Accepted Manuscript

Investigation of chemical modifiers for the determination of cadmium and chromium in fish oil and lipoid matrices using HR-CS GF AAS and a simple 'dilute-and-shoot' approach

Mauana Schneider, Éderson R. Pereira, Daiane P.C. de Quadros, Bernhard Welz, Eduardo Carasek, Jailson B. de Andrade, Javier del Campo Menoyo, Joerg Feldmann

PII: S0026-265X(16)30613-0
DOI: doi: 10.1016/j.microc.2017.03.038
Reference: MICROC 2757

To appear in: *Microchemical Journal*

Received date: 15 November 2016
Revised date: 19 March 2017
Accepted date: 22 March 2017

Please cite this article as: Mauana Schneider, Éderson R. Pereira, Daiane P.C. de Quadros, Bernhard Welz, Eduardo Carasek, Jailson B. de Andrade, Javier del Campo Menoyo, Joerg Feldmann , Investigation of chemical modifiers for the determination of cadmium and chromium in fish oil and lipoid matrices using HR-CS GF AAS and a simple 'dilute-and-shoot' approach. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Microc(2017), doi: 10.1016/j.microc.2017.03.038

Investigation of chemical modifiers for the determination of cadmium and chromium in fish oil and lipoid matrices using HR-CS GF AAS and a simple 'dilute-and-shoot' approach

Mauana Schneider,[a] *Éderson R. Pereira,*[a] *Daiane P.C. de Quadros,*[a] *Bernhard Welz,*[a,b]* *Eduardo Carasek,*[a] *Jailson B. de Andrade,*[b] *Javier del Campo Menoyo,*[c] *Joerg Feldmann*[d]

[a] Departamento de Química, Universidade Federal de Santa Catarina, 88040-900 Florianópolis - SC, Brazil

[b] Instituto Nacional de Ciência e Tecnologia do CNPq – INCT de Energia e Ambiente, Universidade Federal da Bahia, Salvador, BA, Brazil

[c] Golden Omega S.A., Arica, Chile

Trace Element Speciation Laboratory (TESLA), Department of Chemistry, University of Aberdeen, Scotland, UK

*Corresponding Author, e-mail address: w.bernardo@terra.com.br (B.Welz).

Fax: +55 48 3721 6850

Abstract

This work presents simple and reliable methods for the determination of Cd and Cr in fish oil and lipoid samples by high-resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS GF AAS). Only a simple dilution using 1-propanol was adopted as sample preparation for the determination of Cd and Cr in order to reduce the high viscosity of the lipoid samples. Pt was employed as permanent chemical modifier and Pd was used as chemical modifier in solution, added over the sample for the determination of Cd for a better performance and sensitivity. Cr was determined without a chemical modifier. The accuracy of the proposed methods was evaluated by comparison with microwave-assisted digested samples and determination by HR-CS GF AAS, showing good agreement between 2% and 13% for both elements without statistical difference (confidence interval of 95%). The limits of detection for Cd and Cr were 0.5 pg and 7 pg, respectively, corresponding to 0.15 ng g^{-1} and 2 ng g^{-1}, respectively. The developed methods can be described as clean, fast and accurate, which did not require any sample pre-treatment stages, such as digestion or matrix oxidation, and could be extended to different lipoid matrices, demonstrating their robustness.

Keywords: High-resolution continuum source graphite furnace atomic absorption spectrometry; Determination of Cd and Cr; Sample preparation; Dilute-and-shoot approach; Fish oil analysis.

1. Introduction

Fish is an important part of the diet of many people around the world, making it the most common source of OMEGA-3 in the human diet [1]; OMEGA-3 is an essential fatty acid [1] with properties of preventing stroke and neurodegenerative diseases [2], increasing appetite [3], preventing heart disease, sudden death, hypertension [4] and menstrual discomfort [5]. The recommended consumption of two servings of fish per week is enough to get all the benefits of the OMEGA-3 and all the important vitamins and proteins present in fish meat [6]. However, fish also carries the risk of exposure to high concentrations of heavy metals, such as mercury, chromium, cadmium and lead, as well as environmental contaminants, such as organochlorides, accumulated due to biomagnification [7].

An alternative for the benefits of fish meat without the risk of contamination by heavy metals and organochlorides are supplementation products, such as fish oil capsules [8]. The industrial process to obtain the OMEGA-3 ensures safety and neutral organoleptic characteristics by the refining steps, necessary to remove toxic compounds, such as heavy metals, pesticides, non-triglycerides, colorants and smelly compounds [1].

One of the most notorious contaminants in sea food is cadmium [9], a metallic element with a biological half-live between 10 and 30 years that accumulates in the kidney and liver. Intoxication with cadmium causes renal dysfunction and bone demineralization [10]; it is classified as a Group-1 carcinogen by the International Agency for Research on Cancer (IARC) [11]. The control of cadmium levels is mandatory to avoid problems caused by intoxication due to the consumption of contaminated food or supplements. The European Regulation EC No 488/2014, establishes the maximum level of cadmium allowed for food supplements at 1.0 mg kg^{-1} [12]. To ensure the safety of cadmium levels in foodstuffs, the European Standard stipulates a list of methods to determine cadmium at trace levels. Most of the methods employ for sample preparation dry ashing, microwave-assisted acid digestion and posterior determination by flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GF AAS) or anodic stripping voltammetry (ASV) [13]. The British standard stipulates a method for cadmium determination using inductively coupled plasma mass spectrometry (ICP-MS) after pressure digestion [14].

Another metallic element that is also classified as a Group-1 carcinogen by the IARC [15] is chromium. This contaminant can be found in the environment in two different oxidation states, Cr(VI) and Cr(III). Chromium(VI) does not occur naturally, but is a result of anthropogenic and industrial activities [16]. Although Cr(III) occurs naturally, it is insoluble and the great majority of Cr(III) present in the marine environment is found in the sediments. Cr(VI), in contrast, is soluble in water and might become a harmful contaminant for fish and other marine life [17], but it is not commonly found in foodstuff [18]. Cr(III) is an essential trace element for humans, since it regulates the fat metabolism and natural glucose and can be found in whole grains, meat, fish, vegetables and mushrooms [18, 19], with a recommended intake of 40-50 µg per day [20].

There are no data about maximum permissible intakes of Cr(III) due to its low toxicity. Since Cr(III) is an essential trace element, its levels in foodstuff must be specified, to ensure the correct intake. The European Committee for Standardization, through standard No. EN 14082/2003, established dry ashing as sample preparation; and after dilution in HCl the solution is applied to GF AAS to determine chromium in food [21]. Again, the standard method uses hazardous reagents and laborious techniques of sample preparation. The use of dry ashing makes impossible the re-use of the sample solutions for the determination of organic components or volatile elements; in addition, it is very time consuming and exposes the sample to potential contaminants.

