BABEŞ-BOLYAI UNIVERSITY OF CLUJ-NAPOCA

FACULTY OF CHEMISTRY AND CHEMICAL ENGINEERING

CHEMISTRY DEPARTMENT

ANALYTICA RESEARCH CENTER

ABSTRACT OF Ph.D. THESIS

ECO-ANALYTICAL METHODS FOR MERCURY DETERMINATION AND SPECIATION BY OPTICAL EMISSION SPECTROMETRY IN MICROPLASMAS

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Contents

Keywords

Mercury speciation in food of marine origin

Methylmercury determination in food of marine origin

Green derivatization methods for mercury

Eco-scale UV/Vis photo-induced cold vapor generation in formic acid

Mercury extraction from food samples in formic acid

Risk assessment of the population to mercury

Capacitively coupled plasma microtorch

Capacitively coupled plasma microtorch optical emission spectrometry

Analytical methods greenness degree evaluation

Analytical methods whiteness degree evaluation

List of abbreviations

AAS	Atomic absorption spectrometry
AFS	Atomic fluorescence spectrometry
AGREE	Analytical greenness metric
AOAC	Association of Official Analytical Chemists
CCD	Charge coupled device
μCCP-OES	Capacitively coupled microplasma optical emission spectrometry
CRM	Certified reference material
CV-AAS	Cold vapor generation atomic absorption spectrometry
CV-AFS	Cold vapor generation atomic fluorescence spectrometry
CV-ICP-OES	Cold vapor generation inductively coupled plasma optical emission spectrometry
EFSA	European Food Safety Authority
AES	Analytical eco-scale
ETAAS	Electrothermal atomic absorption spectrometry
FAO	Food and Agriculture Organization of the United Nations
GAC	Green analytical chemistry
GAPI	Green analytical procedure index
GC	Gas chromatography
GFAAS	Graphite furnace atomic absorption spectrometry
HG	Hydride generation
HG-AFS	Hydride generation atomic fluorescence spectrometry
HPLC	High performance liquid chromatography
ICP	Inductively coupled plasma
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
LA-ICP-MS	Laser ablation inductively coupled plasma mass spectrometry
LC	Liquid chromatography
LMWOA	Low molecular weight organic acids
LOD	Limit of detection

LOQ	Limit of quantification
MS	Mass spectrometry
NEMI	National environmental methods index
PD-OES	Point discharge optical emission spectrometry
PRSD	Predicted relative standard deviation
PTFE	Tetrafluoroethylene
PTWI	Provisional tolerably weakly intake
RGB-12	Red–Green–Blue 12 procedure
RSD	Relative standard deviation
$SnCl_2$-CV-AFS	$SnCl_2$ cold vapor generation atomic fluorescence spectrometry
$SnCl_2$-CV-μCCP-OES	$SnCl_2$ cold vapor generation capacitively coupled microplasma optical emission spectrometry
$SnCl_2$-CV-ICP-OES	$SnCl_2$ cold vapor generation in inductively coupled plasma optical emission spectrometry
SOP	Standard operating procedure
TD-AAS	Thermal desorption atomic absorption spectrometry
THQ	Target hazard quotient
TMAH	Tetramethylammonium hydroxide
TOC	Total organic carbon
TTHQ	Total target hazard quotient
USEPA	United States Environmental Protection Agency
UV-PVG	UV photochemical vapor generation
UV-PVG-μCCP-OES	UV photochemical vapor generation capacitively coupled plasma microtorch optical emission spectrometry
WAC	White analytical chemistry
WHO	World Health Organization

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Introduction

Thesis motivation

In 2011, the European Commission carried out an interlaboratory study aimed at developing methods for problematic contaminants determination in food, including methylmercury (CH_3Hg^+). Only 8 laboratories from the European Union took part in this study, many of them refusing to participate, as they considered that the methods for determining methylmercury require complicated sample processing and sophisticated and expensive instrumentation, which they do not have. Following this interlaboratory study, the European Commission proposed a simple method for the determination of CH_3Hg^+ in seafood based on double liquid-liquid extraction in the HBr-toluene-L-cysteine system and detection by thermal desorption atomic absorption spectrometry (TD-AAS). At the same time, the European Commission has encouraged the development of new methods that go beyond the difficulties and provide enhanced analytical performance compared to the recommended TD-AAS method. Furthermore, EFSA recommends that the methods developed to be extended to other foods of animal and vegetable origin consumed almost daily. The current standardized methods for the determination of Hg are those based on thermal desorption atomic absorption spectrometry (TD-AAS), atomic absorption spectrometry with cold vapor generation (CV-AAS), and cold vapor atomic fluorescence spectrometry (CV-AFS) using either $SnCl_2$ or $NaBH_4$ as derivatization reagents.

The introduction of the principles of Green Analytical Chemistry (GAC) by Professor Namiesnik's research group in 2012, led to the introduction of the term green atomic spectrometry formulated by Bendicho *et al*. The research directions in green spectrometry are the development of miniaturized instrumentation based on microplasma sources of low Ar and energy consumption, simplification of analytical procedures related to preparation and preconcentration, introduction of environmentally friendly methods for derivatization using green, biodegradable reagents, with low toxicity, that generate as little residue as possible. At present, the coupling between GAC principles, such as generation of chemical vapor by green derivatization with optical emission spectrometry in microplasma sources, is an emerging technology in full development, and is considered a critical approach in atomic spectrometry. Many of the spectrometric methods for the determination and speciation of Hg use classical derivatization with concentrated, harmful and expensive reagents, such as $SnCl_2$, $NaBH_4$ and HCl. As a result, the PhD thesis considered the development of eco-analytical methods for the determination and speciation of Hg using a fully miniaturized instrumentation based on optical emission spectrometry in capacitively coupled plasma microtorch and UV-photo-induced derivatization (UV/VIS-PVG-μCCP-OES). Even if one of the GAC principles requires the avoidance

of derivatization, in the case of Hg high sensitivity cannot be reached in the absence of derivatization. In order to maintain the greenness of the method, a single reagent, namely HCOOH, was used for extraction and derivatization. Mercury was determined with/without *on-line* preconcentration of vapor on Au filament microcollector. Cold vapor generation under UV/Vis irradiation allowed Hg speciation as inorganic (Hg^{2+}) and organic (CH_3Hg^+) species. The methods have been extended, in addition to seafood, to the analysis of various foods of vegetable and animal origin as well as water.

Following the introduction of the 12 GAC principles, new methodologies to objectively assessing greenness were developed, thus eliminating subjective approaches with no clearly defined principles and often left to the developer's decision. Because of that, many methods were evaluated in the past only on a subjective basis and were not properly justified. The level of subjective exaggeration can be easily noticed in the enthusiasm of the authors to present in a better light their methods. Thus, objective methods for greenness assessment have been introduced, of which the most used are the National Environmental Methods Index (NEMI), Analytical Eco-Scale (AES), Green Analytical Procedure Index (GAPI) and Analytical Greenness metric approach (AGREE). As these procedures consider only reagent toxicity and instrumentation power consumption issues, Nowak and colleagues introduced in 2021 the principles of White Analytical Chemistry (WAC) and the Red-Green-Blue Procedure 12 (RGB-12), respectively. This model evaluates the whiteness of a procedure also considering the analytical performance of the method (red) and economical aspects related to the method applicability (blue). Thus, a comprehensive approach based on the 4 procedures for assessing greenness and the RGB-12 model clearly highlights all aspects that contribute to the enhancement of green and white level of some non-chromatographic methods for determining and speciation of Hg by UV/VIS-PVG-μCCP-OES compared to traditional methods, TD-AAS, CV-AFS and CV-ICP-OES. This approach is a novelty at the international level in terms of an objective assessment of a wide range of methods for the determination and speciation of Hg using both miniaturized instrumentation and traditional methods.

Research objectives and methodology

The study of the literature at national and international level regarding the analytical methods used in the determination and speciation of Hg showed that there are several approaches that use microplasma sources, but not capacitively coupled microplasma. Also, at the national level, there are no research teams with interests in the development of instrumentation and eco-analytical methods suitable for the determination and speciation of Hg in foods and environmental samples. Consequently, the general objective of the Ph.D. thesis is an absolute novelty at national level and, in some aspects, even at international level, by developing and

validating non-chromatographic eco-scale methods for the determination and speciation of Hg as CH_3Hg^+ and Hg^{2+} based on UV/Vis-PVG-μCCP-OES, as well as the evaluation of their greenness and whiteness degree for a correct positioning compared to the traditional ones. It has also been considered the comparison of the analytical performance with that required in the legislation of the European Commission regarding the determination of Hg in foods.

The specific objectives were as follows:

1. Determination of CH_3Hg^+ in seafood by photo-induced cold vapor generation and detection by optical emission spectrometry in capacitively coupled plasma microtorch;
2. Speciation of Hg as CH_3Hg^+ and Hg^{2+} in seafood using classical/UV photo-induced cold vapor generation and optical emission spectrometry in a capacitively coupled plasma microtorch;
3. General eco-scale method for the determination of total Hg in food and water by optical emission spectrometry in a capacitively coupled microplasma and UV photo-induced derivatization**;**
4. General eco-scale method for Hg speciation as Hg^{2+} and CH_3Hg^+ in seafood by UV/Vis photochemical vapor generation and detection by optical emission spectrometry in a capacitively coupled microplasma;
5. Assessment of greenness and whiteness of the UV/Vis-PVG-μCCP-OES methods for the determination and speciation of Hg.

In order to achieve these specific objectives, the research methodology consisted of:

1. Study of the coupling between the continuous-flow (*on-line*) UV/Vis photoreactor (laboratory construction) and capacitively coupled plasma microtorch of low power and low Ar consumption for the determination and speciation of Hg as CH_3Hg^+ and Hg^{2+};
2. Development of a method for the determination of CH_3Hg^+ in fish by implementing the sample preparation protocol recommended by the European Commission, based on double liquid-liquid extraction in HBr–toluene–aqueous solution of L-cysteine, UV-PVG derivatization in HCOOH medium and determination by μCCP-OES;
3. Optimization of the new UV/Vis-PVG-μCCP-OES system in terms of the derivatization conditions in diluted HCOOH (concentration of HCOOH in sample and UV irradiation time) and operation of the capacitively coupled plasma microtorch (Ar flow rate, microplasma power and observation height);
4. Study of the extraction of total Hg from foods of animal and vegetable origin, and environmental samples in concentrated HCOOH by sonication, optimization of the extraction conditions (sonication time, sample amount/HCOOH amount ratio);

5. Assessment of the analytical performance of the miniaturized UV/Vis-PVG-μCCP-OES system with/without preconcentration in terms of limit of detection (LOD), limit of quantification (LOQ), precision and accuracy of the method;
6. Examination of the analytical performance of the new UV/Vis-PVG-μCCP-OES methods in relation to the requirements of the European legislation on the determination and speciation of Hg in food and environmental samples set out in Directives 2002/657/CE, 2007/333/CE and 2006/1881/CE, as well as AOAC recommendations;
7. Checking the applicability of the UV/Vis-PVG-μCCP-OES methods on real food samples (fish, meat, vegetables, fruits, cereals, food supplements) and water; comparison of the results provided by the new methods with those obtained with the traditional methods TD-AAS, $SnCl_2$-CV- AFS and $SnCl_2$-CV-ICP-OES using the Bland and Altman statistical test;
8. Assessment of population exposure to total Hg, CH_3Hg^+ and Hg^{2+} *via* fish consumption based on the following approaches: (i) calculation of the weekly intake of total Hg and CH_3Hg^+, and comparison with the Provisional Tolerable Weekly Intake (PTWI) of 4 μg kg^{-1}/body weight and 1.3 μg kg^{-1}/body weight, recommended by EFSA; (ii) calculation of the Target Hazard Quotient (THQ) for non-carcinogenic diseases using the methodology recommended by the Environmental Protection Agency of USA (USEPA), (iii) estimation of the concentration of Hg in blood and hair in accordance with the EFSA study concerning assessment of exposure risk;
9. Comprehensive assessment of greenness of the new methods based on 4 procedures (National Environmental Methods Index (NEMI), Analytical Eco-Scale (AES), Green Analytical Procedure Index (GAPI) and Analytical Greenness metric (AGREE), as well as the Red-Green-Blue Procedure 12 (RGB-12), in comparison with the traditional methods used for Hg determination.

