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Multivariate Optimization in the Direct Determination of Antimony and Chromium in Serum Samples by Graphite Furnace Atomic Absorption Spectrometry

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Abstract: *Methods for direct determination of antimony and chromium in human serum by Graphite Furnace Atomic Absorption Spectrometry (GF AAS) were proposed in this work. The samples were diluted in 1:1 with nitric acid 1% v/v and Triton® X-100 0.1% v/v. The optimization of the instrumental conditions was done using a multivariate approach. A factorial design (2^3) was employed to investigate the tendency of the most intense absorvance signal for each analyte. Permanent zirconium modifier (500 µg) demonstrated better performance for use as a chemical modifier for antimony while for chromium, the best modifier was permanent tungsten. A Central Composed Design (CCD) was used to obtain the optimum conditions through the methodology of surface response, resulting in the best temperatures of 700 ºC and 2400 ºC for pyrolysis and atomization temperatures for antimony and 1600 ºC and 2504 ºC for chromium, respectively. The method allowed the determination of antimony with a curve varying from 0 to 30.0 µg L^{-1}. Recovery studies for three concentration levels (n=7 for each level, prepared and read in three consecutive days) presented results of 101 ± 3 % for Sb and 93 ± 7 % for Cr. The detection limit was 0.3 µg L^{-1} for Cr and Sb. Intra and inter-assay studies, according to coefficients of variation results, were 2.6-2.8 % and 4.3-5.6 % for Sb and 3.0-5.5 % and 6.2-9.9 % for Cr, respectively. The method was applied for the determination of antimony in 60 samples obtaining concentrations from < LOD to 10.9 µg L^{-1} and for Cr with concentrations between < LOD to 41.8 µg L^{-1}.*

Keywords: *Multivariate Optimization, Serum, Antimony, Chromium, Graphite Furnace Atomic Absorption Spectrometry*

I. Introduction

Antimony is a toxic element and known to cause adverse health effects in humans and animals due to environmental contamination and occupational contact. The increase in studies contributing to the analytical chemistry of Sb and its compounds can partially be attributed to the fact that the potentially harmful effects of Sb have been recognized by several authorities and as a result, Sb is listed as a priority pollutant by the US Environmental Protection Agency and by the German Research Community [1]. Antimony drugs are also employed for the treatment of multiple lesions caused by the human leishmaniasis disease [2]. Leishmaniasis is an infectious disease disseminated worldwide with remarkable incidence in Asia, Africa and Central/South Americas. In 1993, the World Health Organization considered Leishmaniasis the second most importance public health disease, caused by protozoa [3]. First described by Pedro Pizarro in 1571, the disease commonly infects hyraxes, canids, rodents, and humans. The genus Leishmania protozoan parasites are the causative agents forming a group of diseases called leishmaniasis, endemic in more than 88 countries, and affecting 12 million people worldwide [4]. In Brazil, leishmaniasis is prevalent in most of the poorest regions of the country, with some 67,000 new cases reported in 2003, classifying the disease as an epidemic [5].

Enrichment of Sb in atmospheric aerosols, plants, soils, sediments, water, as well as alpine and polar snow and ice, suggest that Sb contamination is extensive. Due to its extensive industrial uses, the anthropogenic release of Sb into the environment is significant. Over the last decades, global fluxes of Sb have increased at least 10 fold, resulting in an increase of environmental Sb contamination [6].

Adverse effects of antimony include: arthralgia, myalgia, nausea, vomiting, headache, anorexia, elevated transaminases, alkaline phosphatase, amylase and lipase, leukopenia, widening among other. Other,

less common side effects include an increase of urea and creatinine, cardiac arrhythmia, sudden death, and herpes zoster [7, 8].

Individuals who have had too much of antimony-based drugs, such as meglumine antimoniate, used in leishmaniasis treatment, are sensitive to it once it is injected into their blood or intramuscularly, resulting in adverse health effects. These health effects may include diarrhea, joint and/or muscle pain, vomiting, problems with the blood (anemia) and heart problems (altered electrocardiograms) [9].

The International Association for Cancer Research (IARC) has reported that inhalation of Sb oxides can be carcinogenic in the female rat [10]. Other studies have shown that Sb is a human carcinogen [11, 12].

Several studies were done to measure and determine the effects of heavy metals and trace elements on ecosystem and human. Chromium (Cr) was shown to be associated with some disorders, such as nasal septa defect [13].

Aitio et al. [14] reviewed the results of biological monitoring of exposure to chromium (predominately soluble Cr[VI]) and identified three half-lives for excretion of 7 h, 15–30 days and 3 to 5 years. The best estimates for the sizes of these different compartments are 40%, 50% and 10%, respectively.

If the patient survives for more than about 8 days; the major effects resulting from oral ingestion of toxic doses of chromium are liver and kidney necrosis [15].