The goal of this work was to look for a simple and fast procedure to determine heavy metals in fish oil samples that is suitable for routine analysis in a fish oil factory. This work proposes methods to determine chromium and cadmium, present at trace levels in raw and processed fish oil, by high-resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS GF AAS) using a ‘dilute-and-shoot’ approach. This includes only the dilution in 1-propanol as sample preparation, avoiding the use of highly concentrated acids, oxidant reagents, procedures that include unstable emulsion formation or laborious sample preparation methods, such as dry ashing or microwave-assisted acid digestion.

2. Experimental

2.1. Instrumentation

A high-resolution continuum source atomic absorption spectrometer Model contrAA 600 (Analytik Jena AG, Jena, Germany) was used for all measurements. The spectrometer is equipped with a xenon short-arc lamp with a nominal power of 300 W, operating in a hot-spot mode, emitting a spectral continuum between 190 and 900 nm; a charge-coupled device (CCD) array detector and a high-resolution double monochromator, consisting of a prism monochromator for pre-dispersion of the radiation and an echelle grating monochromator for high resolution. All measurements were performed using the main spectral lines of cadmium at 228.802 nm and chromium at 357.869 nm using the integrated absorbance mode of three pixels (peak volume selected absorbance, PVSA, $A_{\Sigma 3,int}$ [22]).

Pyrolytically coated graphite tubes with PIN platform (Analytik Jena, Part No. 407-A81.025) were used in all experiments. For comparison and to check the accuracy of the developed method, Cd and Cr were also determined after microwave-assisted acid digestion for determination by HR-CS GF AAS. Digestions were carried out using a TOPwave laboratory microwave oven (Analytik Jena) with rotor CX 100 and independent, contact-free temperature and pressure control for each vessel.

2.2 Materials and reagents

Ultrapure water, with a resistivity of 18 MΩ cm was obtained from a Model Mega ROUP (Equisul, Pelotas, Brazil) purification system, and it was used in the dilution and preparation of the standard solutions. Stock standard solutions of 1000 mg L^1 Cd and 1000 mg L^1 Cr (Sigma-Aldrich, St. Louis, USA), were used to prepare the calibration curves. For evaluation of the accuracy, the well-established microwave-assisted digestion with a mixture of concentrated nitric acid (HNO_3, 67%, Fluka, Neu-Ulm, Germany), which was further purified by sub-boiling distillation in a quartz still (Kürner Analysentechnik, Rosenheim, Germany), and hydrogen peroxide (H_2O_2, 30%, Sigma, São Paulo, Brazil), was used. For the development of the simplified fast procedure, 1-propanol (Sigma-Aldrich) was employed as diluent for the oil and lipoid matrices, using 15-mL polypropylene flasks. For the graphite tube coating and thermal stability study, stock standard solutions of 1000 mg L^{-1} Ir, Zr (Fluka), Mg, Pd and Pt (Sigma-Aldrich) were used as permanent modifiers or modifiers in solution.

2.3. Samples

The raw marine fish oil sample, which has been used for all optimizations of the methods, was supplied by Golden Omega S.A. (Arica, Chile). It originated mostly from Peruvian anchovy (*Engraulis ringens*) and Chilean mackerel (*Trachurus murphyi*) from the South Pacific Ocean. The method was applied to the determination of Cd and Cr in the raw fish oil sample and the intermediate products from four different stages of purification of the raw fish oil during the industrial process of Golden Omega in order to ensure the quality and safety of the final oil product. The method was also applied for different lipoid samples, including raw fish oil from a Brazilian fish oil factory and commercial cocoanut butter and milk butter samples acquired in a local supermarket.

2.4. Procedure and sample digestion

For the proposed method, about 1.0 g of fish oil was weighed in polypropylene flasks and filled up to 10 mL with 1-propanol. Thirty µL of the resulting solution was pipetted directly onto the platform of the graphite tube without permanent modifier or previously coated with 400 µg Ir, Pd, Pt or Zr, using ten consecutive injections of 40 µL of the respective modifier stock solution, each injection followed by the temperature program shown in Table 1.

For the microwave-assisted digestion, about 500 mg of fish oil or lipoid sample was weighed directly into each vessel and 5 mL 37% HNO_3 and 3 mL 30% H_2O_2 were added, and the vessels closed for the temperature program shown in Table 2. After cooling, the digested samples were diluted with water to 15 mL and the concentrations of Cd and Cr were determined using HR-CS GF AAS in the same way as described for the samples diluted with 1-propanol. For the statistical calculation, the Graph Pad In Stat (Graph Pad In Stat Software Inc, version 3.00, 1997) software was employed and a 95% significance level was adopted for all comparisons.

3. Results and Discussion

3.1 Temperature program

For Cd and Cr determination using GF AAS, two permanent modifiers and no modifier were studied and compared in this work. For Cd, Ir and Pt were chosen in this

study due their efficiency reported by Bulska et al. [23] and Thomaidis et al. [24], respectively, while, in the case of Cr, Zr and Pd were chosen based on previous studies [25, 26], where Cr was determined in marine sediment and fish feces slurry, respectively by GF AAS.

3.2 Cadmium

The study of the permanent modifiers for this work was performed using 10 µL of an aqueous solution of 1 µg L^{-1} Cd. As can be seen in Figure 1A, when no modifier was employed the highest sensitivity was found around a T_{pyr} of 400 °C and a T_{at} of 1400 °C. These results are coherent with those found by Bulska *et al.* [23] for Cd in aqueous samples, who described losses at pyrolysis temperatures above 300 °C when no modifier was used. For Pt as the permanent modifier, the optimum temperatures for pyrolysis and atomization were 800 °C and 1600 °C, respectively, which is higher than the T_{pyr} of 650 °C found by Thomaidis *et al.* [24] for Cd in sewage sludge. For Ir as the permanent modifier, the T_{pyr} of 800 °C and the T_{at} of 1800 °C were in agreement with data published by da Silva *et al.* [27] for Cd in human serum.

The determination of metals in complex organic matrices demands higher pyrolysis temperatures and longer hold times than in other matrices. Pyrolysis temperatures of at least 800 °C are required to eliminate all the concomitants and prepare the analyte for the atomization. For fish oil samples, temperatures lower than 800 °C are prohibitive since at these temperatures the matrix is not removed sufficiently and causes excessive smoke during atomization, which results in radiation scattering, a higher noise level and a lower precision between measurements. Pyrolysis temperatures above 800 °C and hold times of at least 15 s provided a satisfactory elimination of the organic matrix from the graphite platform and tube, so that no additional cleaning stages whatsoever were required. For that reason, considering the results shown in Figure 1a, the studies without modifier were discontinued, once the analyte losses were significant at higher temperatures.