Statement of the research problem

The research in the PhD thesis was carried out within the Project *Analytical eco-scale methods for the determination and speciation of Hg by UV photo-induced derivatization and detection using a fully-miniaturized experimental spectrometric system (ECOSPEC)* funded by the Romanian National Authority for Scientific Research, code project PN-III-P2-2.1-PED-2016-0135, coordinator Babeş-Bolyai University, Cluj-Napoca, project director Prof. Dr. Tiberiu Frenţiu.

The PhD thesis is structured in two parts, the first part presents the current state of knowledge in the field (chapters 1–2), while the second part discloses the personal contributions (chapters 3–7). The last chapter presents the conclusions and highlights the innovative contributions of the PhD thesis. Chapter 1 presents the principles of Green Analytical Chemistry (GAC) and the procedures for assessing greenness of the analytical methods based on the most

commonly used approaches, namely NEMI, AES, GAPI and AGREE. The principles of White Analytical Chemistry (WAC) and the RGB-12 model are also presented in detail. The second part of the chapter presents a brief overview of several microplasma sources used in the determination and speciation of Hg.

Chapter 2 presents aspects related to the occurrence of Hg in the environment, toxicity and risk of exposure of the population, chromatographic and non-chromatographic methods for the determination and speciation of Hg, as well as classical and eco-scale photo-induced and sono-induced derivatization procedures in the presence of low molecular weight organic compounds. Chapter 3 presents a method for the determination of CH_3Hg^+ in fish after double liquid-liquid extraction in the HBr–toluene–L-cysteine system, followed by photo-induced UV derivatization in 0.6 mol L^{-1} HCOOH and detection by μCCP-OES.

Chapter 4 presents a method for Hg speciation as Hg^{2+} and CH_3Hg^+ in fish by (1) determining the total Hg from the sample digested in a mixture of HNO_3 and H_2O_2, classical derivatization with 20% (m/v) $SnCl_2$ in 5% (v/v) HCl and detection by CV-μCCP-OES; (2) determination of CH_3Hg^+ after double liquid-liquid extraction in the HBr–toluene–L-cysteine system, photo-induced derivatization in HCOOH medium and detection by UV-PVG-μCCP-OES and (3) calculation of the Hg^{2+} concentration by difference. This chapter also presents an assessment of Hg exposure of the population through fish consumption. Chapter 5 presents a method for the determination of total Hg in food samples subjected to ultrasound assisted extraction in concentrated HCOOH, photo-induced derivatization in HCOOH medium and detection by UV-PVG-μCCP-OES with/without preconcentration on a gold filament microcollector. The UV-PVG-μCCP-OES method with preconcentration was also applied to determine the total Hg in water samples adjusted to contain 0.6 mol L^{-1} HCOOH.

Chapter 6 presents a method for Hg speciation as Hg^{2+} and CH_3Hg^+ by UV/Vis-PVG-μCCP-OES in fish samples after extraction in HCOOH. The speciation was performed by selective photo-induced derivatization under UV (lamp on) and Vis (lamp off) irradiation in 0.6 mol L^{-1} HCOOH, to determine the total Hg (sum of Hg^{2+} and CH_3Hg^+) and Hg^{2+}, respectively. In this case the concentration of CH_3Hg^+ was calculated as the difference total Hg - Hg^{2+}. Because the derivatization of Hg^{2+} species under Vis light irradiation occurred much slower, it was necessary to preconcentrate the Hg vapors on a gold filament microcollector to achieve the sensitivity required for the analytical application. Chapter 7 presents the assessment of the green and white level of the methods based on UV/Vis-PVG-μCCP-OES with/without preconcentration using NEMI, AES, GAPI, AGREE and RGB-12 approaches. Chapter 8 lists the general conclusions, the elements of originality and innovation of the PhD thesis.

THEORETICAL PART

1. Green and White Analytical Chemistry – Principles and evaluation methods

Awareness of environmental protection emerged after 1940, following the explosive development of the chemical industry, with major effects on environmental quality. As a result, in 1998, Anastas and Warner[1] launched the concept and principles of Green Chemistry, while in 2012 the research group of Professor Namiesnik[2] formulated the 12 principles of Green Analytical Chemistry (GAC). The principles of GAC consider the following 4 aspects: (1) Elimination or reduction of the chemical reagents used for processing, conservation and analysis of samples; (2) Reduction of energy consumption; (3) Suitable treatment of waste and (4) Increase the operator protection.[2] Unfortunately, the evaluation of the greenness of a method in the absence of clearly defined principles has long been left to the decision of the developer and for this reason the evaluation was often subjective and not properly justified. As a result, objective procedures have been developed to assess greenness of the new analytical methods, of which the most used are: National Environmental Methods Index (NEMI)[3], Analytical Eco-Scale (AES)[4], Green Analytical Procedure Index (GAPI)[5] and Analytical GREEnness metric (AGREE)[6].

Although the assessment of the greenness of analytical methods is based on objective criteria, established at a qualitative and quantitative level, the procedures have proved to be very rigid and limited. The reason is the difficulty to thoroughly assess an analytical method based on the 12 GAC principles, without taking into account the performance and the economic and practical aspects. As a result, Nowak *et al.*[7] introduced in 2021 the 12 principles of White Analytical Chemistry (WAC), which takes into account 3 other criteria highlighted by different colors, namely Red (R, analytical performance), Green (G, reagent toxicity) and Blue (B, economic and practical aspects). It has also been developed a method for assessing the whiteness degree by the Red-Green-Blue (RGB-12) model. Figure 1.1 schematically shows the aspects taken into account in White Analytical Chemistry.

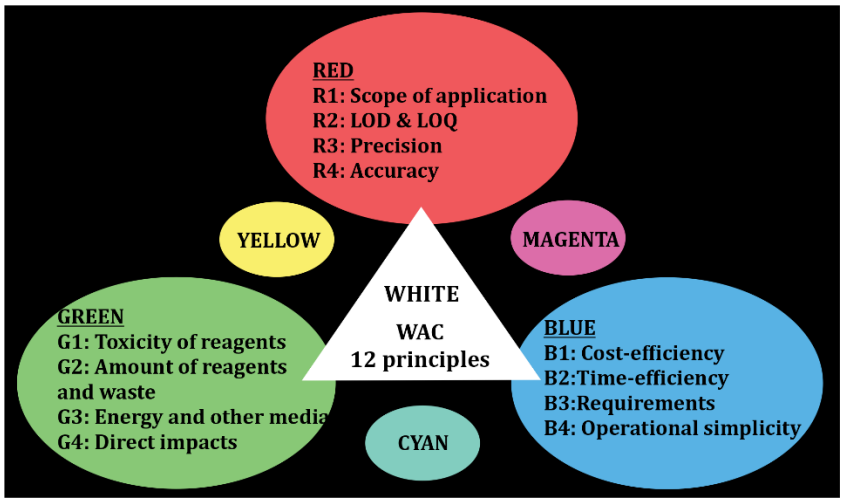


Figure 1.1. Schematic depiction of the principles of White Analytical Chemistry[7,8]

In line with the new principles of green atomic spectrometry introduced by Bendicho *et al.*[9], the research directions aim at developing miniaturized instrumentation based on microplasma sources, simple, fast procedures for sample preparation and analyte preconcentration, and green derivatization methods. A large variety of microplasma sources have been proposed with advantages related to (1) low power (< 100 W) and low Ar (< 1 L min^{-1}) consumption; (2) low size and portability; (3) possibility of interfacing with detection systems based on optical emission spectrometry (OES), atomic fluorescence spectrometry (AFS), atomic absorption spectrometry (AAS) and mass spectrometry (MS); (4) simultaneous multielemental determination in OES and AFS; (5) simple emission spectrum allowing the use of low-resolution spectrometers to record interference-free spectra and (6) analytical performance similar to those achieved with the classical instrumentation based on ICP, provided that the sample is introduced as vapor after chemical or photo-induced derivatization.[9-13] Figure 1.2 presents a classification of the microplasma sources used in the determination and speciation of Hg.

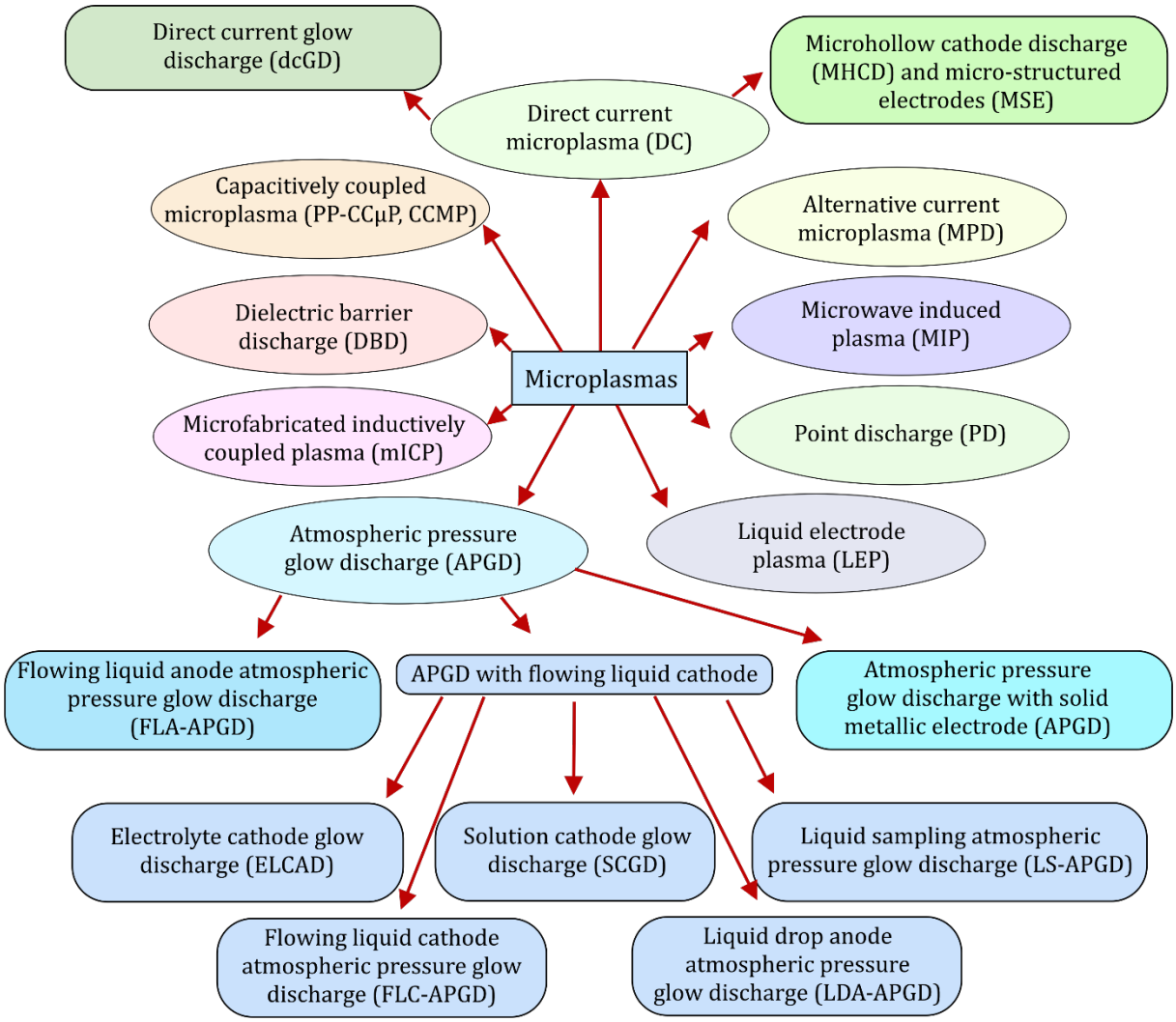


Figure 1.2. Classification of microplasma sources

2. Mercury occurrence in environment and methods for its determination and speciation

Mercury is a ubiquitous element and occurs in the environment in a variety of inorganic and organic species as a result of high bioavailability and various natural and anthropogenic processes. The inorganic species in the environment are elemental mercury (Hg^0), compounds of (Hg^{2+}) and (Hg_2^{2+}), while the best known organometallic compounds are monomethylmercury (MMeHg, CH_3Hg^+), dimethylmercury (DMeHg, $(CH_3)_2Hg^+$), monoethylmercury (MEtHg, $C_2H_5Hg^+$) and diethylmercury (DEtHg, $(C_2H_5)_2Hg^+$).[14] The main natural sources of Hg are vaporization from surface water, soil and vegetation, and volcanic eruption, while the anthropogenic ones are mining, coal-fired power stations, non-ferrous metals industry, cement industry as well as waste incineration.[15]