High Cr absorptions in vivo can result in various diseases, such as fibroproliferative disease, airway hypersensitivity, lung cancer, nasal cancer, and other types of cancer. In addition to adduct formation, exposure to Cr can cause various point mutations in DNA, chromosomal damage, and oxidative changes in proteins [16].

Trace analysis in complex matrices, such as human serum sample preparation, can involve several steps: for example, techniques using a simple introduction system for pneumatic nebulizer, which can require a long sample preparation time with the possibility of loss or contamination. The possibility of sample in-situ digestion, requiring little or no previous sample preparation, has been explored in previous studies using the GF AAS technique [17-32].

The optimization of experimental parameters of univariate analysis by GF AAS (as chemical modifiers, pyrolysis time, pyrolysis and atomization temperatures) requires a great number of time-consuming and costly experiments. Moreover, the interactions between optimized variables are not evaluated. Multivariate optimization seems to be more adequate when many variables are involved. The factorial design is a good and simple statistical tool that can be used to verify the effects of variables and their interactions requiring just a few experiments. Center Composite Design (CCD) is a popular experimental method to adjust quadratic models that generate surface response for optimization of variables useful for building a second order (quadratic) model for the response variable without needing to use a complete three-level factorial experiment [33-35].

In this work, a factorial and CCD design was employed to optimize the experimental conditions for the direct determination of antimony and chromium in diluted human serum samples (1:1) by Graphite Furnace Atomic Absorption Spectrometry (GF AAS) with in-situ matrix removal.

II. Experimental

Instrumentation

All measurements were carried out with a PerkinElmer Aanalyst^{TM} 400, equipped with a graphite furnace (HGA-900), an autosampler (AS-800), and background correction with a continuous arch deuterium source operating under the conditions recommended by the manufacturer (PerkinElmer Life e Analytical Sciences, Shelton, CT, USA). For Sb, an electrodeless discharge lamp (EDL) for Sb from PerkinElmer, operating at 240 mA, with a slit width of 1.35 nm and a wavelength of 217.58 nm was employed. For Cr, a hollow cathode lamp from PerkinElmer operating at 15 mA, with a slit width of 0.8 nm and a wavelength of 357.87 nm was used. Argon 99.996% from White Martins (Belo Horizonte, MG, Brazil) was used as the purge gas with a flow rate of 250 mL min^{-1}. Graphite tubes with integrated platforms (Perkin Elmer, Part Number B3001264 and B3001263) were used for all studies.

Reagents and solutions

Nitric acid – Suprapur®, 65% (Merck, Darmstadt, Germany, part n^o 1.00441.0250); stock antimony solution containing 1 000 ± 2 mg L^{-1} (Titrisol® from Merck, part n^o 109988) in 5% v/v HNO_3 and stock chromium solution containing 1000 ± 2 mg L^{-1} (Titrisol® from Merck) in 5% v/v HNO_3 were used. The following solutions were also used: 1000 mg L^{-1}: $RhCl_3$ in HCL ~ 1M, (Fluka, No. 84033), $IrCL_3$ in HCL ~ 1M, (Fluka, No. 58195), $(NH_4)_2TaF_7$ (Merck, No. 170356) in HCL ~ 1M, $Pd(NO_3)_2$ in HNO_3 ~ 1M (Fluka, No. 76035) and Zr in HCL 5% v/v (Aldrich, No. 274976). In addition to the following solutions, 1 000 mg L^{-1} was prepared in 100 mL in Milli-Q water (HNO_3 1 mol L^{-1}): tungsten was prepared with 0.1794 g of $Na_2WO_4(H_2O)_2$ (Merck) and niobium was prepared with 0.1430 g of Nb_2O_5 (Merck No. 5214816) and both reagents were diluted to 100.0 mL in nitric acid 5% v/v.

Autosampler cups, tips for micropipette and glassware materials were cleaned by soaking in 20% (v/v) HNO_3 for at least one day, rinsing many times with Milli-Q water, and then left to dry. The autosampler washing solution, containing 0.1% (v/v) Triton® X-100 (Merck) plus 0.1% (v/v) nitric acid, was used to avoid analyte adsorption onto the surface of the container and clogging of the capillary sampling tip, in addition to improving sample solution dispersion onto the graphite tube wall.

A solution of Triton® X-100 (Merck) 0.1% (v/v) with nitric acid 1% (v/v) in Milli-Q water was used as a diluent. Triton acts as a detergent to eliminate carbonaceous resides formed inside the graphite tube and also aids in the cleaning of the autosampler capillary between sampling [20, 22, 24, 27-29, 32].

Sampling and Sample Treatment

Initially, blood samples were obtained by venipuncture from 60 adult volunteers using Vacuette non-heparinized tubes (Greiner Bio-One, Arlington Heights, USA). After collection, blood samples were subjected to centrifugation for 15 minutes at 3 000 rpm, and serum samples were separated with the aid of a pipette. Serum samples were stored in a freezer (-80^o C) until ready to use. The only sample preparation was a simple 1:1 dilution with nitric acid 1% v / v Triton® X-100 0.1% v / v, done directly in the autosampler cups of the GF AAS. This solvent was chosen based on good results obtained from previous studies that used aqueous solutions of acid and surfactant as diluents at low concentrations [18-20, 24-29]. The amount of solutions pipetted into the graphite tube was set at 20 µL.

