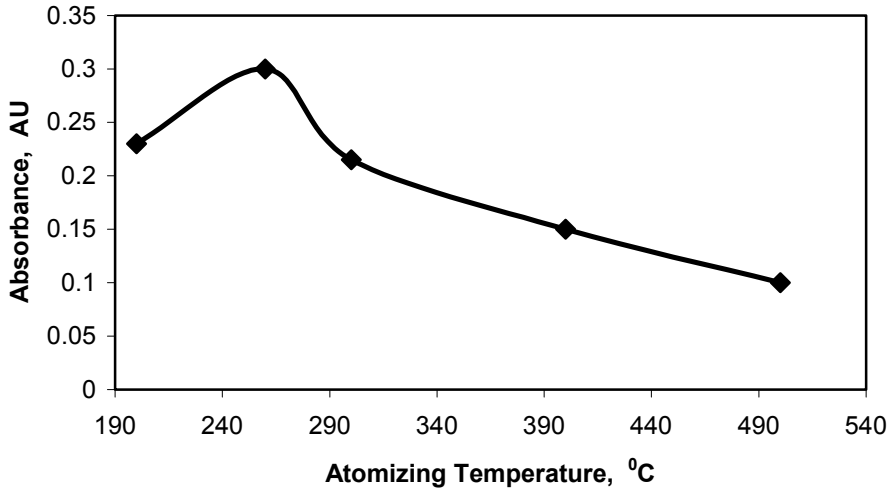

Figure 1. The optimal ashing temperature to measure mercury with H_2O_2 4%+HCl 4% chemical modifier at 1100 °C atomizing temperature.

The maximum mercury absorbance was 0.301 AU and decreased to 0.215 AU for ashing temperature 300 °C.

Atomization temperature between 1000 °C – 1500 °C was investigated. The optimum atomization temperature was found to be 1100 °C when the ashing temperature at 250 °C was used (Figure 2).

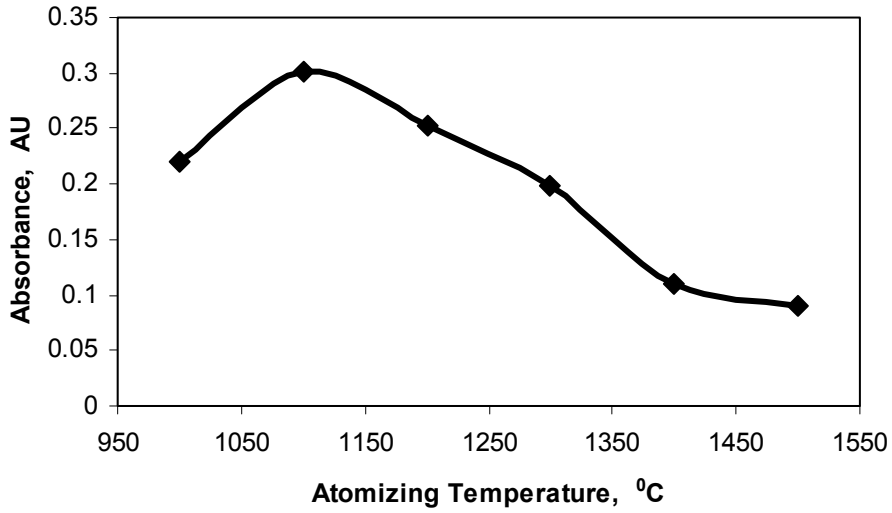

Figure 2. The optimal atomizing temperature to measure mercury with H_2O_2 4%+HCl 4% chemical modifier at 250 °C ashing temperature.

Using the optimum ashing and atomizing temperatures to analyse an organic mercury standard solution with H_2O_2 4% + HCl 4% as chemical modifier, the limit of the detection was 0.1 μg/mL (the limit of detection was set at three-times the standard deviation of the blank). The detection limit was obtained by ten determinations of the blank. The calibration curve was linear for concentration range 0.5 μg/mL-1.5 μg/mL. The relative standard deviation for 1.00 μg/mL organic mercury standard solution was 4.8% (n=6). The concentration of the chemical modifier was selected from literature data.[25]

Mercury determination method by GF-AAS with $PdCl_2$

The optimum ashing temperature was found to be between 250 °C – 300 °C when the atomizing temperature at 1600 °C was used (Figure 3). The maximum mercury absorbance was 0.578 AU.