Mercury in all forms has a toxic effect on plants and animal organisms. The organic Hg species, especially CH_3Hg^+, are the most toxic, and enters the human body *via* seafood, in which more than 85% of Hg is in CH_3Hg^+.[14,16] The main adverse consequences on human health following exposure to CH_3Hg^+ concern the nervous system, with negative cognitive effects on thinking, memory, language, movement, spatial visual ability, cardiovascular and immune system.[14] The high toxicity of CH_3Hg^+ is the result of high absorption through the intestinal tract and lung, and very slow elimination from the body.[17] Because of the multiple negative effects on the human body, the European Commission has set a maximum allowable value of 0.5 mg kg^{-1} total Hg in non-predatory fish and 1 mg kg^{-1} in predatory fish, as well as a limit of 0.1 mg kg^{-1} in food supplements.[18,19] At the same time, EFSA set a provisional tolerable weekly limit (PTWI) of 1.3 µg kg^{-1} body weight / week of CH_3Hg^+ and 4 µg kg^{-1} body weight / week of Hg^{2+} in food.[14]

A study conducted by EFSA showed that at European level the population consumes 10-80 g fish daily, which generates an exposure of 0.1–1.0 µg kg^{-1} Hg body weight/week for an adult of 60 kg, below PTWI. The same study showed that consuming 1–2 servings (150–300 g) fish per week does not pose any risk of exposure, regardless of the variety of fish. In fact, the consumption of fish is beneficial for health due to long-chain polyunsaturated n-3 fatty acids (omega 3), and essential nutrients such as iodine, selenium, calcium and vitamins A, D and E. However, the study recommends moderate consumption of predatory fish, such as tuna, swordfish, shark, whiting fish and pike.[20]

The spectrometric methods for the determination of total Hg and its speciation as Hg^{2+} and CH_3Hg^+ most often involve cold vapor generation (CV). Speciation is achieved by selective derivatization of Hg species or their separation by liquid-liquid extraction based on the difference in polarity of Hg^{2+} and CH_3Hg^+ species, followed by direct measurement or after cold vapor generation. Mercury vapor are purged from the sample matrix and introduced into the spectral

detector with a gas flow. The common methods used for the determination of total Hg and speciation by selective derivatization are cold-vapor atomic absorption spectrometry (CV-AAS),[21,22] cold-vapor atomic fluorescence spectrometry (CV-AFS),[23,24] cold-vapor inductively coupled plasma optical emission spectrometry (CV-ICP-OES)[25] and cold-vapor inductively coupled plasma mass spectrometry (CV-ICP-MS)[26]. The direct spectrometric methods for the determination of total Hg, such as thermal desorption atomic absorption spectrometry (TD-AAS),[27] analyze solid or liquid sample without processing. In the case of speciation, it is necessary a suitable separation procedure, most often the liquid-liquid extraction.

Chemical vapor derivatization consists in converting the initial ionic or non-volatile species from the sample into gaseous derivatives that can be purged from the liquid sample or into volatile forms, which can also be purged or extracted into a non-polar organic solvent and subsequently separated by gas chromatography. Three classical methods for derivatization are used in the determination of total Hg and speciation as Hg^{2+} and CH_3Hg^+: (1) cold vapor generation (CV) with $SnCl_2$; (2) cold vapor/hydride generation with $NaBH_4$ and (3) derivatization by alkylation (ethylation) with $NaBEt_4$.[28-30] Of these 3 methods, those using $SnCl_2$ and $NaBH_4$ are most often used in the non-chromatographic approaches.

Cold vapor generation with $SnCl_2$. The reagent is in this case an acidic solution of $SnCl_2$, which performs a selective derivatization by converting only the Hg^{2+} species to cold vapor. For the determination of total Hg it is necessary a complete digestion of the sample with strong, oxidizing reagents, such as HNO_3 and H_2O_2. The resulting Hg vapor are purged from the reactor and introduced in the spectral detector. The use of $SnCl_2$ alone does not allow Hg speciation. The derivatization reaction with $SnCl_2$ in solution occurs according to the reaction:[31]

$$Sn^{2+} + Hg^{2+} \rightarrow Sn^{4+} + Hg^0 \quad (2.1)$$

Derivatization with $NaBH_4$. In this case the sample containing HCl is mixed with a solution of $NaBH_4$ stabilized in NaOH. The derivatization reactions are:[32]

$$Hg^{2+} + 2NaBH_4 + 6H_2O \rightarrow Hg^0 + 7H_2 + 2H_3BO_3 + 2Na^+ \quad (2.2)$$

$$CH_3Hg^+ + NaBH_4 + 3H_2O \rightarrow CH_3HgH + 3H_2 + H_3BO_3 + Na^+ \quad (2.3)$$

Depending of the $NaBH_4$ concentration, the derivatization process can be selective or non-selective. Thus, a highly dilute solution of $NaBH_4$ achieves selective derivatization of Hg^{2+} species, while a concentrated solution leads to the derivatization of both Hg^{2+} and CH_3Hg^+. In other words, the control of the $NaBH_4$ concentration in solution makes possible Hg speciation by a non-chromatographic method.[33] Also, combination of $NaBH_4$ and $SnCl_2$ is another way for speciation, since $SnCl_2$ converts only Hg^{2+} to cold vapor, while $NaBH_4$ converts both species (sum of Hg^{2+} and CH_3Hg^+).

Among the advantages of using derivatization, in particular in the case of mercury, it should be mentioned: (1) significant improvement of the detection limit due to analyte introduction in gas phase in the atomization/excitation source with 100% efficiency; (2) circumvention of matrix effects by separating the analyte from the sample matrix; (3) opportunity of Hg speciation, either by applying selective derivatization in the case of spectrometric methods or using hyphenated techniques (chromatography-spectrometry).[34]

The conventional derivatization methods require concentrated HCl medium, and unstable and toxic reagents, so that green derivatization methods have been developed, which use low molecular weight organic compounds (formic, propionic, malonic, oxalic acid), alcohols (methanol, ethanol, n-propanol), formaldehyde, acetaldehyde, ionic liquids, etc.,[35] as well as environmentally friendly energy sources, more efficient for derivatization, such as UV/Vis light or ultrasound irradiation. Green derivatization methods are currently considered critical approaches in atomic spectrometry, as substantial improvements have been made in terms of sensitivity, avoidance of non-spectral effects and economic benefits through the use of less expensive reagents, which do not need to be daily prepared.[34-36]

The photo-induced derivatization is based on the generation of highly reactive radicals by LMWOA photolysis, which have the ability to efficiently derivatize Hg^{2+} and CH_3Hg^+. The chemical reactions and details on the determination of total Hg and its speciation using the UV/Vis-PVG procedures are presented in Chapter 3, section 3.4. Compared to conventional derivatization, the UV photo-induced approach avoids the use of inorganic, unstable and toxic reagents, simplifies sample preparation, makes possible Hg speciation as Hg^{2+} and CH_3Hg^+ by UV/Vis-PVG using a single reagent (HCOOH) and considerable decreases the amount of the resulted H_2 that could cause microplasma instability.[34,35] Figure 2.1 presents the scheme of a continuous flow photoreactor used in the determination and speciation of Hg.

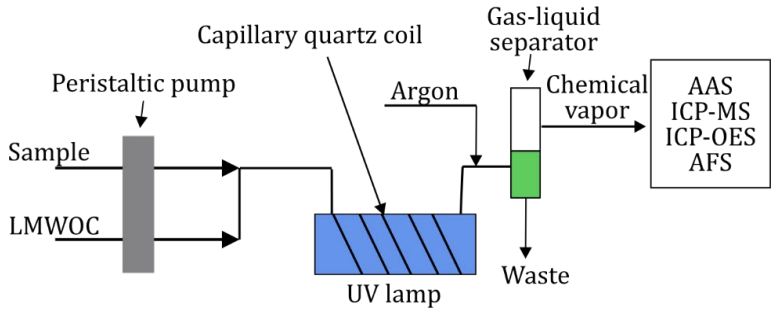


Figure 2.1. Scheme of a continuous flow photoreactor used in the determination and speciation of Hg[35]

PERSONAL CONTRIBUTIONS

3. Determination of CH_3Hg^+ in seafood by photo-induced cold vapor generation and detection by optical emission spectrometry in capacitively coupled plasma microtorch

3.1. Status at international level. Working hypotheses and objectives.

Among Hg species, the organic forms, such as methylmercury (CH_3Hg^+), exhibit the highest toxicity to the human body, with long-lasting harmful effect on the nervous, cardiovascular and immune systems.[37] In a study conducted by the European Food and Safety Authority (EFSA) in 20 European countries over the period 2004–2011, it was highlighted that the exposure of the population to Hg occurs primarily through fish consumption, in which more than 75% of total Hg is present as CH_3Hg^+.[14] However, the European Commission does not distinguish between the organic and inorganic Hg species in their Decisions[18], although it is much more important to know the content of CH_3Hg^+. Only PTWI values are established for CH_3Hg^+ and Hg^{2+} by WHO [38] and EFSA[14].

The determination of Hg species by non-chromatographic methods is usually achieved by a double liquid-liquid extraction and/or selective derivatization to cold vapor using harmful, corrosive and unstable reagents, such as $SnCl_2$ or $NaBH_4$. For this reason, there has been an increasing interest for new methods for sample preparation and Hg determination in line with the Green Analytical Chemistry (GAC) principles,[2] based on ultrasound,[39] UV[40] or microwave[41] assisted derivatization in the presence of formic acid or acetic acid [42] and detection using microplasma sources, such as PD-OES[43] or μCMP-OES[44]. In 2011 the European Commission organized an interlaboratory study aimed at developing methods for the determination of several problematic contaminants in food, including arsenic and methylmercury.[45] Unfortunately, only eight laboratories from the European Union participated in this study, most of them refusing the invitation, wrongly considering that the determination of CH_3Hg^+ requires a complicated sample preparation for routine analysis laboratories, as well as sophisticated instrumentation.[45] Based on these hypotheses, the general objective of the study was the development of a non-chromatographic method for the determination of CH_3Hg^+ in seafood using UV photo-induced derivatization and detection by optical emission spectrometry in a capacitively coupled plasma microtorch of low power and low Ar consumption (UV-PVG-μCCP-OES). Sample preparation was performed in accordance with the Standard Operation Procedure (SOP),[46] proposed by the European Commission for the determination of CH_3Hg^+ by TD-AAS. Compared to this procedure, the novelty of the study consists in the eco-scale UV photo-induced derivatization of the CH_3Hg^+ species in HCOOH medium and detection by μCCP-OES using a low resolution microspectrometer, with low operating cost.

3.2. Sample preparation for the determination of CH_3Hg^+ by UV-PVG-µCCP-OES

The preparation of CRMs (4 samples) and of the freeze-dried fish fillet test samples (12 samples) was performed in agreement with the SOP,[46] proposed by the European Commission (Figure 3.1). The extractions were carried out in 50 mL plastic vials using 200 mg sample moistened with 0.5 mL ultrapure water, then 10 mL of 47% (w/w) HBr were added and the vials were manually shaken for 2–3 min. The CH_3Hg^+ species captured in HBr was further successively reextracted in 20 mL and 15 mL toluene, respectively, by vigorous shaking for 10 min. The two fractions were separated by centrifugation, while the supernatants were transferred into a centrifuge tube containing 6 mL of 1% (w/v) L-cysteine solution. Re-extraction of CH_3Hg^+ from the organic phase into the aqueous solution of L-cysteine was carried out by vigorous shaking in a vortex system. The aqueous extract was transferred with a Pasteur pipette in a glass vial with cap. Along with the samples, blank solutions were prepared following the same procedure. For the determination of CH_3Hg^+ by UV-PVG-µCCP-OES, aliquot volumes of 1–5 mL aqueous extracts were diluted to 50 mL to reach a final concentration of 0.6 mol L^{-1} HCOOH. Determinations by TD-AAS were performed directly on the L-cysteine aqueous extracts.