In the studies using a permanent modifier, the platform of graphite tubes was treated by applying 50 µL of 1 000 mg L^{-1} of the desired modifier and submitting the tube to a specific temperature program as published elsewhere [36, 37]. This procedure was repeated 10 times in order to obtain a permanent modifier deposit of 500 µg. A random serum sample was selected from the group of volunteers to be used in the developed method (reference sample). The reference sample, diluted 1:1 with nitric acid 1% v / v Triton® X-100 0.1% v/v, was contaminated with 30 µg L^{-1} of Sb and with 5 µg L^{-1} of Cr and used in the optimization.

Initially, temperature and drying time of the reference sample, were optimized based on the conditions recommended by the manufacturer, until no bubbling was observed in the sample graphite furnace. Then, using pyrolysis and atomization temperatures and times, also recommended by the manufacturer, different graphite tubes were tested to select the two that presented the best performance (highest absorbance for each studied metals, lower RDS < 5% with background corrected) to be used in a factorial design, those being: tubes with platform treated with permanent chemical modifiers (W, Rh, Ru, Ir, Zr, Nb and Ta), and a platform tube with untreated modifier. After thah, a 2^3 factorial design was constructed for preliminary evaluation of the variables pyrolysis and atomization temperatures and use of chemical modifier for antimony and for chormium. Next, a CCD plan was conducted to determine optimal values of pyrolysis and atomization temperatures for both analytes. For all of experiments, the cleaning temperature was maintained at 2600 °C for 5 seconds. The experimental data were processed using the software Statistica 6.0 [38].

III. Results And Discussion

Antimony

Optimization procedure

Tubes with platforms treated with permanent modifiers Zr and Ta obtained higher absorbance readings and background corrected in relation to other studies for antimony (all about the platform with 500 µg of each modifier). After that, we used a 2^3 factorial design to obtain a Pareto chart (Table 1, Figure 1), The results shows that all variables and the interactions of second order had a significant effect on response (integrated absorbance, s) at a 95% confidence level. The best results were 700 °C (-), 2400 °C (+) and Zr (-) for pyrolysis and atomization temperatures and use of modifier, respectively. Planning CCD was performed to determine the optimal values for the pyrolysis and atomization temperatures as described in Table 2. The response surface obtained by this design can be seen in Figure 2. The optimum values for the pyrolysis and atomization temperatures were obtained by deriving the mathematical equation (1).

$$Abs = - 0.0000008T_a^2 - 0.0000008T_p^2 - 0.0000002T_a^2T_p^2 + 0.0034T_a + 0.0018T_p - 4.1786 \quad (1)$$

where,

Abs = Absorbance; T_a = Atomization Temperature and T_p = Pyrolysis Temperature.

After reaching optimal conditions, an undesirable accumulation of carbonaceous residues was deposited on the wall of the tube after a few heating cycles. To minimize the accumulation of these residues, a pre-injection of 10 µ L solution of Triton® X-100 0.1% was performed, followed only by the drying stage.

By reducing the sample preparation steps, preparation time and use of reagents, this method helped eliminate or reduced cross contamination of the samples.

The graphite tube lifetime, using permanent modifier without a decrease in sensitivity, is more than 1000 cycles [25, 36, 37], however, this was not investigated in this study. The optimized furnace program for Sb determination in serum samples was presented in Table 3.

Calibration for antimony

Aqueous calibration curves (n=3) were compared with standard addition calibration curves (n=3) for antimony. The studied linear range was between 0 to 30 μg L^{-1}, presenting a coefficient of determination always greater than 0.99 for all curves. Statistical analysis (F- and Student's t tests) of the slopes of the curves showed no significant difference between them at a 95% confidence interval. Therefore, subsequent studies were conducted using aqueous calibration. The linear regression equation was Abs = (0.0029 ± 0.0003) C_{Sb} + (0.0006 ± 0.0006) as shown in Table 4.

Analytical Figures of Merit

The limit of detection (LOD) at the optimized conditions was 0.3 μg L^{-1} calculated according the IUPAC: three times the standard deviation for 10 distinct measurements of the blank, divided by the slope of the calibration curve. The characteristic mass of 13 ± 1 pg was obtained as an average of the values for each point on the calibration curve. Some figures of merit are presented in Table 4. It was observed that the determined characteristic mass was almost half that recommended by the manufacturer for standard antimony in water described a method for the direct determination of antimony in rat serum after univariate optimization of important variables measured by GF AAS [32]. In this study, using a mixture of Zr and Rh, as a permanent modifier, we obtained a characteristic mass of 30 pg and LOD of 0.6 μg L^{-1}. For the determination of Sb (III) and Sb (V) in liver and blood by FI-HG ASA, Pena et al. [39] reported a LOD of 1.0 μg L^{-1} for Sb (III) and a LOD of 0.5 μg L^{-1} for Sb (V).