Atomizing temperature between 1200 °C – 2000 °C was investigated and the optimum atomizing temperature was found to be 1800 °C when the ashing temperature at 250 °C was used (Figure 4).

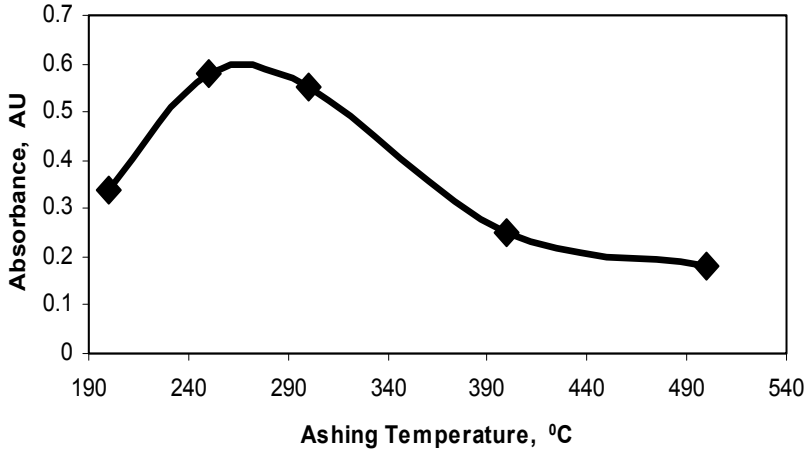

Figure 3. The optimal ashing temperature to measure mercury with $PdCl_2$ 10% chemical modifier at 1600 °C atomising temperature.

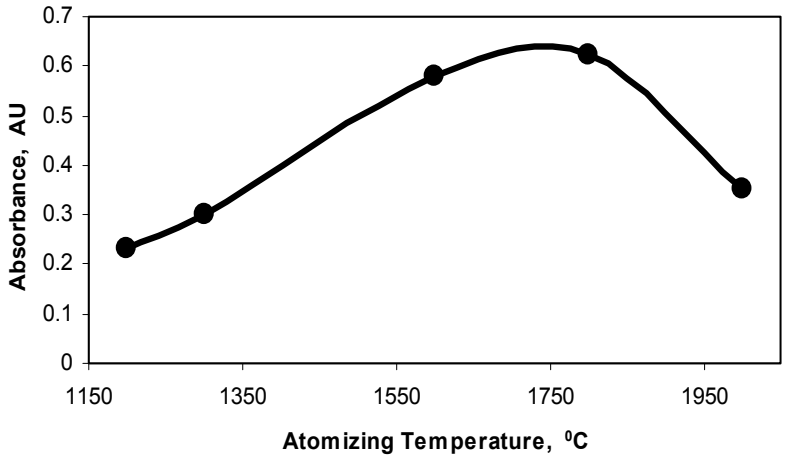

Figure 4. The optimal atomizing temperature to measure mercury with $PdCl_2$ 10% chemical modifier at 250 °C ashing temperature.

The temperature program for the determination of mercury with $PdCl_2$ 10% as chemical modifier by GF-AAS is listed in Table 2.

Table 2. Temperature program for the determination of mercury with $PdCl_2$ 10% as chemical modifier by GF-AAS.

Step	Temperature ˚C	Ramp ˚C/s	Time s	Gas flow L/min
Drying	105	10	30	3.00
Ashing	250	1	20	3.00
Cooling	20	1	15	3.00
Atomizing	1800	0	3	0.00
Cleaning	2600	1	5	3.00

It was necessary to optimise the concentration of palladium chloride solution. To study the effect of palladium chloride concentration on absorbance, the mercury standard (0.5 μg/mL) with 2.5, 5.0, 10.0 and 12.0% palladium chloride solution was analysed by GF-AAS. Results are shown in Figure 5. The mercury absorbance increases with palladium chloride amounts up to 1mg and then remains stable.