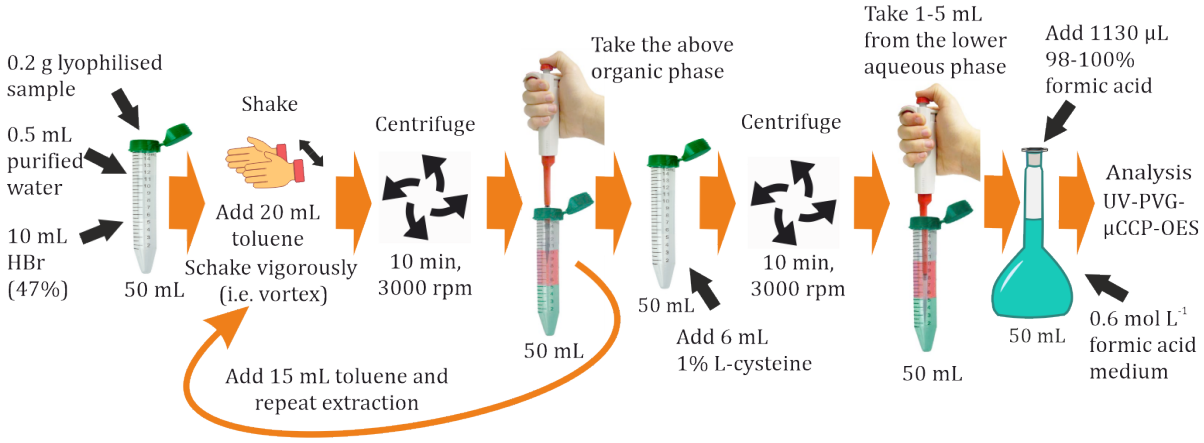


Figure 3.1. Procedure for the preparation of fish fillet samples by double liquid-liquid extraction for the determination of CH_3Hg^+ by UV-PVG-µCCP-OES[46,47]

3.3. UV-PVG-µCCP-OES instrumentation

The set-up of the UV-PVG-µCCP-OES experimental system is presented in Figure 3.2, while the operating conditions in Table 3.1. The photoreactor (Babeş-Bolyai University, Cluj-Napoca, Romania) consisted of a PTFE tube (1 mm i.d. x 1.5 mm o.d. x 120 cm length, cut-off 185 nm), wrapped around the quartz tube of the UV 705 Digester Metrohm (Herisau, Switzerland) equipped with a high-pressure Hg lamp (500 W). The sample was pumped through the photoreactor in the gas-liquid separator (Babeş-Bolyai University, Cluj-Napoca, Romania) by the peristaltic pump MasterFlex L/S Model 7535-04 Cole Parmer (Montreal, Canada). Mercury vapor was purged from the solution by an Ar stream (Ar 5.0, Linde, Cluj-Napoca, Romania) and

introduced into the plasma microtorch through 4 holes with 75 µm in diameter. The microtorch consisted of a quartz tube (5 mm i.d., 1.25 mm wall thickness, 25 mm length, cut-off 160 nm) placed in a PTFE support, in the center of which there was the Mo tip electrode (1.25 mm diameter) for RF power coupling.[47]

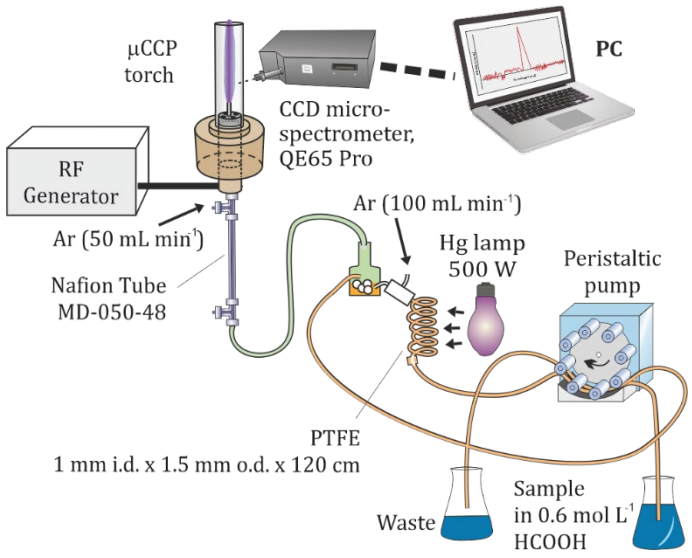


Figure 3.2. Experimental set-up of the UV-PVG-µCCP-OES system used for the determination of CH_3Hg^+ [47]

A low power radio frequency generator (10-30 W, 13.56 MHz, 15 x 17 x24 cm^3, Technical University, Cluj-Napoca, Romania) was used for plasma generation. The microplasma developed as a diffuse blue discharge at the tip of the Mo microelectrode. Drying of the Ar stream containing the mercury vapor purged from the liquid sample was achieved with a Nafion Perma Pure MD-050-48 membrane (120 cm length, Chromoservis, Praha, Czech Republic) interposed between the gas-liquid separator and the plasma microtorch, with an efficiency of up to 90% (Dew point -8 ° C). Drying was necessary to remove water vapor, which had a negative influence on the stability of the low power discharge and efficiency of Hg atoms excitation. The Ocean Optics QE65 Pro microspectrometer (190-380 nm spectral range, 0.4 nm FHWM, Dunedin, USA), equipped with a Peltier cooling system (-20 ° C) was used to record plasma spectrum and Hg emission at 253.652 nm. The emission signal observed radially through a 10 mm focal lens was recorded using the SpectraSuite software of the microspectrometer. The determination of CH_3Hg^+ by UV-PVG-µCCP-OES was performed using external calibration with Hg^{2+} standard solutions over the range 0–5 µg L^{-1} Hg^{2+} in 0.6 mol L^{-1} HCOOH (n=7 standards). Between-sample memory effect was removed by pumping the 0.6 mol L^{-1} HCOOH blank solution through the photoreactor for 40 s.

The results obtained by UV-PVG-µCCP-OES were compared with those of the TD-AAS method, recommended by the European Commission for the determination of CH_3Hg^+, directly in the 1% L-cysteine aqueous extract.[46] The measurements by TD-AAS were performed on solid and

liquid samples using Hydra IIC-Mercury Analyzer, Teledyne Instruments (Leeman Labs, Hudson, New Hampshire, USA).

Table 3.1. Working conditions for the UV-PVG-μCCP-OES set-up used for the determination of CH_3Hg^+ by external calibration[47]

Photo-induced cold vapor generation of Hg	
Concentration of HCOOH (mol L^{-1})	0–1.4; optimal 0.6
UV irradiation time (s)	3–33; optimal 5
Sample uptake (mL min^{-1})	1.5–13.5; optimal 10
Plasma generation	
Ar flow rate (mL min^{-1})	50–200; optimal 100
Power (W)	10–20; optimal 15
Hg emission signal measurement	
Wavelength (nm)	253.652
Signal processing	Peak height
Integration time (s)	10
Observation height in plasma (mm)*	0–2.6, optimal 1.6
Background correction	Linear, two-point

3.4. Optimization of the UV-PVG-μCCP-OES analytical system

The working conditions for the UV-PVG-μCCP-OES system were optimized to achieve the best sensitivity of the method. Thus, the conditions for the photo-induced derivatization of the CH_3Hg^+ species to Hg vapor were taken into account, such as the formic acid concentration, time of sample UV-light irradiation, as well as plasma operating conditions, such as operation power, Ar flow rate and observation height.[47]

The influence of the HCOOH concentration on the Hg emission following cold vapor generation by 1.00 ± 0.03 μg L^{-1} Hg^{2+} and 1.00 ± 0.10 μg L^{-1} Hg^{2+} as CH_3Hg^+ extracted from DOLT-4 CRM was investigated over the range 0–1.4 mol L^{-1}. It was observed that cold vapor generation occurred with similar efficiency for Hg^{2+} and CH_3Hg^+ species, regardless of the HCOOH concentration, thus making it possible the derivatization of both species in the presence of the UV light by photochemical reactions.[48-50] As a results, the determination of CH_3Hg^+ by the UV-PVG-μCCP-OES method is achievable using external calibration with Hg^{2+} standard solutions in an optimal concentration of 0.6 mol L^{-1} HCOOH.[47]

The photolysis of the formic acid under UV-light irradiation occurs according to the following reactions:[48]

$$HCOOH \xrightarrow{h\nu} HCO^{\bullet} + {}^{\bullet}OH \quad (3.1)$$

$$HCOOH \xrightarrow{h\nu} {}^{\bullet}H + {}^{\bullet}COOH \quad (3.2)$$

$$HCO^{\bullet} + {}^{\bullet}OH \xrightarrow{h\nu} CO^{\bullet} + H_2O \quad (3.3)$$

The sequence of reactions for cold vapor generation from CH_3Hg^+ in the presence of HCOOH is:

$$CH_3Hg^+ + HCOOH \xrightarrow{h\nu} CH_3HgCOOH + H^\bullet \xrightarrow{h\nu} Hg^{2+} + HCOO^\bullet + {}^\bullet CH_3 + {}^\bullet H \xrightarrow{h\nu} Hg^0 + CH_4 + CO_2 + 1/_2H_2 \quad (3.4)$$

The overall cold vapor generation reaction is:

$$CH_3Hg^+ + HCOOH \xrightarrow[+1e^-]{h\nu} Hg^0 + CH_4 + CO_2 + 1/_2H_2 \quad (3.5)$$

The proposed reactions for the cold vapor generation from Hg^{2+} species in formic acid under UV-irradiation are:[48]

$$Hg^{2+} + 2\,{}^\bullet COOH \xrightarrow{h\nu} Hg^0 + 2CO_2 + H_2 \quad (3.6)$$

$$Hg^{2+} + 2\,HCO^\bullet \xrightarrow{h\nu} Hg^0 + 2CO + H_2 \quad (3.7)$$

The overall cold vapor generation reaction is:

$$Hg^{2+} + HCOOH + 2e^- \xrightarrow{h\nu} Hg^0 + CO_2 + H_2 \quad (3.8)$$

According to the thermodynamic data from the literature, it was highlighted that the photo-induced derivatization reaction of CH_3Hg^+ species in HCOOH medium is exothermic and spontaneous at 298 °K (25 °C).[48-50] Also, the Gibbs free energies for the two reactions in the presence of formic acid are similar (-204.5 kJ mol^{-1} for Hg^{2+}, eq. 3.8 and -206.4 kJ mol^{-1} for CH_3Hg^+, eq.3.5), which leads to the idea that both reactions occur at similar rates under similar derivatization conditions.

The occurrence of the Hg emission signal even in the absence of formic acid is the result of water molecule photolysis under UV-irradiation generating ·OH and ·H radicals, able to accomplish the derivatization of Hg^{2+} and CH_3Hg^+ species to cold vapor.[49] The corresponding reactions are:

$$H_2O \xrightarrow{h\nu} {}^\bullet OH + {}^\bullet H \quad (3.9)$$

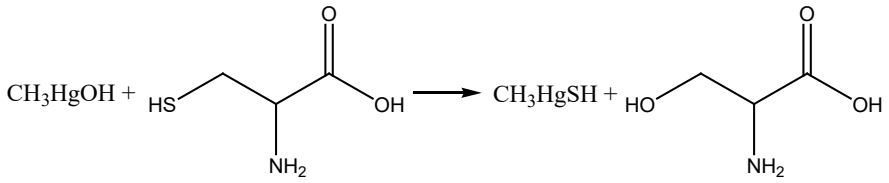
$$Hg^{2+} + 2\,{}^\bullet H \xrightarrow{h\nu} Hg^0 + H_2 \quad (3.10)$$

$$Hg^{2+} + 2\,{}^\bullet OH \xrightarrow{h\nu} Hg^0 + H_2O_2 \quad (3.11)$$

The overall reaction is:

$$2Hg^{2+} + 2H_2O + 4e^- \xrightarrow{h\nu} 2Hg^0 + H_2O_2 + H_2 \quad (3.12)$$

According to the stability constant of CH_3Hg^+ in aqueous solution[50], these species occur in the CH_3HgOH hydrolyzed form, while in the presence of L-cysteine (2-amino-3-sulfhydrylpropanoic acid) the following ion exchange reaction takes place:



(3.13)

This chemical reaction is consistent with the higher stability of CH_3HgSH species, compared to CH_3HgOH, suggesting that the use of L-cysteine reagent for extraction in aqueous solution is due to the increased stability of the CH_3Hg^+ species, which increases the yield of its extraction in toluene.