Gadhari et al. [2] proposed an electrochemical method based on potentiometric stripping analysis (PSA) employing a hexathia 18C6 (HT18C6) and rice husk (RH) modified carbon paste electrode (HT18C6–RH-CPE) for the subnanomolar determination of antimony. A detection limit of 2.11×10^{-11} M for Sb (III) was achieved. According to the authors, the PSA method presents advantages such as high sensitivity, very low detection limit with high reproducibility, easy handling, resistance against surface fouling, and low cost. The proposed method has been successfully employed to analyze antimony in pharmaceutical formulations, human hair, sea water, urine and blood serum samples.

Samadi-Maybodi and Rezaei [40] reported a simple, sensitive and reliable method using cloud point extraction of antimony and spectrophotometric detection. The method can be used for the determination of Sb at a range of 0.8 - 95 μg L^{-1} with a detection limit of 0.23 μg L^{-1} and a preconcentration factor of 200. The method compared favorably to other methods and was applied to determine antimony in seawater, anti-leishmania drugs (Glucantime®) and human serum.

Subramanian et al. [41] developed a method using Transversely Heated Graphite Furnace Atomization-Atomic Absorption Spectrometry (THGA-GF AAS) for the determination of Sb in leachate water samples exposed to copper plumbing joined with Sn/Sb solders and serum of rats sub chronically exposed to this metalloid in the form of potassium antimony tartrate-dosed drinking water. The AAS method is based on the concept of stabilized temperature platform furnace atomization (STPF) using a transversely heated graphite atomizer (THGA) furnace, longitudinal Zeeman-effect background correction, and matrix modification with palladium nitrate-magnesium nitrate-nitric acid.

Souza and Tarley [42] presented a Sb(III) and Sb(V) preconcentration and speciation method, optimized by multivariate design using cloud point extraction (CPE) with determination by graphite furnace atomic absorption spectrometry. It is based on the formation of Sb(III) complexes with ammonium O,O- diethyl dithiophosphate (DDTP) in acid medium in the presence of Triton X-114. The method provides a linear calibration range from 0.25 to 16.0 μg L^{-1} ($r>0.994$) and limits of detection and quantification of 0.08 μg L^{-1} and 0.25 μg L^{-1}, respectively. According to the authors, the proposed analytical approach can be easily implemented for monitoring Sb(III) and Sb(V) in various kinds of water, as well as in blood samples. Finally, the optimization using factorial designs was successfully applied as a rigorous and simple method to find the adequate conditions to produce the best analytical response involving those factors that play a fundamental role in both CPE and the GF-AAS heating program.

In our study, the intra-assay precision was evaluated by the relative standard deviation (RSD, %) of seven distinct serum replicates added to 12.5, 17.5 and 22.5 μg L^{-1} of Sb, and the inter-assay precision, using RSD, %, also for seven different replicates of the same concentrations, but measured over three different days. The observed range of the relative standard deviation for intra- and inter-assay precision were, respectively, 2.6 and 2.8% and 4.3 to 5.6% (Table 5). The results are consistent with the criteria established by The International Association of Official Analytical Chemists [43], where the coefficient of variation (RSD) might vary from 15-30%, depending on the concentration range used (1-100 μg g^{-1}). The accuracy was assessed by recovery studies

of serum samples added to Sb in the same three concentration levels of the inter-assay precision study (n=21 spiked samples). The recovery values are presented in Table 5. The recovery was 101 ± 3%, respecting the satisfactorily limit included in the 80-120% range [44], demonstrating a good method of accuracy.

Determination of antimony in human serum samples

Antimony concentrations of serum samples from 60 volunteers were determined using the optimized experimental conditions. Table 6 presents the analyzed antimony level samples. The Sb levels in subjects' (n= 60 individuals') serum samples ranged between LOD to 10.9 μg L^{-1}.

Chromium

Optimization procedure

The tubes treated with platforms with the permanent modifiers Ru and W (500μg), obtained higher absorbance readings and corrected background in relation to the other studied modifiers, and therefore, the 2^3 factorial designs was used (Table 7). The Pareto chart (Figure 7), generated by factorial design, shows that all the variables and their interactions, demonstrated a significant response effect (integrated absorbance, s) at a 95% confidence level, and that best results were 1600 °C (-), 2504 ^oC (+) and W (+) for pyrolysis and atomizing temperatures and modifier, respectively. A planning CCD design, using W as permanent modifier, was conducted to determine the optimum values for pyrolysis and atomization temperatures as shown in Table 8. The response surface obtained by this design is presented in Figure 8. The optimal values for the pyrolysis and atomization temperatures were obtained by deriving the mathematical equation below (1).