To evaluate the thermal behavior of Cd in the sample using Ir or Pt coated platforms, the same study was carried out using raw fish oil diluted with 1-propanol. The pyrolysis and atomization curves are shown in Figure 1b. When Pt was employed as permanent modifier, the highest sensitivity was obtained for pyrolysis and atomization temperatures of 800 °C and 1600 °C, respectively, which is in agreement with the data

found for aqueous standard solutions. This behavior might also be an indicative for the possibility of using aqueous standard solutions for calibration to quantify fish oil samples. For Ir, the highest sensitivity was obtained at pyrolysis and atomization temperatures of 800 °C and 1700 °C, respectively. The integrated absorbance values obtained with Ir as the permanent modifier were slightly lower than those for Pt, and for this reason, Pt was adopted as permanent modifier for the next studies. Although the mechanism of action of permanent modifiers is still a point of discussion, it was speculated that platinum acts as a catalyst in breaking C-C and C-H bonds of organic compounds [28]. This catalytic role of Pt could be a reason for the higher sensitivity achieved when Pt is used as permanent modifier.

Chemical modifiers in solution are occasionally also applied in addition to a permanent modifier to improve the thermal stability of the analyte, making possible higher pyrolysis temperatures without losses. Pd and the mixture of Pd/Mg were chosen for the evaluation of the thermal stability of Cd in fish oil diluted with 1-propanol. Using a 1 g L^{-1} solution of chemical modifiers, 10 µg of Pd or 10/15 µg of Pd/Mg, were injected with the sample on the Pt coated graphite platform, and the results are shown in Figure 2a. The mixture of Pd/Mg is commonly used as a chemical modifier for Cd determination. Good results were reported by Oliveira *et al.* [29] for Cd determination in oil shale by-products, where pyrolysis temperatures of up to 800 °C could be used without significant losses using only the mixture of Pd/Mg as chemical modifier in solution. In the same way, Bulska *et al.* [23], using aqueous standards of Cd, added Pd as modifier in solution and found good results, and pyrolysis up to 900 °C could be applied. In this investigation, Pd or the mixture of Pd/Mg presented improvement in stability and sensitivity when compared with the results obtained without a chemical modifier in solution, making possible pyrolysis temperatures of up to 1000 °C without significant losses. Pd showed higher stability at higher temperatures than Pd/Mg and a more symmetrical signal was obtained at an atomization temperature of 1700 °C. The temperatures for Pd as chemical modifier found in this work were slightly higher than those found by Bulska *et al*. [23] when Pd was used as chemical modifier in solution and Ir as permanent modifier. However, best results were found in this work using Pt instead of Ir as permanent modifier, since Pt can provide a

thermal stability at higher temperatures compared with Ir, even without a chemical modifier in solution.

To investigate the possibility of calibration with aqueous standard solutions, the study of the chemical modifiers in solution was made with 10 µL of an aqueous solution of Pd or a Pd/Mg mixture. As can be seen in Figure 2b, the effect of chemical modifiers in solution for Cd in fish oil samples is not the same as that obtained in aqueous solution (refer to Figure 2a). Since fish oil is a complex matrix, composed mainly of lipoid chains, and PGM can act in the degradation of such matrices [30], the improvement of sensitivity is plausible when the chemical modifiers in solution are applied to organic matrices. The thermal behavior of Cd applying Pd/Mg, or no chemical modifier in solution, were significantly different between fish oil and aqueous standard solutions. However, Pd in solution as chemical modifier, when applied to aqueous samples shows the same thermal behavior as when applied to fish oil samples, with a symmetrical and sensitive signal achieved at pyrolysis and atomization temperatures of 1000 °C and 1700 °C, respectively.

Due to the sensitivity and signal profile, Pt was chosen as permanent modifier and Pd as chemical modifier in solution; pyrolysis and atomization temperatures of 1000 °C and 1700 °C, respectively, were selected. The temperature program is shown in Table 3.

3.3 Chromium

No modifier and Zr and Pd as permanent modifiers have been investigated for the determination of Cr, using an aqueous solution of 10 µg L^{-1} Cr. When no modifier was used, the highest sensitivity was obtained with pyrolysis and atomization temperatures of 1100 °C and 2500 °C, respectively. When Pd or Zr was used as permanent modifier, the highest sensitivities were found for pyrolysis and atomization temperatures of 1300 °C and 2500 °C, respectively. These results showed good agreement with data reported by Oliveira *et al.* [31] who found best results at 1200 °C / 2600 °C with Pd as the permanent modifier. Bermejo-Barrera *et al.* [32] used pyrolysis temperatures up to 1300 °C without significant losses using a Zr coated graphite tube. Our results are shown in Figure 3a. It has to be noted that the sensitivity obtained with both permanent modifiers was about a factor of two lower than that without a modifier.

For comparison, the same measurements were also carried out with a solution of 0.5 g mL^{-1} fish oil in 1-propanol. The thermal behavior was evaluated without modifier and with a tube coated with Pd or Zr as a permanent modifier, as shown in Figure 3b. To evaluate the pyrolysis curves, the atomization temperature was fixed at 2400 °C for all modifiers and no modifier, and for atomization, the pyrolysis temperature was fixed at 1100 °C without modifier and at 1300 °C for Zr and Pd as the modifiers. When no modifier was used, the highest sensitivity was obtained with a pyrolysis temperature of 1100 °C and an atomization temperature of 2500 °C. The temperatures are in agreement with those found for the aqueous solution. Zr also showed values comparable with the aqueous solution, with a pyrolysis temperature of 1300 °C and an atomization temperature of 2600 °C. Only for Pd, the optimum conditions were slightly different from those found for the aqueous solution. The highest sensitivity was found for a pyrolysis temperature of 1000 °C and an atomization temperature of 2600 °C. As in the study with an aqueous solution, the use of these permanent modifiers resulted in significant losses of sensitivity compared to the situation without a permanent modifier at the same temperatures. Based on these results, the use of a permanent modifier was discarded for the determination of Cr in fish oil samples.

The lipoid matrix of the fish oil can be a challenge for the determination of volatile elements, such as Cd, which need to be stabilized at high temperatures, making necessary the use of chemical modifiers, permanent and/or in solution. Although Cr is not volatile, the use of chemical modifiers in solution has been investigated to ensure the thermal stability, since the fish oil matrix needs a long exposure time to high temperatures to eliminate all organic constituents. Pd/Mg and Pd, respectively, have been chosen as chemical modifiers in solution to study the thermal behavior of Cr in a tube without permanent modifier. The combination of Pd and Mg is known as a "universal chemical modifier" [30] and its efficiency was verified for more than 20 elements. Bermejo-Barrera *et al.* [32] studied the effect of Pd alone or in mixtures as chemical modifier and obtained thermal stability even at high temperatures. As can be observed in Figure 4a, the use of chemical modifiers in solution improved the thermal stability, keeping the signal almost constant up to 1400 °C. However, after a great number of cycles have been performed, the signals increased, probably due to a memory effect.