The UV-light irradiation time was optimized based on the emission signals obtained from a Hg^{2+} standard solution of 1.00 ± 0.03 μg L^{-1} and a solution containing 100 ± 0.10 μg L^{-1} Hg^{2+} as CH_3Hg^+ coming from CRM DOLT-4. It was observed an exponential decrease of the emission signal with the increase of the irradiation time for both Hg species. An irradiation time of 5 s was found to be optimal for an efficient derivatization of both Hg species. The very short sample exposure to UV light prevented the loss of Hg vapor by reabsorption in the sample stream or adsorption on the PTFE capillary walls. At the same time, the short irradiation time is a clear advantage of the UV-PVG-μCCP-OES method, as it ensures an easy continuous flow derivatization and measurement of the Hg emission, with benefits in terms of sample throughput and repeatability, as well as sensitivity of the developed analytical method.[47]

The optimization study of the microplasma parameters indicated an optimal 100 mL min^{-1} Ar flow rate, 15 W plasma power and 1.6 mm observation height above the tip electrode. These working conditions provided maximum Hg emission signals and best LOD for CH_3Hg^+.[47]

3.5. Characterization and validation of the UV-PVG-μCCP-OES method by analyzing CRMs and evaluation of the analytical performance

The UV-PVG-μCCP-OES method was characterized in terms of analytical performance, namely LOD, LOQ, precision and accuracy.[51] According to the results presented in Table 3.2, LOD for CH_3Hg^+ by UV-PVG-μCCP-OES was 2 μg kg^{-1} in solid, about 2 times better than by TD-AAS of 5 μg kg^{-1}. Because the LOD and LOQ for CH_3Hg^+ by UV-PVG-μCCP-OES were 250, and 85 times, respectively, below the maximum admitted value for Hg in fish fillet (0,5 mg kg^{-1}),[18] the developed method fulfills the demands of the European Commission in the Decision 2007/333/CE[52] regarding the determination of CH_3Hg^+ in seafood.

Table 3.3 presents the results obtained for the accuracy of the UV-PVG-μCCP-OES method, evaluated based on certified reference materials (CRMs) analysis, in comparison with those obtained by the traditional TD-AAS method. Recoveries of CH_3Hg^+ obtained by UV-PVG-μCCP-OES were in the range 97–102%, fulfilling the requirement in the Decision 2002/657/CE[53] to be 90–110%, for 95% confidence level. Thus, it can be stated that the UV-PVG-μCCP-OES method does not show systematic error in the determination of CH_3Hg^+.

The accuracy of the UV-PVG-μCCP-OES method was evaluated by analyzing CRMs and the results were compared with those provided by the traditional TD-AAS. Data are presented in Table 3.3. Recovery in the UV-PVG-μCCP-OES method ranged between 97–102%, fulfilling the requirement in the Decision 2002/657/CE[53] to be 90–110%, for 95% confidence level. Thus, it can be stated that the UV-PVG-μCCP-OES method does not show systematic error in the determination of CH_3Hg^+.

Table 3.2. Characteristics of the calibration curves, LOD and LOQ for CH_3Hg^+ by UV-PVG-μCCP-OES and TD-AAS [47]

	Method	
	UV-PVG-μCCP-OES	**TD-AAS**
Calibration range	0–5 (μg L^{-1}) (n=7)	0–0.025 (μg) (n=5)
Slope of the calibration curve	2150 ± 9 (peak height/μg L^{-1})	11780 ± 81 (peak height/μg)
Intercept	3.5	8640
Analytical sensitivity (γ)[a]	238	145
$s_{y/x}$[b]	6.2	3005
Correlation coefficient (r)	0.9999	0.9999
LOD[c] (ng L^{-1})	7	-
(μg kg^{-1})	2[d]	5[e]
LOQ[f] (μg kg^{-1})	6	10

[a] Analytical sensitivity expressed as the ratio *Slope of the calibration curve/Standard deviation of slope*; [b] $s_{y/x}$ is the residual standard deviation; [c] Limit of detection (LOD)in liquid; [d] LOD in solid sample by UV-PVG-μCCP-OES calculated for 200 mg sample subjected to extraction and dilution of 5 mL aqueous extract to 50 mL; [e] LOD by TD-AAS calculated for an aliquot of 200 μL solution and 200 mg sample extracted in 6 mL 1% (w/v) L-cysteine; [f] LOQ in solid sample considered as 3xLOD.

The selectivity of the UV-PVG-μCCP-OES method was checked through recovery of CH_3Hg^+ after 10-50-fold dilution of the sample and quantification of concomitants, namely anions (Br^-, Cl^-, NO_3^-, PO_4^{3-}, SO_4^{2-}, F^-), metals (Na, K, Ca, Mg, Fe, Cu, Zn, Cr) and total organic carbon (TOC). The results indicated that the presence of anions in the range 0.1–5.2 mg L^{-1}, metals within <LOD – 0.6 mg L^{-1} and TOC of 0.12–0.65 mg L^{-1} did not cause non-spectral interference in the determination of CH_3Hg^+.[47] Anions quantification was performed with the 761 Compact Ion Chromatograph (Metrohm, Herisau, Switzerland*),* metals were determined by ICP-OES using the Spectro CIROS^{CCD} spectrometer with axial viewing (Kleve, Germany), while TOC with the Multi N/C2100S Analyzer (Analytik Jena, Jena, Germany).

Table 3.3. Results (mg kg^{-1}) obtained for CH_3Hg^+ in CRMs by UV-PVG-μCCP-OES and TD-AAS [47]

CRM	Certified value ± U[a]	Found Value ± C.I.[b,c]		Recovery ± C.I.[c]	
		UV-PVG-μCCP-OES	TD-AAS	UV-PVG-μCCP-OES	TD-AAS
TORT-2	0.152 ± 0.013	0.152 ± 0.014	0.152 ± 0.015	100 ± 9	100 ± 10
DOLT-4	1.33 ± 0.12	1.34 ± 0.13	1.36 ± 0.14	101 ± 10	102 ± 10
BCR-463	3.04 ± 0.16	3.03 ± 0.22	2.97 ± 0.26	100 ± 7	98 ± 9
ERM-CE464	5.50 ± 0.17	5.36 ± 0.33	5.38 ± 0.35	97 ± 6	98 ± 7
Pooled recovery ± C.I.[d]				99 ± 8	99 ± 9

[a] U is expanded uncertainty; [b] C.I. is the confidence interval for 95% confidence level; [c] n = 5 complete extractions for each sample; [d] C.I. is the pooled recovery for the CRMs

3.6. Use of the UV-PVG-μCCP-OES method for the determination of CH_3Hg^+ in real samples of fish fillet

Table 3.4. presents the concentration of CH_3Hg^+ in real samples of fish fillet analyzed by UV-PVG-μCCP-OES and the corresponding results achieved by TD-AAS. The found values were in the range 0.044–0.208 mg kg^{-1} CH_3Hg^+ by UV-PVG-μCCP-OES and 0.041–0.202 mg kg^{-1} by TD-AAS. The maximum tolerance limit of total Hg in fishery products in Europe (0.5 mg kg^{-1}) was not exceeded in any of the examined samples.[18]

Table 3.4. Results (mg kg^{-1}) obtained for CH_3Hg^+ in fish fillet by UV-PVG-μCCP-OES and TD-AAS [47]

Sample	UV-PVG-μCCP-OES			TD-AAS		
	Mean ± C.I.[a]	RSD[b] (%)	HorRat[c]	Mean ± C.I.	RSD (%)	HorRat
1	0.176 ± 0.017	4.9	0.26	0.165 ± 0.018	5.5	0.29
2	0.208 ± 0.019	4.6	0.24	0.198 ± 0.021	5.2	0.27
3	0.129 ± 0.020	7.9	0.42	0.121 ± 0.021	8.6	0.45
4	0.195 ± 0.021	5.4	0.28	0.202 ± 0.022	5.5	0.29
5	0.097 ± 0.018	9.4	0.49	0.089 ± 0.019	10.4	0.55
6	0.115 ± 0.016	7.2	0.38	0.120 ± 0.017	7.1	0.37
7	0.154 ± 0.025	8.0	0.42	0.155 ± 0.021	6.9	0.36
8	0.100 ± 0.008	4.0	0.21	0.097 ± 0.008	4.0	0.21
9	0.044 ± 0.003	3.9	0.21	0.041 ± 0.005	6.4	0.34
10	0.117 ± 0.007	2.8	0.15	0.118 ± 0.009	3.7	0.19
11	0.099 ± 0.005	2.7	0.14	0.097 ± 0.007	3.5	0.18
12	0.172 ± 0.017	4.8	0.25	0.169 ± 0.025	7.5	0.39
Minimum	0.044 ± 0.003	2.7	0.14	0.041 ± 0.005	3.5	0.18
Maximum	0.208 ± 0.019	9.4	0.49	0.202 ± 0.022	10.4	0.55
Mean	0.134 ± 0.016	5.5	0.29	0.131 ± 0.017	6.2	0.32

[a] C.I. is the confidence interval for 95% confidence level and k=2 (5 complete extractions); [b] RSD (%) is the relative standard deviation; [c] HorRat index for 19% PRSD

The precision of the method from repeated measurements of test samples expressed as RSD was in the range 2.7–9.4% (Table 3.4) and complied with the provision established by the Association of Official Analytical Chemists (AOAC)[54] and European Commission in the Decision 2007/333/EC[52].

The Bland and Altman analysis is summarized in Table 3.5. The results obtained by UV-PVG-μCCP-OES and TD-AAS did not differ significantly and the differences fall in within the limits of confidence of 0.026–(-0.020) mg kg^{-1} CH_3Hg^+. The slight positive bias (0.003 ± 0.007 mg kg^{-1}) towards the UV-PVG-μCCP-OES method was random, since the confidence interval included the zero value, and its level was much lower than the concentration of CH_3Hg^+ in the fish fillet. The within- and between-method standard deviations demonstrated good repeatability of measurements, so that the differences were considered random. In conclusion, the Bland and Altman test proves that the UV-PVG-μCCP-OES method using external calibration with Hg^{2+} standards is able to provide the accurate measurement of CH_3Hg^+ in seafood, comparable to TD-AAS.

Table 3.5. Results obtained in the Bland and Altman test for the determination of CH_3Hg^+ (m = 5 parallel measurements for each sample) in fish fillet for 95% confidence level [47]

Concentration range for Hg as CH_3Hg^+ (mg kg^{-1})	**Sample size**	**$s_{UV\text{-}PVG\text{-}\mu CCP\text{-}OES}$[a] (mg kg^{-1})**	**$s_{TD\text{-}AAS}$[a] (mg kg^{-1})**	**$s_{B_{X-Y}}$[b] (mg kg^{-1})**	**Bias[c] (mg kg^{-1})**	**Limits of agreement[d] (mg kg^{-1})**
0.043–0,203	12	0.008	0.008	0.012	0.003 ± 0.007	0.026 ± 0.008 -0.020 ± 0.008

[a] Within-method standard deviation for UV-PVG-μCCP-OES and TD-AAS.
[b] Between method standard deviation.
[c] Systematic error (bias) for 95% confidence level.

3.7. Conclusions

The results obtained from the development of the UV-PVG-μCCP-OES method for the determination of CH_3Hg^+ in fish fillet led to the following conclusions:

1. The UV-PVG-μCCP-OES method meets the requirements of the European Commission in terms of analytical performance (LOD, LOQ, accuracy, precision) for the determination of Hg in seafood;
2. The figures of merit of the UV-PVG-μCCP-OES method are similar to those of the TD-AAS method recommended by EC; the Bland and Altman statistics showed that there is no significant difference between them and, consequently, UV-PVG-μCCP-OES could be considered an alternative to TD-AAS;
3. The UV-PVG-μCCP-OES method is sensitive and can be considered eco-friendly due to the simple sample preparation procedure, without additional oxidation of the CH_3Hg^+ species, avoidance of expensive and unstable derivatization reagents and the use of fully miniaturized instrumentation with low energy and argon consumption;
4. The instantaneous derivatization to cold vapor under UV-light irradiation with similar efficiency for Hg^{2+} and CH_3Hg^+ led to the development of an on-line method for the

determination of CH_3Hg^+ using external calibration with Hg^{2+} standard solutions without non-spectral interferences;

5. The analytical approach represents an advance for CH_3Hg^+ quantification in terms of sample preparation, and determination using the photo-induced derivatization and external calibration with Hg^{2+} standards; consequently, the UV-PVG-μCCP-OES method could be considered innovative in line with the principles of the green atomic spectrometry.