Abs =

$$- 0.00000289T_a^2 - 0.000000076T_p^2 – 0.000000491T_a^2T_p^2 + 0.0159T_a + 0.0018T_p + 0.0152T_p – 21.730 \quad (1)$$

Where,

Abs = Absorvance; T_a = Atomization Temperature and T_p = Pyrolysis Temperature.

Even after reaching the optimal conditions, an undesirable buildup of carbonaceous residues was deposited on the tube wall after a few heating cycles. To minimize the accumulation of these residues a pre-injection of 10 μL solution of Triton ® X-100 0.1% was done, followed by the drying step only. Table 9 presents the optimized furnace program for chromium determination.

Calibration for chromium

Aqueous calibration was compared with standard addition calibration curves derived from spiking Cr level serum samples (n=3 for each calibration curve). The studied linear range was from 0 to 12 μg L^{-1} with a determination coefficient always greater than 0.99 for all curves. Statistical analysis (F test and Student t) of the curves of the slopes showed no significant difference between them for a 95% confidence interval. Therefore subsequent studies were conducted using aqueous calibration.

Analytical Figures of Merit

The limit of detection (LOD) and quantification (LOQ) at the optimized conditions were of 0.3 and 1.0 μg L^{-1}, respectively, calculated according the IUPAC: Three times the standard deviation for 10 distinct measurements of the blank, divided by the slope of the calibration curve for the LOD calculation and ten times for LOQ calculation. The characteristic mass of 3.2 ± 0.1 pg was obtained as an average of the values found for each point of the calibration curve. Some figures of merit are shown in Table 10. Observe that the determined characteristic mass is almost half the manufacturer's recommended standard for chromium in water (recommended characteristic mass of 3.0 pg).

The intra-assay precision was evaluated by the relative standard deviation (RSD %) of seven different replicates of whey added to 3.0, 7.0 and 11.0 μg L^{-1} of Cr, while the inter-assay precision of RSD%, also by seven different replicates of the same concentrations, but were prepared and read on three different days. The resulting tracks of the relative standard deviation for the intra-and inter-assay precision were 3.0 to 5.5% and 6.2 to 9.9%, respectively (Table 11). The results are consistent with the criteria established by AOAC [43], where the coefficient of variation (RSD) might vary from 15-30%, depending on the concentration range used (1 - 100 μg g^{-1}). The accuracy was assessed by recovery studies on serum samples of Cr added in the same three levels of concentration of the inter-assay precision study. The recovery values can be seen in Table 11. The recovery was found to be 92.7 ± 7.3% (n = 21), respecting the limit included in the satisfactory range of 80 - 120%, thus showing a good accuracy for this method [44].

Determination of chromium in human serum samples

The concentrations of chromium in the serum of sixty volunteers were determined using the optimized experimental conditions. Table 12 shows the levels of chromium in the analyzed samples. Cr levels found in the serum of these subjects included a range of <LOQ to 41.8 μg L^{-1}.

IV. Conclusions

The presented methods require minimal sample preparation procedures, which involves a simple dilution of the serum samples (1:1) with a solution containing Triton X-100 0.1% v/v and HNO_3 1% v/v sample and the removal of the in-situ matrix for the determination of antimony and chromium by GF AAS. The best modifier for antimony, was permanent zirconium (500μg), while for chromium, was permanent tungsten reducing analytical time and analysis cost. The use of a fragmental factorial design, followed by a CCD design, is a simple and fast procedure to evaluate the GF AAS analytical conditions for antimony and chromium determination in diluted serum samples. A response surfaces analysis allowed for the identification of a maximum absorbance for both analytes. A pre-injection of Triton X-100 0.1% v/v solution resulted in a more efficient tube cleaning, and with the contribution of permanent modifier, a higher firing cycle was achieved. The resulting figures of merit are very adequate for GF AAS determinations for antimony and chromium. The Sb levels in subjects' (n = 60 individuals') serum samples ranged between LOD to 10.9 μg L^{-1} and for Cr, the levels were between <LOQ to 41.75 μg L^{-1}. The simplicity of method preparation, in addition to the set of instrumental and analytical conditions, permitted an adequate determination of antimony and chromium in human serum samples.

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Table 1. Matrix of the factorial design experiments for antimony (2^3).

Experiment	Atomization temperature (°C)	Pyrolysis temperature (°C)	Modifier	Integrated absorbance (n=3)
1	2000 (-)	800 (-)	Zr (-)	0.229
2	2400 (+)	800 (-)	Zr (-)	0.182
3	2000 (-)	1500 (+)	Zr (-)	0.145
4	2400 (+)	1500 (+)	Zr (-)	0.123

5	2000 (-)	800 (-)	Ta (+)	0.221
6	2400 (+)	800 (-)	Ta (+)	0.193
7	2000 (-)	1500 (+)	Ta (+)	0.057
8	2400 (+)	1500 (+)	Ta (+)	0.052

Table 2. Matrix of the CCD planning experiments using a tube with a zirconium treated platform (500 µg) for antimony determination.