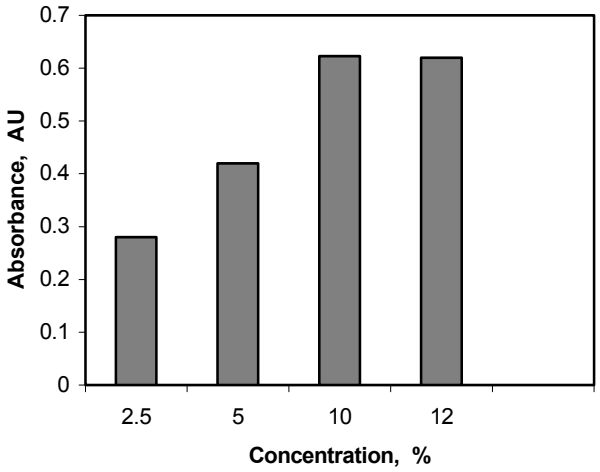

Figure 5. Effect of palladium chloride concentration on mercury measurement (0.5μg/mL organic mercury standard solution).

Using the temperature program shown in Table 2, the limit of detection for analysis of an organic mercury standard solution with $PdCl_2$ 10% as chemical modifier, was 0.02 μg/mL (3 σ of the blank) and calibration curve was linear for concentration range 0.1 μg/mL-1.0 μg/mL. The equation of the regression line of the calibration curve was y = 0.2549x-0.001 with correlation coefficient of r = 0.991.

In conclusion, when palladium chloride is used as chemical modifier, the increased atomization temperature increases the sensitivity and lowers the detection limit and has the advantage of removing some interferences, which may be present in pharmaceutical. The proposed mercury determination method by GF-AAS with $PdCl_2$ 10% as chemical modifier was applied for assay of thimerosal in pharmaceutical samples. The values obtained for six batches are shown in Table 3 and they demonstrate that the results obtained with the proposed method, are in agreement with the results obtained by the official method.[24] This method can be applied to any routine laboratory analysis used for quality control of thimerosal samples.

Table 3. The total mercury content in pharmaceutical thimerosal samples.

Batch	Thimerosal amount, mg	mercury amount		
		Theoretical amount, mg	Found, mg	
			By proposed method	By method [23]
B01	50.1	24.85	24.80	24.82
B02	49.7	24.66	24.62	24.59
B03	49.5	24.56	24.56	24.54
B04	50.2	24.91	24.88	24.91
B05	49.9	24.76	24.69	24.70
B06	49.4	24.51	24.49	24.53

The technique was found to be repeatable and reproducible with relative standard deviations of 1.82% (n=6) and 3.21% (n=4, two analysts), respectively.

The accuracy of the analytical method was evaluated by determination of mercury in thimerosal standard reference material and by quantitative recovery studies of amounts of mercury added to the sample. The data for the recovery studies are presented in Table 4.

Table 4. Recovery of mercury, from thimerosal standard reference material.

Batch	mercury present, mg	mercury added, mg	mercury found, mg	Recovery, %
B03	24.56	10.0	35.22	101.90
B04	24.91	11.2	36.41	100.83
B05	24.76	15.6	38.74	96.00
B06	24.51	15.2	39.70	99.97

The method for determination of mercury in thimerosal, was applied to gamaglobulinic vaccine samples. Accuracy of the method was established by analysis of four batches of vaccine. The results are shown in Table 5.

Table 5. Recovery of total mercury from gamaglobulinic vaccine samples.

Batch	mercury present µg/mL	mercury added µg/mL	mercury found µg/mL	Recovery %
B01	0.424	0.200	0.627	100.48
B02	0.398	0.200	0.596	99.66
B03	0.517	0.200	0.708	98.74
B04	0.368	0.200	0.556	97.82

The influence of matrix interferences on the mercury determinations in gamaglobulinic vaccine was studied. Figures 6 and 7 show the time profile of atomization signal for thimerosal and gamaglobulinic vaccine. Interferences from the matrix appeared to be negligible, as verified by the standard addition method which was performed on representative vaccine samples.

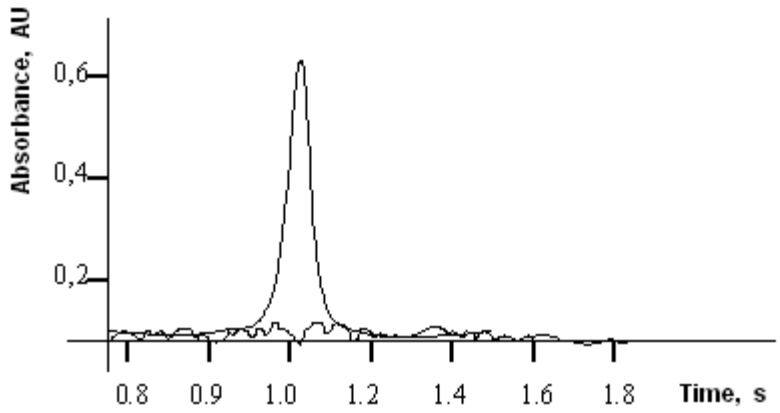

Figure 6. Time profile of atomization signal for a thimerosal (0.5 µg/mL Hg).