To investigate this behavior, a complete atomization cycle with a previously used tube, but without the addition of sample or standard solution was carried out, and no signal of the analyte was detected. As described by Quadros and Borges [33], this increase of the signal might be attributed to 1-propanol as the diluent. 1-propanol makes it possible that the analyte penetrates into the graphite pores and is only gradually released at high temperatures in the atomization stage. Since Cr is not a carbide forming element, all the Cr is released in the atomization stage in the absence of chemical modifiers. In the presence of a solution of Pd or Pd/Mg as chemical modifier, due to the thermal stability, the analyte remains in the graphite pores even after the cleaning stage. In a study of the chemical modifier in aqueous solution, Cr did not show any trace of this effect; therefore, it is quite likely that 1-propanol is the responsible reagent permitting the analyte to penetrate the graphite pores. The thermal behavior of Cr in aqueous solution was in agreement with the results obtained with the fish oil sample. To avoid this effect and once the chemical modifiers in solution did not improve the analytical signal, no modifier at all was used for Cr determination. These results are shown in Figure 4b.

Due to the highest sensitivity and the good signal profile, a pyrolysis temperature of 1100 °C and an atomization temperature of 2500 °C were chosen for all further measurements. No permanent or chemical modifier in solution was used. The temperature program for this work is shown in Table 4.

3.4. Figures of Merit

The figures of merit, such as the limit of detection (LOD), limit of quantification (LOQ) and the linear correlation coefficient (R^2) were evaluated and can be seen in Table 5. The LOD was calculated as $3\sigma/S$ ($n = 10$), where σ is the standard deviation of 10 measurements of a blank and S is the slope of the calibration curve; the LOQ was calculated as $10\sigma/S$ of the same data set. The LOD was 0.5 pg (0.15 ng g^{-1}) for Cd and 7 pg (2 ng g^{-1}) for Cr. The characteristic mass, m_0, which is defined as the analyte mass that provides an integrated absorbance of 0.0044 s, was 1.5 pg for Cd and 6 pg for Cr. The linear range was determined in order to ensure the working range and showed linearity up to 330 pg for Cd and 1080 pg for Cr. To prove the compatibility of the calibration with

aqueous standards, a standard addition calibration was made, and the slopes were compared with the calibration using aqueous standard solutions. The difference between the slopes was 10% for Cd and 4% for Cr, which might be considered an acceptably accurate correlation between matrix and aqueous solution, and the calibration could be carried out using aqueous standard solutions.

3.5 Analysis of lipoid samples

The developed methods have been applied to samples from four stages of the industrial refining process of fish oil from Chile, to a raw fish oil sample from Brazil and commercially available samples of cocoa butter and milk butter. In the same way, the method was also applied to commercial fish oil capsules acquired in Brazil and Germany; however, none of the capsules contained fish oil with a detectable concentration of Cd or Cr, so that the results are not shown in Table 6.

In order to establish the trueness of the results, the samples were also submitted to a microwave-assisted acid digestion, and the digested samples were analyzed using the same HR-CS GF AAS equipment and the same temperature program as for the samples diluted with 1-propanol. The LOQs for microwave-assisted digested samples were 1 ng g^{-1} and 13 ng g^{-1} for Cd and Cr respectively. The comparison is based on a t-test of two experimental means with 95% confidence and the results obtained by the proposed methods were not significantly different from those obtained after microwave-assisted digestion.

The proposed methods achieve LOQs low enough to be compatible with European legislation [14], which stipulates a maximum value of 1.0 µg g^{-1} Cd, to guarantee the safe consumption of supplements. For Cr, the low values can be an indicative for the absence of pollutants, since Cr(VI) is in the environment due to anthropogenic activities [17]. The results are shown in Table 6.

Among the evaluated fish oil samples, only the raw oil (stage 1) showed a significant concentration of Cd (about 1.4 µg g^{-1}) using the proposed direct determination and microwave-assisted digestion. To find a relatively high concentration of Cd in this raw oil demonstrates that Cd is not in its ionic form, Cd^{2+}, because if it were, Cd could be removed at the early stages of the oil extraction from fish, where all the meat is boiled in water, and would not remain in the lipoid fraction. However, Cd remains in the oil phase

and can form interesting complexes with stable lipoid structures. We cannot proof this information until now, but probably these results will open a new way for the studies involving lipoid matrices and will answer questions about the Cd metabolism and the route of transport through the cells in biological systems.

There is a lack of information about Cd in biological systems and fish oil. Beniwal *et al.* [34] showed that the increase in Cd concentration can cause alterations in the lipoid profile of developing mustard seed, causing regularly a decrease in the total and non-polar lipids. The authors report a significant correlation between the increase of saturated fatty acids and the Cd concentration, while unsaturated fatty acids showed a significant decrease when the Cd concentration was positive. These two statements allowed the interesting conclusion that Cd induced increase in the saturated/unsaturated ratio, since the activity of olelyl-CoA desaturase enzyme was affected when the Cd concentration was increased. Similarly, the same observation was made by Chia et al. [35] in studies of the lipoid composition in microalgae in the presence of different positive concentrations of Cd and phosphate.

In the other stages of the fish oil cleaning (stage 2-4, Table 6), the Cd concentration was below the LOD of the method (0.15 ng g^{-1}). This shows that the removing process, adopted by the company between the first and the second stage, which uses the adsorbent bentonite for clean-up, is successful. Among the other samples, just cocoanut butter showed a low concentration of Cd (0.007 ± 0.001µg g^{-1}), and using the proposed method, it was possible to quantify this concentration, since the dilution factor is low compared with microwave-assisted digestion. Furthermore, it was one more evidence how sensitive the developed method using HR-CS GF AAS and the dilute-and-shoot approach are. Regarding the microwave-assisted digestion procedure, the concentration found using this approach was below the method's LOD (1 ng g^{-1}).

In addition, Cr has been found in the oil A with concentration of 1.3 ± 0.1 µg g^{-1}, oil from stage 1 (1.1 ± 0.1 µg g^{-1}), cocoanut butter (0.071 ± 0.02 µg g^{-1}), and milk butter (0.051 ± 0.01 µg g^{-1}), while the other samples had no detectable Cr concentration (<2 ng g^{-1}). Regarding the significant concentration of Cr found in the oil samples (A oil and stage 1), we expect to find only Cr(III) in the lipid phase since its hexavalent form is reduced by the organic matter, which makes Cr(VI) a harmful contaminant for fish and other marine

life. Generally, in the environment, the Cr(VI) compounds are reduced to Cr(III), because they are strong oxidizing agents and highly corrosive [36]. As in the case of Cd, these results open a new way for studies involving Cr in lipoid matrix, such as fish oil, and further efforts will be necessary.