Experiment	Pyrolysis temperature (°C)	Atomization temperature (°C)	Integrated absorbance (n=3)
1	800 (-1)	1800 (-1)	0.270
2	800 (-1)	2200 (1)	0.224
3	1000 (1)	1800 (-1)	0.245
4	1000 (1)	2200 (1)	0.184
5	760 (-1.414)	2000 (0)	0.262
6	1040 (1.414)	2000 (0)	0.203
7	900 (0)	1720 (-1.414)	0.245
8	900 (0)	2280 (1.414)	0.203
9 (CP*)	900 (0)	2000 (0)	0.221
10 (CP)	900 (0)	2000 (0)	0.231
11 (CP)	900 (0)	2000 (0)	0.230
12 (CP)	900 (0)	2000 (0)	0.226
13 (CP)	900 (0)	2000 (0)	0.224

*CP = central point.

Table 3. Optimized furnace program for the determination of antimony in human serum by GF AAS.

Step	Temperature/°C	Ramp/s	Hold/s	Ar flow rate (mL min^{-1})
Drying	100	10	20	250
Drying	140	20	20	250
Pyrolysis	200	10	30	250
Pyrolysis	700	10	30	250
Atomization	2400	0	6	0
Clean	2600	1	5	250

Table 4. Analytical figure of merits for the determination of antimony in human serum under optimized experimental conditions by GF AAS.

Parameters	Results
Regression equation (n=3)	Abs = (0.0029 ± 0.0003)C_{Sb} + (0.0006 ± 0.0006)
R^2 (n=3)	0.9977 ± 0.0005
Linear range (µg L^{-1})	0 – 30
LOD (µg L^{-1})	0.3
LOQ (µg L^{-1})	1.0
Characteristic mass (n=6, pg)*	13 ± 1

* characteristic mass recommended by the manufacturer for the determination of Sb in the antimony standard = 22 pg.

Table 5. Precision and accuracy results for the developed method for antimony determination in human serum samples.

Sb concentration (µg L^{-1})	CV intra-assay (%, n=7)	CV inter-assay (%, n=21)	Recovery (%, n=21)
12.5	2.8	5.1	102
17.5	2.6	4.3	101
22.5	2.6	5.6	100
Mean	2.7 ± 0.1	5.0 ± 0.6	101 ± 3

Table 6. Antimony concentration in sixty human serum samples analyzed by the proposed method.

Subject	Sb level (µg L^{-1})	Subject	Sb level (µg L^{-1})	Subject	Sb level (µg L^{-1})
1	5.8 ± 0.4	21	< LOD	41	< LOD
2	< LOD	22	2.1 ± 0.9	42	4.9 ± 0.9
3	< LOD	23	7.1 ± 1.2	43	< LOD
4	7.6 ± 0.8	24	< LOD	44	< LOD
5	5.8 ± 0.4	25	< LOD	45	9.1 ± 0.0
6	< LOD	26	< LOD	46	< LOD
7	< LOD	27	7.2 ± 0.4	47	< LOD
8	< LOD	28	3.1 ± 0.8	48	7.4 ± 1.2
9	6.7 ± 0.8	29	< LOD	49	< LOD
10	6.1 ± 0.8	30	9.6 ± 0.4	50	2.0 ± 0.4
11	< LOD	31	9.1 ± 1.5	51	6.4 ± 0.4
12	< LOD	32	< LOD	52	< LOD
13	< LOD	33	< LOD	53	1.4 ± 0.5

14	5.4 ± 0.0	34	7.6 ± 0.8	54	< LOD
15	7.9 ± 0.4	35	< LOD	55	< LOD
16	< LOD	36	1.9 ± 1.2	56	2.1 ± 0.5
17	< LOD	37	6.4 ± 1.2	57	1.6 ± 0.0
18	10.9 ± 1.2	38	< LOD	58	< LOD
19	8.9 ± 0.4	39	< LOD	59	< LOD
20	< LOD	40	7.9 ± 1.2	60	< LOD

Table 7. Factorial design matrix for Cr experiments (2^3): preliminary evaluation of optimized variables.

Experiment	Atomization temperature (°C)	Pyrolysis temperature (°C)	Modifier	Integrated absorbance*
1	2100 (-)	1600 (-)	Ru (-)	0,049
2	2500 (+)	1600 (-)	Ru (-)	0,166
3	2100 (-)	2000 (+)	Ru (-)	0,003
4	2500 (+)	2000 (+)	Ru (-)	0,067
5	2100 (-)	1600 (-)	W (+)	0,026
6	2500 (+)	1600 (-)	W (+)	0,284
7	2100 (-)	2000 (+)	W (+)	0,013
8	2500 (+)	2000 (+)	W (+)	0,259

*Average of triplicates.

Table 8. Matrix experiments for central composite design (CCD) for Cr using W (500 µg) as a permanent chemical modifier.