4. Speciation of Hg as CH_3Hg^+ and Hg^{2+} in seafood by classical and photo-induced cold vapor generation and optical emission spectrometry in capacitively coupled plasma microtorch

4.1. Status at international level. Working hypothesis and objectives

The data collected by EFSA regarding the presence of Hg in food and feed marketed in 20 European countries during 2004-2011 showed that: (1) the main source of human exposure to Hg is seafood, which represents between 17.6–36.8% in human nutrition; (2) more than 98% of the data collected by EFSA refer to total Hg in such foods, and less than 2% to Hg speciation as CH_3Hg^+ and Hg^{2+}.[14] In another study EFSA addressed the risk of exposure to Hg together with the benefits of the long-chain polyunsaturated (n-3) fatty acids (omega 3 and omega 6) by fish consumption. It has been shown that 1–4 servings of fish per week, depending of their variety, do not pose any risk of exposure to Hg when its concentration is below the maximum level of 0.5 mg kg^{-1} Hg.[18] At the same time, fish consumption has beneficial effects on the cardiovascular system.[20]

As shown in the study of the European Commission mentioned in the previous chapter, many laboratories consider the determination and speciation of Hg quite complicated because of the laborious sample preparation and sophisticated instrumentation required for the detection.[45] On the other hand, the determination of total Hg/CH_3Hg+ in seafood, and not only, is essential in order to establish the maximum admitted level for CH_3Hg^+ from that of total Hg. To this end, the development of new, simpler, not expensive and affordable methods is of great interest and encouraged by the European Commission. Based on these considerations, the main objective of the present study was to develop a method for Hg speciation as CH_3Hg^+ and Hg^{2+} in fish fillet using fully miniaturized instrumentation with capacitively coupled plasma microtorch and detection by optical emission spectrometry. Mercury speciation consisted in: (i) Determination of the total Hg in samples digested in HNO_3 and H_2O_2 and cold vapor generation with 20% (w/v) $SnCl_2$ in 15% (v/v) HCl; (ii) Determination of the CH_3Hg^+ species by UV-photo-chemical vapor generation in 0.6 mol L^{-1} HCOOH after selective liquid-liquid extraction in the system HBr–toluene–L-cysteine solution, and (iii) Calculation of the concentration of Hg^{2+} species as the difference between total Hg and CH_3Hg^+. The novelty consists in the development and validation of a simple method for Hg speciation based on optical emission spectrometry in a capacitively coupled microplasma equipped with a low resolution microspectrometer, which offers economic benefits through low consumption of energy and Ar for plasma support.

4.2. Sample preparation for Hg speciation as CH_3Hg^+ and Hg^{2+} in seafood by $SnCl_2$-CV-µCCP-OES and UV-PVG-µCCP-OES

The analyzed samples were 4 CRMs for checking the accuracy of the $SnCl_2$-CV-µCCP–OES and UV-PVG-µCCP-OES methods, and 15 test samples of fish fillet, of different varieties (8 wild species and 7 farmed species). For the determination of total Hg, samples were mineralized in a mixture of 8 mL HNO_3 and 2 mL H_2O_2 in the Berghof MWS3+ digester (Berghof, Germania), while the CH_3Hg^+ determination was performed after double liquid-liquid extraction in the system HBr–toluene–L-cysteine, presented in Chapter 3, section 3.2. The total fat content in fish fillet was determined according to the gravimetric method after Soxhlet extraction as described in SR ISO 1443:2008.[55]

4.3. The $SnCl_2$-CV-µCCP-OES and UV-PVG-µCCP-OES instrumentation

The components of the instrumentation used for Hg speciation were similar to those presented in Chapter 3, section 3.3, with the following difference. The capacitively coupled plasma microtorch was interfaced both with the continuous flow generation system HGX-200 for the determination of total Hg (Fig. 4.1, left) and the photoreactor presented in Chapter 3 (Fig. 4.1, right) for the quantification of CH_3Hg^+.

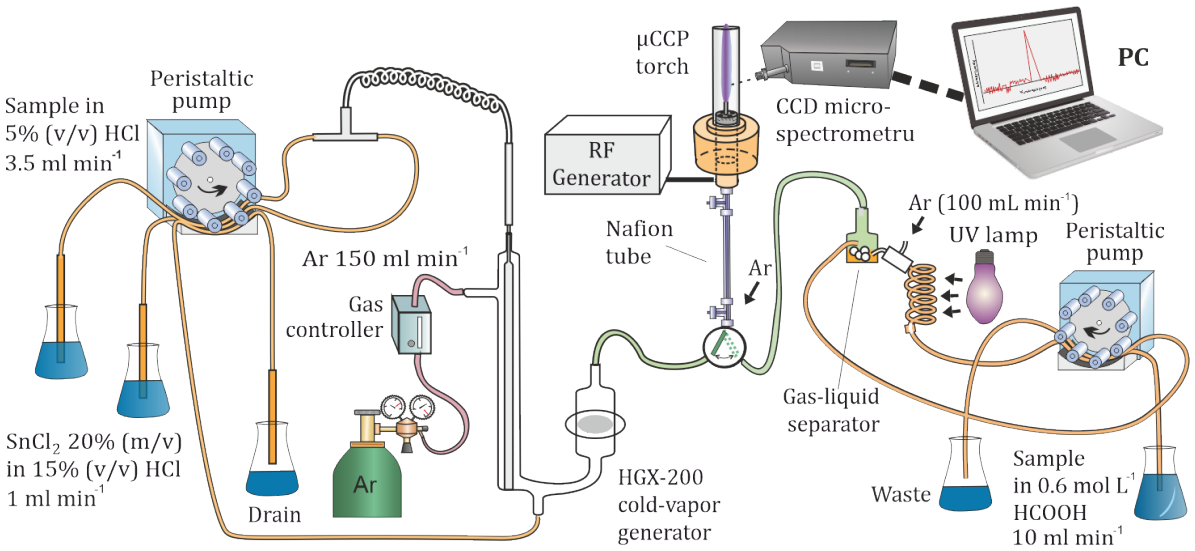


Figure 4.1. Scheme of the experimental model $SnCl_2$-CV-µCCP-OES (left)/UV-PVG-µCCP-OES (right) used for the speciation of Hg as CH_3Hg^+ and Hg^{2+} [56]

The introduction of standards/samples into the hydride generator HGX-200/photoreactor was performed using the peristaltic pump MasterFlex L/S (Model 7535-04 Cole Palmer, Montreal, Canada). The hydride generator HGX-200 (Teledyne Cetac Technologies, Nebraska, SUA) achieved cold vapor generation by mixing the continuous sample stream with the derivatization reagent, a solution of 20% (w/v) $SnCl_2$ in 15% (v/v) HCl. Mercury vapor was separated in the gas-liquid

separator and introduced into the plasma with an Ar flow of 150 mL min^{-1} through a Nafion membrane tubing needed for drying. The emission signal corresponding to total Hg was measured at 253.652 nm using the Ocean Optics QE65 Pro microspectrometer. The optimal working conditions for the quantification of total Hg by $SnCl_2$-CV-μCCP-OES and CH_3Hg^+ by UV-PVG-μCCP-OES are provided in Table 4.1.

Table 4.1. Optimal working conditions for the determination of total Hg in seafood by $SnCl_2$-CV-μCCP-OES and CH_3Hg^+ by UV-PVG-μCCP-OES[56]

	Total Hg $SnCl_2$-CV-μCCP-OES	**CH_3Hg^+ UV-PVG-μCCP-OES**
Cold vapor generation system	HGX - 200	Photoreactor, PTFE tube (1 mm i.d. x 1.5 mm o.d. x 120 cm length) wrapped around the quartz tube of the UV 705 digester equipped with a 500 W Hg lamp (Metrohm, Switzerland)
Medium for sample/CRM/standards	5% (v/v) HCl	0.6 mol L^{-1} HCOOH
Blank	5% (v/v) HCl	0.6 mol L^{-1} HCOOH
Derivatization reagent	20% (w/v) $SnCl_2$ in 15% (v/v) HCl	0.6 mol L^{-1} HCOOH
Sample flow rate (mL min^{-1})	3.5	10
Derivatization reagent flow rate (mL min^{-1})	1	-
UV irradiation time (s)	-	5
Plasma power (W)	10	15
Ar flow rate (mL min^{-1})	150	100
Observation height in plasma (mm)	1.8	1.6
Wavelength (nm)	253.652	253.652
Signal processing	Peak height	Peak height
Integration time (s)	10	10
Background correction	Linear, two-point model	Linear, two-point

4.4. Characterization and validation of the UV-PVG-μCCP-OES method by CRMs analysis and evaluation of the analytical performance

LOD and LOQ for CH_3Hg^+ and total Hg in fish by UV-PVG-μCCP-OES and $SnCl_2$-CV-μCCP-OES are presented in Table 4.2. The LODs are 166 and 250 times respectively, lower than the maximum level of total Hg in fish fillet (0.5 mg kg^{-1} according to the European Commission, 2006/1881/EC[18]), while LOQs are 55 and 83 times respectively, lower, so that the new method for Hg speciation meets the requirements of the Decision 2007/333/CE[52] and could be used successfully for Hg speciation in seafood.

Table 4.2. Limit of detection (LOD) and limit of quantification (LOQ) in seafood achieved for CH_3Hg^+ and total Hg by UV-PVG-μCCP-OES and $SnCl_2$-CV-μCCP-OES[56]

Method (Species)	Calibration range (μg L^{-1})[a]	Correlation coefficient (r)	LOD (μg kg^{-1})	LOQ (μg kg^{-1})
UV-PVG-μCCP-OES (Hg as CH_3Hg^+)	0–5	0.9999	2[b]	6
$SnCl_2$-CV-μCCP-OES (total Hg)	0–5	0.9999	3[c]	9

[a] n=7 calibration Hg^{2+} standard solutions;
[b] LOD in solid sample calculated for 200 mg sample subjected to extraction and diluted 1:10;
[c] LOD in solid sample calculated for 200 mg sample subjected to extraction and diluted to 50 mL.

The results on the accuracy of the speciation method based on μCCP-OES by analyzing CRMs are given in Table 4.3. Recovery values of total Hg, CH_3Hg^+ and Hg^{2+} were in the range 100 ± 10%, 100 ± 8% and 102 ± 13% respectively, so that the proposed method complies with the Decision 2002/657/CE[53] and does not show systematic errors.

Table 4.3. Results obtained for Hg speciation in CRMs using $SnCl_2$-CV-μCCP-OES and UV-PVG-μCCP-OES[56]

CRM	Certified value ± C.I.[a] (mg kg^{-1})			Found value ± C.I.[a,b] (mg kg^{-1})			Recovery ± C.I.[a,b] (%)		
	Total Hg	CH_3Hg^+	Hg^{2+}	Total Hg	CH_3Hg^+	Hg^{2+}	Total Hg	CH_3Hg^+	Hg^{2+}
DOLT-4 [e]	2.58 ± 0.22	1.33 ± 0.12[c]	1.25 ± 0.16	2.58 ± 0.08	1.34 ± 0.13	1.25 ± 0.13	100 ± 3	101 ± 10	100 ± 10
BCR-463 [f]	2.85 ± 0.16	3.04 ± 0.16[d]	0.02 ± 0.002	2.84 ± 0.15	3.03 ± 0.22	0.02 ± 0.002	99 ± 5	100 ± 7	100 ± 10
ERM CE-464 [f]	5.24 ± 0.10	5.50 ± 0.17[d]	0.12 ± 0.004	5.23 ± 0.25	5.49 ± 0.33	0.12 ± 0.009	100 ± 5	99 ± 6	100 ± 8
Tort-2 [g]	0.27 ± 0.06	0.152 ± 0.013[c]	0.118 ± 0.028	0.28 ± 0.05	0.152 ± 0.014	0.128 ± 0.026	104 ± 18	100 ± 9	108 ± 20
Pooled recovery ± C.I.							101 ± 10	100 ± 8	102 ± 13

[a] C.I. – the confidence interval for 95% confidence level; [b] m=5 extractions/analysis for each sample; [c] Content expresses as Hg; [d] Content expressed as CH_3Hg^+; [e] Shark liver; [f] Tuna fish; [g] Lobster hepatopancreas.

4.5. Use of the speciation method in the analysis of real samples of fish fillet

The results in Table 4.4 for Hg speciation in fish fillet indicated that the total Hg in the analyzed samples was below the maximum level of 0.5 mg kg^{-1} set by the EC (Decision 2006/1881/EC)[18], and from this point of view there is no risk of exposure for fish consumers. Concentrations ranged between 0.094–0.308 mg kg^{-1} total Hg, 0.021–0.259 mg kg^{-1} CH_3Hg^+, and 0.011–0.136 mg kg^{-1} Hg^{2+} species. The highest concentrations of total Hg and CH_3Hg^+ were found in oceanic fish (Atlantic cod, Atlantic mackerel), while the lowest in farmed fish (trout, carp, Alaska salmon and Atlantic salmon). In terms of species distribution, the CH_3Hg^+ form accounted for an average of 69.3% of the total Hg. In the same time the CH_3Hg^+ species was found to be dominant

in oceanic fish, while Hg^{2+} species prevailed in farmed fish. According to data in the Table 4.4 no correlation was found between total fat content and content of the CH_3Hg^+ species or its weight in total.