Cocoa butter and milk butter showed concentrations of 0.071 ± 0.02 μg g^{-1} and 0.051 ± 0.01 μg g^{-1}, respectively, showing a good agreement with the well-established method employing microwave-assisted digestion and determination by HR-CS GF AAS. For the other samples (stages 2-4), the concentrations were below the LOQ of the proposed method (2 ng g^{-1}) and the microwave-assisted digestion procedure (13 ng g^{-1}).

4. Conclusions

Rapid and sensitive methods have been developed for Cd and Cr determination in lipoid matrices by HR-CS GF AAS. The samples were just diluted using 1-pronanol and injected onto the platform of the GF, coated with Pt and using Pd as chemical modifier in solution when Cd was determined, while no modifier was used in the Cr determination. The sample preparation does not require the use of acids or oxidant reagents, reducing the volume of residues and the associated risks, and making needless the use of secondary instrumentation. The dilution in 1-propanol reduces the viscosity of the fish oil to a degree that injection with an autosampler became feasible, and no additional rinsing was necessary. The accuracy of the proposed HR-CS GF AAS methods was verified by comparing the ‘dilute-and-shoot’ method with microwave-assisted acid digestion, and no significant difference was found between the two methodologies. From the results found in the Cd determination using pooled oil (stage 1), an interesting hypothesis was proposed in the literature [34] about organo-cadmium compounds, which were found in the lipoid phase (oil phase) and could influence the structure of the lipoid profile.

Acknowledgments

The authors are grateful to the Conselho Nacional de Desenvolvimento Científico and Tecnológico (CNPq), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support and scholarships. The present research was mostly

financed through Project no. CNPq 406877/2013-0. The authors are also grateful to Analytik Jena for financial support and the donation of the contrAA 600.

References

1. Rubio-Rodriguez N, Beltran S, Jaime I, de Diego SM, Sanz MT, Carballido JR. (2010). Production of omega-3 polyunsaturated fatty acid concentrates: A review. Innovative Food Science and Emerging Technologies. DOI:10.1016/j.ifset.2009.10.006.

2. Dellatre AM, Staziaki PV, Ferraz AC. Effects of OMEGA-3 on Neurodegenerative Diseases and Stroke in: Watson RR (Eds.), Foods and Dietary Supplements in the Prevention and Treatment of Disease in Older Adults. Elsevier Inc., London, 2015, p. 187-201.

3. Damsbo-Svendsen S, Ronsholdt MD, Lauritzen L. Fish oil-supplementation increases appetite in healthy adults. A randomized controlled cross-over trial. (2013) Appetite. DOI:10.1016/j.appet.2013.02.019.

4. Sidhu KS. Health benefits and potential risks related to consumption of fish or fish oil. (2003). Regulatory Toxicology and Pharmacology. DOI: 10.1016/j.yrtph.2003.07.002.

5. Deutch B, Jorgensen EB, Hansen JC. Menstrual discomfort in Danish women reduced by dietary supplements of omega-3 PUFA and B-12 (fish oil or seal oil capsules). (2000).Nutrition Resource. DOI: 10.1016/S0271-5317(00)00152-4.

6. Lara JJ, Economou M, Wallace AM, Rumley A, Lowe G, Slater C et al. Benefits of salmon eating on traditional and novel vascular risk factors in young, non-obese healthy subjects. (2007).Atherosclerosis. DOI: 10.1016/j.atherosclerosis.2006.06.018.

7. Cheung MS, Wang WX. Analyzing biomagnification of metals in different marine food webs using nitrogen isotopes. (2008). Marine Pollution Bulletin. DOI: 10.1016/j.marpolbul.2008.09.004.

8. Bays HE. Safety considerations with omega-3 fatty acid therapy. (2007). American Journal of Cardiology. DOI:10.1016/j.amjcard.2006.11.020.

9. Okyere H, Voegborlo RB, Agorku SE. Human exposure to mercury, lead and cadmium through consumption of canned mackerel, tuna, pilchard and sardine. (2015). Food Chemistry. DOI: 10.1016/j.foodchem.2015.01.038

10. Jan A, Diane B, Cockburn A, Cravedi J-P, Dogliotti E, Domenico AD, Fernandez-Cruz ML, Furst P, Fink-Gremmels J, Galli CL, Grandjean P, Gzyl J, Heinemeyer G, Johansson N, Mutti A, Schlatter J, va Leeuwen R, van Peteghem C, Verger P. Scientific Opinion of the Panel on Contaminants in the food Chain. (2009). The EFSA Journal. 980, 1-39.

11. I.A.R.C. Summaries & evaluations: Cadmium and cadmium compounds (Group 1). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. (1993) 58, 119-239.

12. European Commission. COMMISSION REGULATION (EU) No 488/2014 of 12 May 2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of cadmium in foodstuffs, Official Journal of the European Union. (2014) 57-62.

13. European Commition Joint Research Centre. Official methods for the determination of heavy metals in feed and food, (2012) 1-3.

14. British Standards. Foodstuffs - Determination of trace elements - Determination of arsenic, cadmium, mercury and lead in foodstuffs by inductively coupled plasma mass spectrometry (ICPMS) after pressure digestion, BS EN 15763:2009 (2009) pp. 1-19.

15. I.A.R.C. Summaries & evaluations: Chromium and Chromium compounds. Chromium[VI] (Group 1). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. (1990) 49, 49-214.

16. Sadeghi P, Kazerouni F, Savari A, Movahedinia A, Safahieh A, Ajdari D. Application of biomarkers in Epaulet grouper (Epinephelus stoliczkae) to assess chromium pollution in the Chabahar Bay and Gulf of Oman.(2015). Science of the Total Environment. DOI:10.1016/j.scitotenv.2015.03.017.

17. Velma V, Vutukuru SS, Tchounwou PB. Ecotoxicology of Hexavalent Chromium in Freshwater Fish: A Critical Review. Rev on Environ Health. 24 (2009) 129-145.

18. European Comission. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Trivalent Chromium, (2003).

19. Guerrero-Romero F, Rodriguez-Moran M. Complementary therapies for diabetes: The case for chromium, magnesium, and antioxidants. (2005). Archieves of Medical Research. DOI:10.1016/j.arcmed.2005.01.004.

20. Anderson RA. Chromium, glucose intolerance and diabetes. J Am Coll Nutr. 17 (1998):548-55.

21. European Standards. Foodstuffs - Determination of trace elements - Determination of lead, cadmium, zinc, copper, iron and chromium by atomic absorption spectometry (AAS) after dry ashing. (2003) pp.1-4.