Experiment	Pyrolysis temperature (°C)	Atomization temperature (°C)	Integrated absorbance*
1	1300	2530	0.183
2	1700	2530	0.202
3	1300	2670	0.218
4	1700	2670	0.209
5	1500	2500	0.192
6	1500	2700	0.206
7	1220	2600	0.218
8	1780	2600	0.225
PC	1500	2600	0.207
PC	1500	2600	0.218
PC	1500	2600	0.224
PC	1500	2600	0,221
PC	1500	2600	0,223

*Average of triplicates

Table 9. Optimized furnace program for determination of chromium in human blood serum by GF AAS.

Step	Temperature/°C	Ramp/s	Hold/s	Ar flow rate (L min^{-1})
Drying	110	10	20	250
Drying	140	20	20	250
Drying	200	10	30	250
Pyrolysis	1600	10	20	250
Atomization	2504	0	10	0.0 (read)
Cleaning	2700	1	5	250

Table 10. Analytical characteristics for determination of chromium in human blood serum under optimized conditions by GF AAS.

Regression equation (n=3)	Abs = (0.027 ± 0.001)C_{Cr} + (0.0017 ± 0.0041)
R^2 (n=3)	0.9947 ± 0.0008
Linear range (µg L^{-1})	0 – 12.0
LOD (µg L^{-1})	0.3
LOQ (µg L^{-1})	1.0
Characteristic mass (pg)*	3.2 ± 0.1
*Recommended value for standard solution of chromium = 3.0 pg	

Table 11. Precision and accuracy results for the developed method for determination of Cr in human blood serum under optimized conditions by GF AAS.

Concentration (µg L^{-1})	Intra-assay precision (%, n = 7)	Inter-assay precision (%, n = 21)	Recovery (%, n = 21)
3.0	5.5	9.9	99.4
7.0	4.6	6.2	89.6
11.0	3.0	7.7	89.1

Table 12. Chromium concentration in sixty human serum samples analyzed by chromium with the proposed method.

Individual	Level of Cr (µg L^{-1})	Individual	Level of Cr (µg L^{-1})	Individual	Level of Cr (µg L^{-1})
1	3.0	21	5.0	41	8.7
2	3.9	22	<LOQ	42	2.4
3	23.6	23	29.0	43	6.8
4	3.7	24	<LOQ	44	4.5
5	<LOQ	25	17.8	45	9.4
6	<LOQ	26	2.0	46	3.8
7	14.6	27	26.0	47	3.8
8	2.2	28	7.2	48	2.8
9	3.7	29	<LOQ	49	2.8
10	<LOD	30	<LOQ	50	2.3
11	2.2	31	<LOQ	51	3.3
12	1.4	32	26.7	52	7.3
13	<LOQ	33	4.4	53	3.0
14	<LOQ	34	5.4	54	4.9
15	<LOQ	35	41.8	55	4.5
16	4.5	36	<LOQ	56	6.9
17	<LOQ	37	<LOQ	57	9.7
18	12.1	38	27.7	58	3.2
19	13.87	39	<LOQ	59	1.78
20	10.30	40	<LOQ	60	2.91

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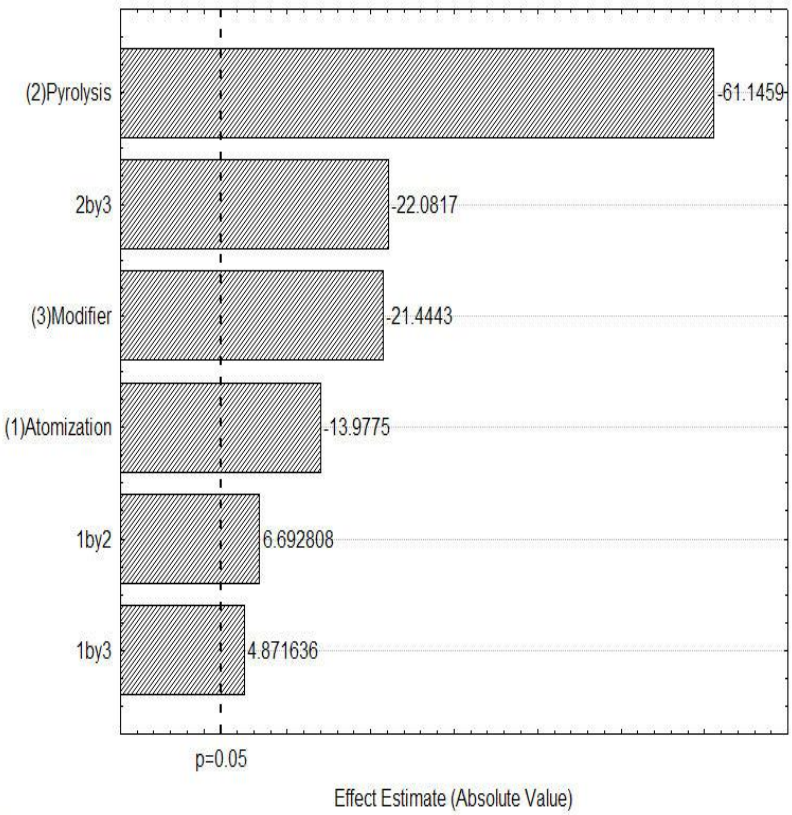

Fig. 1. Pareto chart results for the factorial design for Sb determination.