The precision of the UV-PVG-μCCP-OES and $SnCl_2$-CV-μCCP-OES methods used for Hg speciation expressed as RSD from parallel measurements in the analysis of real samples of fish fillet was in the range 2.4–14.0% for total Hg, CH_3Hg^+ and Hg^{2+} and fulfilled the demands of AOAC[54]. The RSD corresponding to Hg^{2+} species was higher, since it was a cumulative value, calculated considering both precisions of total Hg quantification and CH_3Hg^+ respectively.

4.6. The risk exposure of the population to Hg via fish consumption

The assessment of the risk from exposure to Hg of the population via fish consumption based on the procedure recommended by EFSA, which takes into account the relatively weekly inputs of CH_3Hg^+ and Hg^{2+} compared to PTWI [14], has shown that there is no health risk for a 60 kg adult/15 kg child at a weekly consumption of a serving of 150 g/20 g, since the relative percentage input of CH_3Hg^+, Hg^{2+} and total Hg is below 60%. However, the exposure was higher in the case of oceanic fish consumption. Also, the exposure risk was higher to CH_3Hg^+ than Hg^{2+}, with relative input weights in the range 22–93% and 7–78% respectively for adults, and 2–27% and 0–5% in the case of children. Considering the relative percentages of Hg input by fish consumption and PTWI as 100%, it has resulted that an adult may consume 2–3 servings per week of 150 g oceanic fish (mackerel, hake, cod), 3–5 servings of salmon and 7 servings of farmed fish (carp, pangasius, trout). In the case of children, it has been estimated that they may consume 3-10 servings per week.

Exposure risk assessment based on the target hazard quotient (THQ) for a particular contaminant and the total THQ (TTHQ)[204] has indicated that there is no risk to human health in terms of non-carcinogenic diseases, since values were below 1.

It was also estimated the total Hg in blood and hair using the procedure recommended by EFSA[14] and compared with different threshold values above which different non-carcinogenic diseases may occur. The results did not show any risk of exposure to coronary heart disease (myocardial infarction), and neurological disease (dementia, impaired cognitive function, etc.) for the weekly consumption of 1–2 servings (1 serving around 150 g) of farmed fish (trout, pangasius, carp or salmon), since the estimated levels were 1.37–1.85 μg L^{-1} in blood and 0.34–0.46 mg kg^{-1} in hair, below the corresponding reference values of 2–3 μg L^{-1} and 0.5–0.7 mg kg^{-1}, respectively.[14]

Table 4.4. Results for the Hg speciation analysis in fish tissue by $SnCl_2$-CV-μCCP-OES and UV-PVG-μCCP-OES[56]

Fish species	Source [b]		Total fat (%)	Average concentration ± C.I.[a] / mg kg^{-1}			Weight/ %	
				Hg total	CH_3Hg^+	Hg^{2+}	CH_3Hg^+	Hg^{2+}
Atlantic Mackerel 1	Wild	FAO 27	6.12	0.293 ± 0.016	0.259 ± 0.014	0.034 ± 0.003	88	12
Atlantic Mackerel 2	Wild	FAO 27	4.94	0.152 ± 0.010	0.123 ± 0.006	0.029 ± 0.002	81	19
Pacific Hake 1	Wild	FAO 67	1.25	0.231 ± 0.015	0.201 ± 0.016	0.030 ± 0.003	87	13
Pacific Hake 2	Wild	FAO 67	0.72	0.197 ± 0.015	0.167 ± 0.017	0.030 ± 0.004	85	15
Atlantic Hake	Wild	FAO 34	0.24	0.149 ± 0.017	0.117 ± 0.007	0.032 ± 0.004	79	21
Atlantic Cod	Wild	FAO 34	1.78	0.308 ± 0.015	0.172 ± 0.017	0.136 ± 0.015	56	44
Alaska Pollock-Cod	Wild	FAO 61	1.62	0.177 ± 0.010	0.130 ± 0.008	0.047 ± 0.004	73	27
Alaska Salmon	Wild	FAO 67	2.82	0.165 ± 0.018	0.154 ± 0.025	0.011 ± 0.002	93	7
Atlantic Salmon 1	Ocean-farmed	FAO 27 Norway	1.94	0.126 ± 0.013	0.108 ± 0.012	0.018 ± 0.003	86	14
Atlantic Salmon 2	Ocean-farmed	FAO 27 Norway	1.85	0.116 ± 0.009	0.100 ± 0.008	0.016 ± 0.002	86	14
Carp	Farmed	Romania	14.06	0.110 ± 0.013	0.099 ± 0.005	0.011 ± 0.001	90	10
Pangasius 1	Farmed	Vietnam	2.81	0.119 ± 0.012	0.056 ± 0.008	0.063 ± 0.011	47	53
Pangasius 2	Farmed	Vietnam	3.14	0.127 ± 0.014	0.044 ± 0.003	0.083 ± 0.011	35	65
Trout 1	Farmed	Turkey	10.13	0.103 ± 0.016	0.030 ± 0.005	0.073 ± 0.017	29	71
Trout 2	Farmed	Romania	9.02	0.094 ± 0.016	0.021 ± 0.005	0.073 ± 0.021	22	78
Minimum			0.24	0.094	0.021	0.011	22	7
Maximum			14.06	0.308	0.259	0.136	93	78
Average			4.16	0.164	0.119	0.046	69	31
Standard deviation			4.00	0.067	0.066	0.051	25	25

[a] C.I. is the confidence interval for 95% confidence level;
[b] FAO – Food and Agriculture Organization of the United Nations; FAO 27 – Atlantic North-East; FAO 34 – Atlantic Eastern Central; FAO 61 – Pacific North West; FAO 67- Pacific North East

However, there is a risk of exposure to myocardial infarction at a consumption of more than 3–4 portions of 150 g per week of oceanic fish, such as mackerel, cod and hake, since in this case the estimated Hg concentration in blood would exceed the limit value of 12 μg L^{-1}. Thus, a moderate consumption of fish brings health benefits due to the content of long-chain polyunsaturated fatty acids such as Omega 3 (n-3 PUFA).[20]

4.7. Conclusions

The results obtained in the development and characterization of the Hg speciation method based on optical emission spectrometry in a capacitively coupled plasma microtorch after classical and photo-induced derivatization and detection using a microspectrometer led to the following conclusions:

1. The method provided suitable characteristics (limit of detection, precision, accuracy) to be used for the analysis of fish fillet of different varieties, of oceanic or aquaculture origin;
2. The method ensures Hg speciation in fish as CH_3Hg^+ and Hg^{2+} with good accuracy and precision even when the organic species represents more than 98% of the total Hg and the concentration of Hg^{2+} is below 0.01 mg kg^{-1};
3. The distribution of CH_3Hg^+ and Hg^{2+} species was depending on the fish variety and source. Thus, the oceanic(capture) fish exhibited a more elevated Hg concentration and higher weight of the CH_3Hg^+ species than fish coming from aquaculture, in which the Hg^{2+} species was dominant;
4. It has been pointed out that a realistic assessment of the exposure risk of the population to Hg via fish consumption requires the speciation analysis because CH_3Hg^+ exhibits a much higher toxicity than Hg^{2+}. Thus, it would be necessary a revision of EC Decision 2006/1881/EC[18], which regulates only the total Hg content, and updating it with the maximum admitted concentration of the CH_3Hg^+ species.
5. Although CH_3Hg^+ is responsible for the bioaccumulation of Hg in fish fillet, there is no risk of exposure of adults and children to the neurotoxic action of CH_3Hg^+ via moderate consumption (2–3 servings) of oceanic fish compared to farmed fish, which can be consumed almost daily (3–7 servings);
6. Taking into account the different PTWI values for CH_3Hg^+ and Hg^{2+} and their very different toxicities, it is recommended to assess the risk based on the input of both species using THQ and TTHQ, instead of considering the total Hg in fish fillet.
7. It is useful to assess the risk of Hg exposure via fish consumption by estimating the total content in blood and hair according to the EFSA procedure. In other words, an integrated risk assessment for Hg exposure should be performed taking into account the PTWI

values for CH_3Hg^+ and Hg^{2+}, their inputs in the human body, THQ and TTHQ, and the estimated concentrations in blood and hair;

8. The results obtained in the thesis following this study are important for the social community in terms of the level of the exposure risk of the population to Hg/ CH_3Hg^+ and Hg^{2+} via fish consumption; thus, no risk was recognized for a moderate consumption of fish fillet;
9. The proposed speciation approach has real advantages in terms of analytical performance and use of fully miniaturized instrumentation based on optical emission spectrometry in a capacitively coupled plasma microtorch with economical operation; the method is deeply innovative and could be a robust and reliable alternative to more expensive and sophisticated approaches based on ICP-OES, ICP-MS and chromatographic methods coupled with spectral detectors;
10. The knowledge and options related to Hg speciation analysis in seafood have been extended by using miniaturized instrumentation, as a starting point for other, more advanced speciation approaches, which will be discussed in the next chapters of the thesis.

5. General eco-scale method for the determination of total Hg in food and water by optical emission spectrometry in a capacitively coupled plasma and UV photo-induced derivatization

5.1. Situation at international level. Working hypothesis and objectives.

As shown in the previous chapters, food with high Hg content are fish meat and fishery products[16], that are the most common way of Hg to enter in the human body. More than 85% of total Hg exists as CH_3Hg^+ species. Because of the high toxicity, the determination of total Hg and speciation as CH_3Hg^+ and Hg^{2+} in food and environmental samples still remain challenges for the scientific community[37,57-60].

The classical methods for Hg determination involve cold vapor generation using $SnCl_2$ or $NaBH_4$ after the decomposition of the organic matrix of the sample with strongly oxidizing reagents (mixture of HNO_3 and H_2O_2), and detection by CV-ICP-OES, CV-ICP-MS, CV-AAS, CV-ETAAS and CV-AFS.[61-69] The green methods for sample preparation and derivatization use low molecular weight organic acids (LMWOA) (formic acid, acetic acid, etc.), and eco-friendly energy sources (ultrasonic and UV irradiation), coupled with classical spectrometric methods for detection.[34,35,70-76] Sturgeon *et al.*[48] demonstrated for the first time that biological samples can be solubilized in concentrated HCOOH in an ultrasonic bath and showed that Hg vapor generation can occur in 2.5% (v/v) HCOOH under UV irradiation (15 W lamp). This method has only been used for seafood, without reference to other foods of animal origin, vegetables or environmental samples. Thus, coupling the sample solubilization in LMWOA, usually HCOOH, with photo-induced derivatization in HCOOH medium could be an advanced eco-scale approach for the determination and speciation of Hg. Based on this consideration, the aim of this study was to develop and characterize a general eco-scale method, with high greenness degree, for the determination of total Hg in seafood, vegetables, food of animal origin, water, environmental samples, and detection by μCCP-OES with low operating costs. Only HCOOH is to be used for both sample extraction and UV photochemical cold vapor generation with/without *on-line* preconcentration of Hg vapor.

5.2. Sample preparation for the determination of total Hg

Procedure for extraction in concentrated HCOOH medium for the determination of total Hg by UV-PVG-μCCP-OES. The procedure was similar to that described by Scriver *et al.*[77,78]. An amount of 200 mg CRM/lyophilized test sample was subjected to extraction in 10 mL of 98–100% (w/w) HCOOH in the ultrasonic bath SONOREX SUPER RK 102H (Bandelin Sonorex, Berlin, Germany) at 50 °C for 3 h. The supernatant was separated by centrifugation at 4500 rpm for 15 min. Then, aliquots of 0.2–1 mL were diluted to 25 mL to contain 0.6 mol L^{-1} HCOOH for derivatization. Total

Hg in the extracts of CRMs and test samples of fish fillet and mushroom was determined by UV-PVG-μCCP-OES without preconcentration, while for the extracts of vegetables and food of animal origin a preconcentration step was necessary because of the low Hg concentration.

Procedure for solid sample mineralization for the determination of total Hg by $SnCl_2$-CV-μCCP-OES, $SnCl_2$-CV-AFS and $SnCl_2$-CV-ICP-OES was that described in Chapter 4, Section 4.2.3. Finally, a 1:10 dilution was performed.

Procedure for the preparation of water samples for the determination of total Hg by UV-PVG-μCCP-OES with preconcentration involved a simple dilution of an aliquot volume of up to 20 mL sample to 25 mL in a volumetric flask, and the addition of an appropriate volume of 98–100% (w/w) HCOOH to achieve 0.6 mol L^{-1}.