22. Heitmann, U., Welz, B., Borges, D. L. G., & Lepri, F. G. Feasibility of peak volume, side pixel and multiple peak registration in high-resolution continuum source atomic absorption spectrometry. (2007). Spectrochimica Acta Part B: Atomic Spectroscopy. DOI: 10.1016/j.sab.2007.10.011.

23. Bulska E, Liebert-Ilkowska K, Hulanicki A. Optimization of electrochemical deposition of noble metals for permanent modification in graphite furnace atomic absorption spectrometry. (1998). Spectrochimica Acta Part B: Atomic Spectroscopy. DOI: 10.1016/S0584-8547(98)00126-8.

24. Nikolaos S. Thomaidis EAP, Panayotis A. Siskos. Comparison of three digestion methods for the determination of the aqua regia soluble content of lead, cadmium and chromium in sewage sludges by ETAAS. (1995). Microchimica Acta. DOI: 10.1007/BF01244002.

25. Pereira LA, Amorim I, da Silva JBB. Determination of cadmium, chromium and lead in marine sediment slurry samples by electrothermal atomic absorption spectrometry using permanent modifiers. (2006). Talanta. DOI:10.1016/j.talanta.2005.06.013.

26. Silva FA, Padilha CCF, Pezzato LE, Barros MM, Padilha PM. Determination of chromium by GFAAS in slurries of fish feces to estimate the apparent digestibility of nutrients in feed used in pisciculture. (2006). Talanta. DOI:10.1016/j.talanta.2005.12.008.

27. da Silva JBB, Bertilia M, Giacomelli O, de Souza IG, Curtius AJ. Iridium and rhodium as permanent chemical modifiers for the determination of Ag, As, Bi, Cd, and Sb by electrothermal atomic absorption spectrometry. (1998). Microchemical Journal. DOI: 10.1006/mchj.1998.1670.

28. Somorjai GA, Blakely DW. Mechanism of Catalysis of Hydrocarbon Reactions by Platinum Surfaces. (1975). Nature. DOI: 10.1038/258580a0.

29. Oliveira HR, Mesko MF, Vale MGR, Silveira CAP, Picoloto RS, Becker EM. Development of methods for the determination of cadmium and thallium in oil shale by-products with graphite furnace atomic absorption spectrometry using direct analysis. (2014). Microchemical Journal. DOI: 10.1016/j.microc.2014.04.006.

30. Schlemmer G, Welz B. Palladium and Magnesium Nitrates, a More Universal Modifier for Graphite-Furnace Atomic-Absorption Spectrometry. (1986). Spectrochimica Acta Part B: Atomic Spectroscopy. DOI: 10.1016/0584-8547(86)80175-6.

31. Oliveira EP, Santelli RE, Cassella RJ. Combined use of Pd and HF as chemical modifiers for the determination of total chromium in produced waters from petroleum exploration by ET AAS. (2008). Microchemical Journal. DOI: 10.1016/j.microc.2008.01.002

32. Bermejo-Barrera P, Moreda-Pineiro A, Moreda-Pineiro J, Bermejo-Barrera A. Comparative study on the use of Ir, W and Zr-coated graphite tubes for the determination of chromium in slurries of human scalp hair by electrothermal atomic absorption spectrometry. (1998). Fresenius Journal of Analytical Chemistry. DOI: 10.1007/s002160050787.

33. Quadros DPC, Borges DLG. Determination of Trace Elements in B5-diesel Oil by HR-CS ET AAS Using a Simple Dilute-and-Shoot Approach. (2013). Journal of Brazilian Chemical Society. DOI: 10.5935/0103-5053.20130255.

34. Beniwal; V, Nehra; KS, Chhoka; V. Cadmium induced alteration in lipid profile of developing mustard (Brassica juncea L.) seed. (2015). Biocatalysis and Agricultural Biotechnology. DOI: 10.1016/j.bcab.2015.06.003

35. Chia; MA, Lombardi; AT, Melão; MdGG, Parrish; CC. Lipid composition of Chlorella vulgaris (Trebouxiophyceae) as a function of different cadmium and phosphate concentrations. (2013). Aquatic Toxicology. DOI: 10.1016/j.aquatox.2012.12.004

36. National Toxicity Program. Chromium hexavalent compounds: Department of Health and Human Services. Report on Carcinogens third ed. 2014, pp. 1-4.

FIGURE CAPTIONS

Figure 1. Pyrolysis and atomization curves for Cd using Pt or Ir as permanent chemical modifier.

a) 10 pg Cd in an aqueous solution; (●) without modifier; T_{at} for pyrolysis curve = 1400 °C; T_{pyr} for atomization curve = 400 °C. (▲) 400 µg Pt as permanent modifier; (■) 400 µg Ir as permanent modifier; for both modifiers:T_{at} for pyrolysis curve = 1700 °C; T_{pyr} for atomization curve = 800 °C.

b) 30 µL of fish oil in 1-propanol; (■) 400 µg Ir as permanent modifier; (▲) 400 µg Pt as permanent modifier; for both modifiers: T_{at} for pyrolysis curve = 1700 °C; T_{pyr} for atomization curve = 800 °C.

Figure 2. Pyrolysis and atomization curves for Cd using a Pt coated platform and in addition a chemical modifier in solution.

a) 20 µL of a solution of fish oil in 1-propanol: (■) without a chemical modifier in solution; (♦) 15 µg/10 µg of Pd/Mg in solution as chemical modifier; (★) 10 µg of Pd in solution as chemical modifier; in all three cases: T_{at} for pyrolysis curve = 1700 °C; T_{pyr} for atomization curve = 800 °C.

b) 10 pg Cd in aqueous solution: (■) without chemical modifier in solution; T_{at} for pyrolysis curve = 1700 °C; T_{pyr} for atomization curve = 800 °C; (♦) 15 µg/10 µg of Pd/Mg in solution as chemical modifier; T_{at} for pyrolysis curve = 1700 °C; T_{pyr} for atomization curve = 800 °C; (★) 10 µg Pd in solution as chemical modifier; T_{at} for pyrolysis curve = 1700 °C; T_{pyr} for atomization curve = 800 °C.

Figure 3. Pyrolysis and atomization curves for Cr using Pd or Zr as permanent chemical modifier.

a) 200 pg Cr in an aqueous solution (■) without a modifier; T_{at} for pyrolysis curve = 2400 °C; T_{pyr} for atomization curve = 1100 °C. (►) 400 µg Zr as permanent chemical modifier; (●) 400 µg Pd as permanent modifier; in both cases: T_{at} for pyrolysis curve = 2400 °C; T_{pyr} for atomization curve = 1300 °C.

b) 30 µL of a solution of 0.5 g mL^{-1} fish oil in 1-propanol. (■) without modifier; T_{at} for pyrolysis curve = 2400 °C; T_{pyr} for atomization curve = 1100 °C. (●) 400 µg Pd as permanent modifier; (►) 400 µg of Zr as permanent modifier; in both cases: T_{at} for pyrolysis curve = 2400 °C; T_{pyr} for atomization curve = 1300 °C.