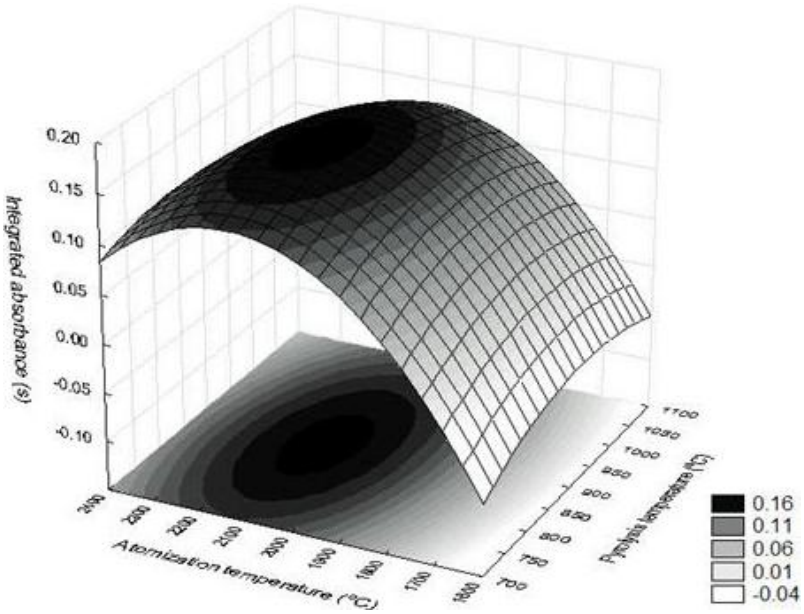

Fig. 2. Response surface results for the CCD design for Sb in serum.

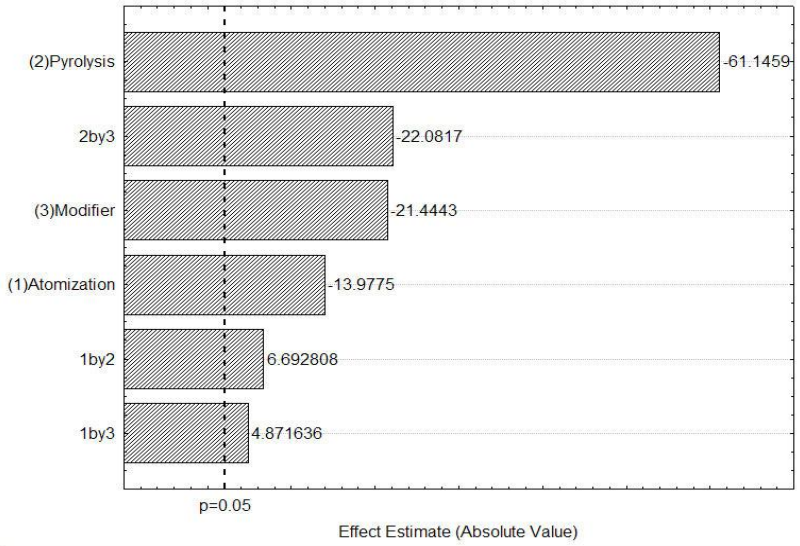

Fig. 3. Pareto chart obtained in factorial design for Cr in serum samples.

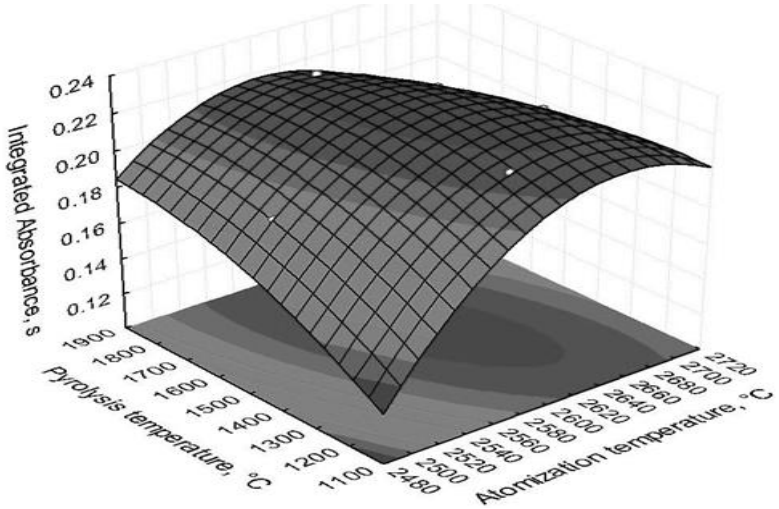

Fig. 4. Response surface obtained in the CCD design for Cr.