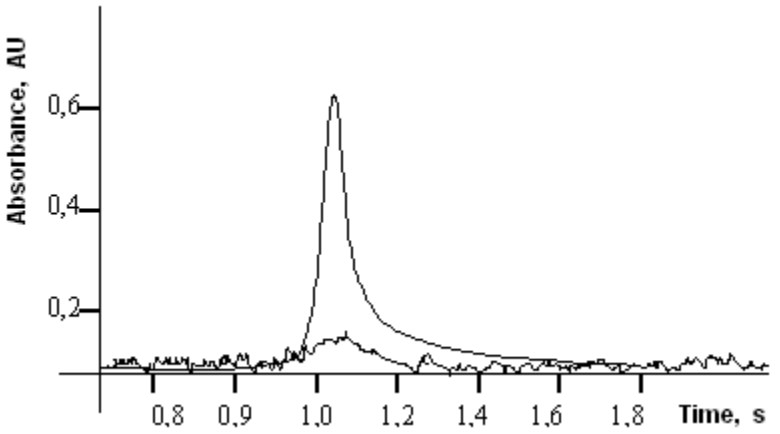

Figure 7. Time profile of atomization signal for a gamaglobulinic vaccine (0.517 µg/mL Hg).

The repeatability of the proposed method for determination of mercury in gamaglobulinic vaccine is characterized by relative standard deviation of 3.48% calculated from 6 consecutive measurements of B03 sample (0.517 µg/mL mercury). Reproducibility of the method was estimated from the results for four B03 sample solution prepared by two analysts. The relative standard deviation was 3.70%.

Conclusions

It has been shown that a method based on the application of the palladium chloride as chemical modifier can be applied successfully to the analysis of mercury in thimerosal samples and gamaglobulinic vaccine. The optimum ashing temperature and atomizing temperature were found to be 250 °C and 1800 °C, respectively. This method was applied for determination of mercury in real samples such pharmaceutical solutions that contain thimerosal as preservative and for determination of mercury in thimerosal samples. The proposed method is rapid, precise and sensitive, with limit of detection of 0.02 μg/mL, the linearity range 0.10 μg/mL-1.0 μg/mL and recovery range 96%-102%.

The presented work demonstrates that the results obtained with the proposed method are in agreement with the results obtained by the USP official method. The proposed method is rapid and because of require low consumption of reagents, the level of the contamination is very low.

Statistical evaluation of the results shows that the thimerosal concentration in gamaglobulinic vaccine is under maximum limits (0.5 μg Hg/dose) required by WHO.

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Povzetek

Koncentracije živega srebra v farmacevtskih preparatih so običajno zelo nizke. Zato lahko dosegamo visoko natančnost in zanesljivost meritev samo s primernimi analiznimi tehnikami. Določevanje živega srebra z ET-AAS pogosto zahteva uporabo kemijskih modifikatorjev s katerimi zagotovimo večjo stabilnost analita ali lažjo uparitev matriksa. V pričujoči raziskavi smo proučevali učinke dodatkov HCl+H_2O_2 ali $PdCl_2$ na občutljivost določevanja živega srebra z ET-AAS. Najboljše rezultate smo dosegli z uporabo 10% $PdCl_2$ ali raztopine 4% HCl + 4% H_2O_2. S 4% HCl + 4% H_2O_2 je bila meja detekcija (3σ) za določevanje živega srebra 0.1 μg/mL z 10% $PdCl_2$ pa 0.02 μg/mL, pri čemer je bila temperatura sežiga 250 °C temperatura atomizacije pa 1800 °C. Metodo s $PdCl_2$ smo uspešno uporabili za določevanje živega srebra v farmacevtskih vzorcih v katerih smo dosegali izkoristke v območju 96 do 102 %.