Figure 4. Pyrolysis and atomization curves for Cr using different modifiers in solution.
a) 30 µL of a solution of 0.5 g mL^{-1} fish oil in 1-propanol: (♦) without chemical modifier; (■) 15 µg/10 µg of Pd/Mg in solution as chemical modifier; (★) 10 µg Pd in solution as chemical modifier; in all three cases: T_{at} for pyrolysis curve = 2300 °C; T_{pyr} for atomization curve = 1100 °C.
b) 200 pg of Cr in aqueous solution: (■) without chemical modifier; (♦) 15 µg/10 µg of Pd/Mg in solution as chemical modifier; (★) 10 µg Pd in solution as chemical modifier; in all three cases: T_{at} for pyrolysis curve = 2500 °C; T_{pyr} for atomization curve: = 1100 °C.

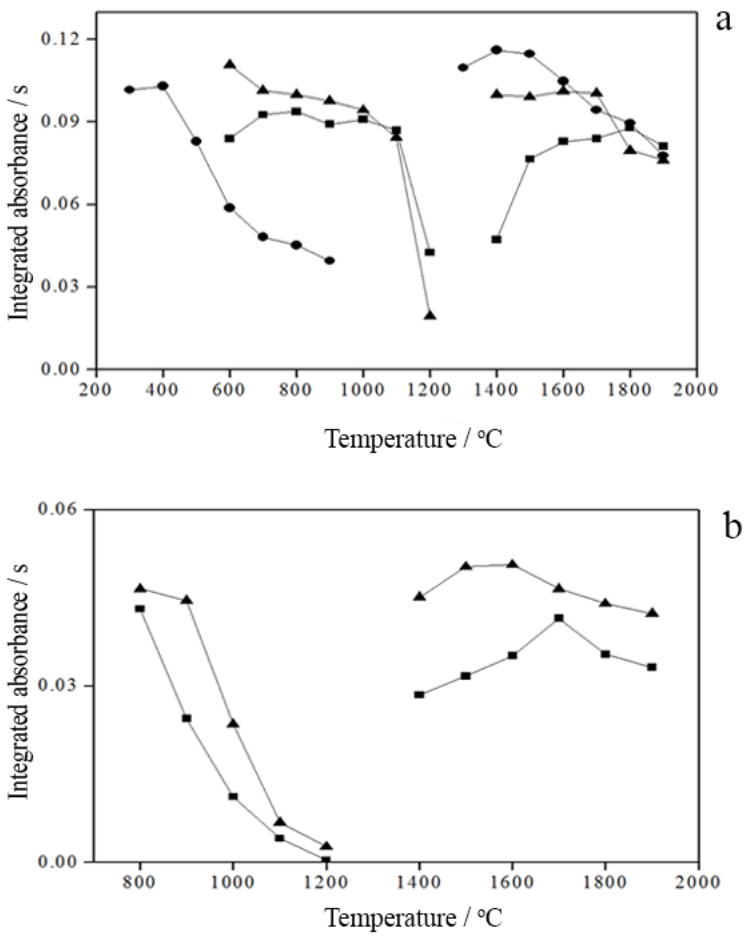

Fig. 1

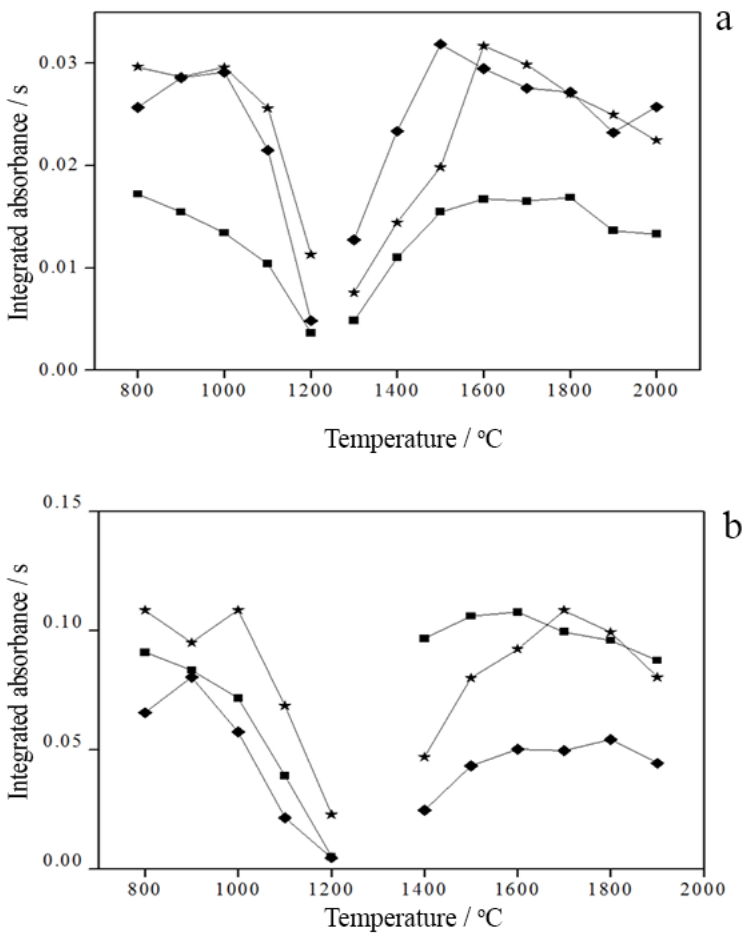

Fig. 2

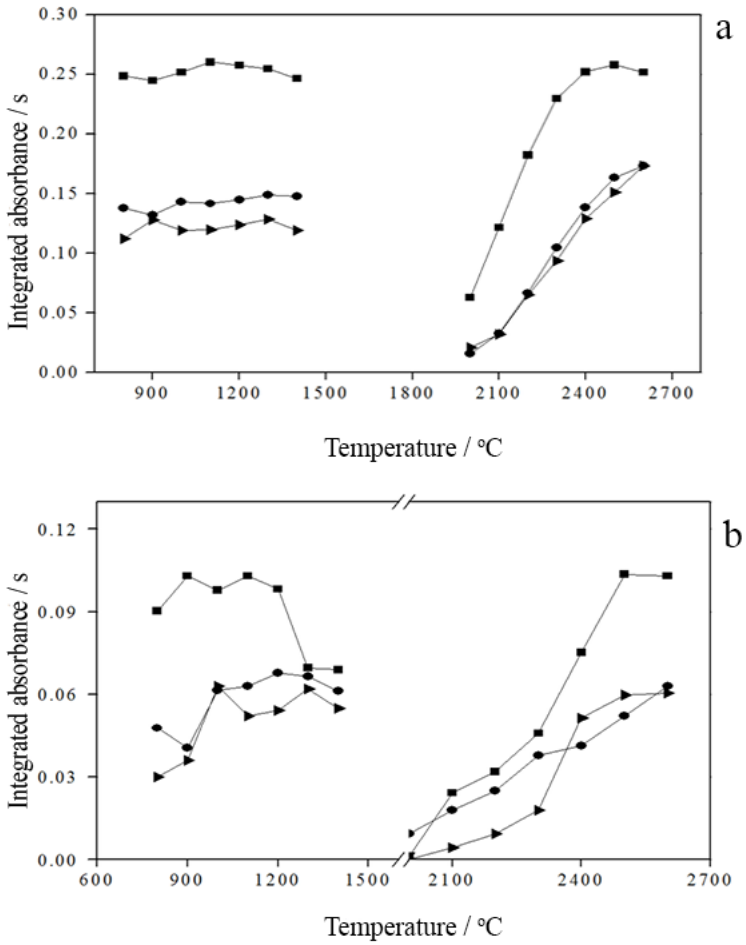

Fig. 3

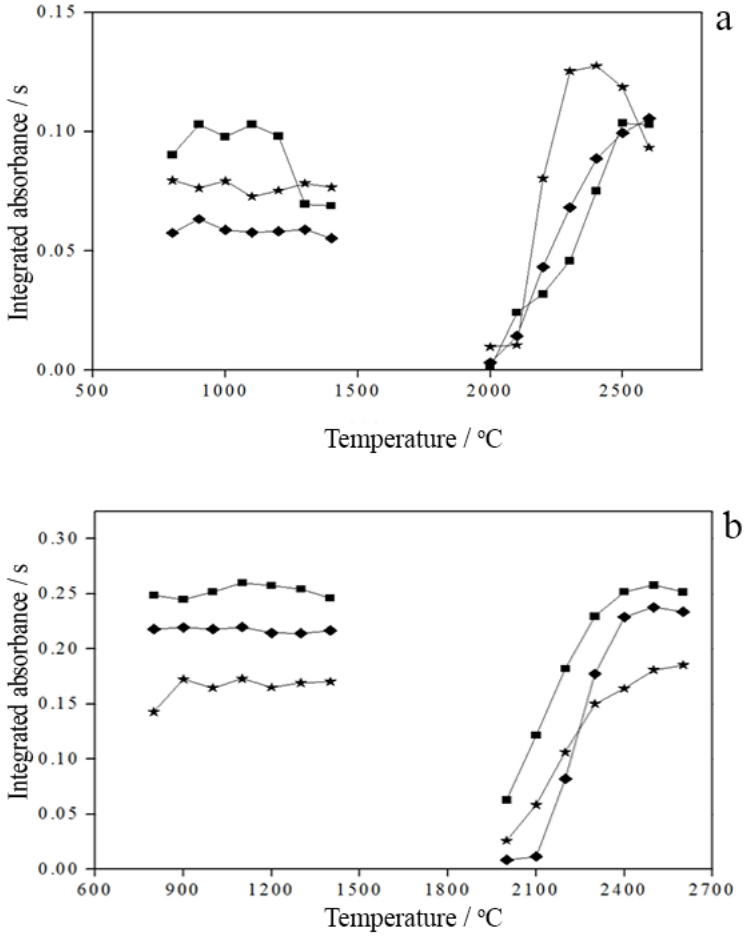

Fig. 4

Table 1. Temperature program for Ir, Pd, Pt or Zr coating of the PIN platform.

Step	T / °C	Ramp / °C s^{-1}	Hold Time / s
1	90	5	40
2	110	1	40
3	130	1	40
4	1200	300	25
5	2100	500	10
6	2100	0	5

Table 2. Program for microwave-assisted digestion of fish oil and other lipoid samples.

Temperature / °C	Pressure / bar	Ramp time / min	Hold time / min
145	4	2	10
170	40	5	5
200	40	2	20
50	0	0	10
50	0	0	1

Table 3. Temperature program for Cd determination in fish oil and lipoid samples by HR-CS GF AAS after dilution with 1-propanol. Permanent modifier: Pt, and Pd added as modifier in solution.

Stage	T / °C	Ramp / °C s^{-1}	Hold Time / s
Drying 1	150	50	15
Drying 2	250	50	20
Pyrolysis	1000	100	15
Atomization	1700	3000	5
Cleaning	2100	1000	4

Table 4. Temperature program for the determination of Cr in fish oil and lipoid samples by HR-CS GF AAS after dilution with 1-propanol. No permanent modifier or modifier in solution was used.

Stage	T / °C	Ramp / °C s^{-1}	Time / s
Drying 1	150	50	15
Drying 2	250	50	20
Pyrolysis	1100	100	15
Atomization	2500	3000	5
Cleaning	2600	1000	4

Table 5. Figures of merit for the determination of Cd and Cr in fish oil and lipoid samples by the proposed method using HR-CS GF AAS.

Figure of merit	Cd	Cr
Slope (s pg^{-1})	0.1043	0.0381
R^2	0.998	0.999
m_o (pg)	1.5	6
LOD (pg)	0.5	7
LOD (ng g^{-1})	0.15	2
LOQ (ng g^{-1})	0.5	7
Linear range (pg)	1.5 – 360	23 – 1080

Table 6. Determination of Cd and Cr in fish oil and other lipoid samples by HR-CS GF AAS using just a dilution in 1-propanol, in comparison with microwave-assisted digestion . The values represent the mean of five measurements ± standard deviation (SD).

Sample	1-propanol dilution / µg g^{-1}		Microwave-assisted digestion / µg g^{-1}	
	Cd	Cr	Cd	Cr
Oil A	ND	1.3 ± 0.1	ND	1.2 ± 0.05
Stage 1	1.4 ± 0.2	1.1 ± 0.1	1.3 ± 0.2	1.3 ± 0.04
Stage 2	ND	ND	ND	ND
Stage 3	ND	ND	ND	ND
Stage 4	ND	ND	ND	ND
Cocoanut butter	0.007 ± 0.001	0.071 ± 0.02	ND	0.082 ± 0.03
Milk butter	ND	0.051 ± 0.01	ND	0.050 ± 0.01

ND: Not detected.

HIGHLIGHTS

- A simple method was developed for Cd and Cr determination in fish oil.
- The "dilute-and-shoot "procedure involves just the dilution of the sample in 1-propanol.
- Cd and Cr can be determined using aqueous standard solutions for calibration.
- Low limits of quantification can be achieved due the low volume of solvent compared to digestion.