5.3. $SnCl_2$-CV-μCCP-OES and UV-PVG-μCCP-OES instrumentation

The scheme of the UV-PVG-μCCP-OES instrumentation with/without preconcentration used for the determination of total Hg is presented in Figure 5.1 (a), while the microcollector with gold filament used for the *on-line* preconcentration of Hg vapor in Figure 5.2 (b).

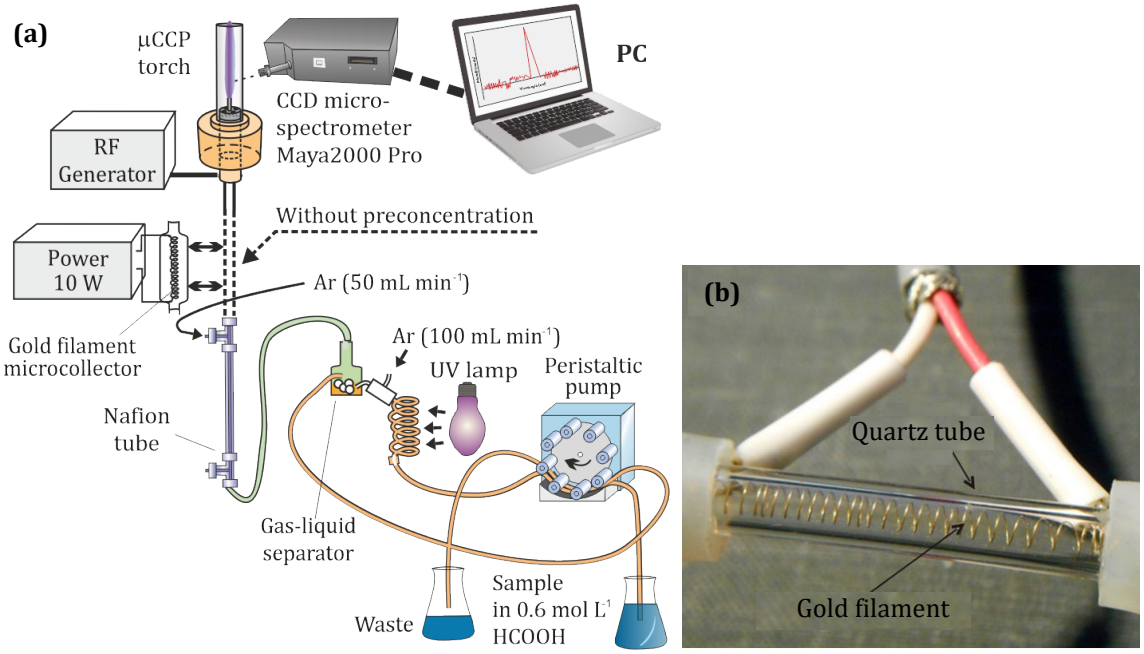


Figure 5.1. The experimental set-up of UV-PVG-μCCP-OES instrumentation(a)[79] and gold filament microcollector (b)[80]

The components of the UV-PVG-μCCP-OES set-up were similar to those presented in the previous chapters, excepting the microspectrometer and gold filament microcollector. In this study, the emission of Hg was registered using the low resolution microspectrometer Ocean Optics Maya2000 Pro (Dunedin, SUA). The microcollector for Hg vapor concentration consisted of a gold coiled-filament, 99.99% purity (Goodfellow, Cambridge, UK), 24 cm length, 43 turns, mounted in a quartz capillary.[81] The UV-PVG-μCCP-OES system with preconcentration was

operated in two steps: (1) trapping of Hg vapor generated by UV photo-induced derivatization from 25 ml sample on the gold filament, and (2) fast thermal desorption of Hg vapor upon heating the filament by supplying a voltage/current of 5 V/1.8 A from the HM 7042-5 source (Hameg Instruments, Mainhausen, Germany). Twenty emission spectra at an integration time of 1 s/episode were recorded during filament heating. The total emission signal of Hg was obtained by summing the net episodic spectra after background correction using the linear two-point model. The optimal working conditions for the determination of total Hg by UV-PVG-μCCP-OES with/without preconcentration and the reference methods $SnCl_2$-CV-μCCP-OES, $SnCl_2$-CV-AFS and $SnCl_2$-CV-ICP-OES are presented in Table 5.1.[79]

The determination of total Hg by $SnCl_2$-CV-ICP-OES was performed using the ICP-OES Spectro CIROS^{CCD} spectrometer (Spectro, Kleve, Germania) interfaced with the HGX-200 hydride generation system (Teledyne Cetac Technologies, Nebraska, SUA), while by $SnCl_2$-CV-AFS using the Direct Hg Analyzer Hydra-AF (Teledyne Leeman Instruments, SUA).

5.4. Characterization and validation of the UV-PVG-μCCP-OES method with/without preconcentration by analyzing CRMs and evaluation of the analytical performance

The parameters of the calibration curves, LOD and LOQ achieved for total Hg by UV-PVG-μCCP-OES with/without preconcentration compared to the reference methods $SnCl_2$-CV-μCCP-OES, $SnCl_2$-CV-ICP-OES and $SnCl_2$-CV-AFS, without preconcentration, are presented in Table 5.3. LOD in the UV-PVG-μCCP-OES method with/without preconcentration of 9/0.25 μg kg^{-1} is 2000/56 times lower than the maximum admitted level in fish meat, while LOQ is accordingly 667/14 times lower[18], so that the method fulfils the demands in the Decision 2007/333/EC [52].

The accuracy of the UV-PVG-μCCP-OES method without preconcentration was assessed by analyzing seven CRMs and it was found that the requirement in the Decision 2002/657/CE[53] was complied as the pooled recovery R (%) was 101 ± 7% (Table 5.3). The accuracy of the UV-PVG-μCCP-OES with preconcentration was checked by analyzing spiked water samples and R (%) values were in the range 97 ± 12% (Table 5.4). The results were similar to those in the reference methods. The good accuracy of the UV-PVG-μCCP-OES method proved the lack of non-spectral interference from elements and organic matter of the matrix.

The method was found to be free from non-spectral interference caused by ions of alkali (Na, K), earth-alkali (Ca, Mg), transitional (Al, Cr, Cu, Fe, Zn) metals, and NO_3^-, SO_4^{2-} and PO_4^{3-} anions provided that the sample is diluted at least 1:50. Instead, the Cl^- anion could cause non-spectral interference in the determination of Hg by UV-PVG-μCCP-OES when the original sample exhibits a high concentration of Cl^-, as could be the case of water samples.

Table 5.1. Optimal conditions for the determination of total Hg by UV-PVG-μCCP-OES, $SnCl_2$-CV-μCCP-OES, $SnCl_2$-CV-ICP-OES and $SnCl_2$-CV-AFS[79]

	UV-PVG-μCCP-OES without preconcentration	**UV-PVG-μCCP-OES with preconcentration**	**$SnCl_2$-CV-μCCP-OES**	**$SnCl_2$-CV-ICP-OES**	**$SnCl_2$-CV-AFS**
Derivatization stage					
Derivatization reagent	0.6 mol L^{-1} HCOOH	0.6 mol L^{-1} HCOOH	20% (m/v) $SnCl_2$ in 15% (v/v) HCl	20% (m/v) $SnCl_2$ in 15% (v/v) HCl	2% (m/v) $SnCl_2$ in 10% (v/v) HCl
Blank	0.6 mol L^{-1} HCOOH	0.6 mol L^{-1} HCOOH	5% (v/v) HCl	5% (v/v) HCl	5% (v/v) HCl
Sample flow rate (mL min^{-1})	10	10	3.5	5	5
Derivatization reagent flow rate (mL min^{-1})	-	-	1	2	1
UV-light irradiation (s)	5	5	-	-	-
Sample volume subjected to preconcentration (mL)	-	25	-	-	-
Plasma operation, fluorescence cell and detection					
R.f. (MHz)/Power (W)	13.56/15	13.56/15	13.56/15	27.12/1400	-
Ar flow rate (L min^{-1})	0.10	0.10	0.15	Outer Ar: 12; CV Ar gas: 0.7; auxiliary Ar: 0.6	0.7
Potential (V)/current (A) for heating the Au filament in the desorption step	-	5/1.8	-	-	-
Heating time of Au filament for Hg desorption (s)	-	20	-	-	-
Observation height in plasma (mm)	1.6	1.6	1.6	Axial viewing, ICP torch position (mm) X=-3.9; Y=+3.6; Z=+2.6.	-
Wavelength (nm)	253.652	253.652	253.652	191.770	253.652
Signal measurement	Peak height	Peak height	Peak height	Peak height	Steady-state
Integration time (s)	1	1 (20 episodic spectra)	1	48	15
Calibration range (μg L^{-1})	0–1	0–0.1	0–1	0–10	0–1
Background correction	Linear two-point model	Linear two-point model	Linear two-point model	Linear two-point model	-

Table 5.2. Parameters of the calibration curves, LODs and LOQs for total Hg by UV-PVG-μCCP-OES with/without preconcentration compared to the reference methods $SnCl_2$-CV-μCCP-OES, $SnCl_2$-CV-ICP-OES and $SnCl_2$-CV-AFS, without preconcentration[79].

Parameter	Method				
	UV-PVG-μCCP-OES without preconcentration	**UV-PVG-μCCP-OES with preconcentration**	**$SnCl_2$-CV-μCCP-OES without preconcentration**	**$SnCl_2$-CV-AFS**	**$SnCl_2$-CV-ICP-OES**
Calibration range (μg L^{-1}) (n=6)	0–1	0–0.1	0–1	0–1	0–10
Slope (signal a.u. mL ng^{-1})	5905 ± 9	753000 ± 114	5899 ± 11	24737 ± 57	495 ± 1
Intercept	-1 ± 6	0 ± 411	0 ± 8	0 ± 105	0 ± 2
Signal-to-noise ratio (SNR)[a]	740	7500	590	495	495
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999	0.9994
$s_{y/x}$ (signal a.u.)	7	25	7	129	3
LOD (ng L^{-1})	3.5	0.1[b]	3.5	5	19
LOQ (ng L^{-1})[e]	10.5	0.3	10.5	15	57
LOD (μg kg^{-1})	9[c]	0.25[c]	9[d]	12.5[d]	49[d]
LOQ (μg kg^{-1})[e]	27	0.75	27	38	147

[a] Corresponding to 1 μg L^{-1} Hg^{2+} (slope-standard deviation ratio); [b] LOD obtained after Hg preconcentration from 25 mL sample in 0.6 mol L^{-1} HCOOH; [c] LOD in solid calculated for 200 mg sample subjected to extraction in 10 mL 98-100% (w/w) HCOOH followed by 1:50 dilution to achieve 0.6 mol L^{-1} HCOOH in the sample; [d] LOD in solid calculated for 200 mg sample in 50 mL solution followed by 1:10 dilution; [e] Limits of detection calculated for 200 mg sample in 50 mL solution without subsequent dilution.

Table 5.3. Results obtained for total Hg in CRMs using the UV-PVG-μCCP-OES method with formic acid extraction, and the reference methods $SnCl_2$-CV-μCCP-OES, $SnCl_2$-CV-ICP-OES and $SnCl_2$-CV-AFS after mineralization in HNO_3–H_2O_2 without preconcentration[79]

CRM	Certified value ± U[a] (mg kg^{-1})	Recovery ± C.I. (%)			
		UV-PVG-μCCP-OES	**$SnCl_2$-CV-μCCP-OES**	**$SnCl_2$-CV-ICP-OES**	**$SnCl_2$-CV-AFS**
Tort-2 lobster hepatopancreas	0.27 ± 0.06	93 ± 8	93 ± 8	104 ± 7	104 ± 7
BCR-463 tuna fish	2.85 ± 0.16	100 ± 6	101 ± 9	109 ± 9	108 ± 9
ERM CE-464 tuna fish	5.24 ± 0.10	100 ± 6	95 ± 6	97 ± 4	101 ± 5
ERM BB422 fish muscle	0.601 ± 0.030	100 ± 2	101 ± 9	101 ± 2	98 ± 5
SRM 2976 mussel tissue	0.0610 ± 0.0036	103 ± 7	102 ± 8	95 ± 10	105 ± 5
CS-M-3 mushroom powder *Boletus edulis*	2.849 ± 0.104	101 ± 10	98 ± 3	101 ± 2	98 ± 3
ERM CA713 waste water[b]	1.84 ± 0.11	100 ± 10	101 ± 7	98 ± 8	102 ± 7
Pooled recovery ± C.I.		101 ± 7	100 ± 7	100 ± 7	102 ± 6

[a] U is expanded uncertainty;

[b] concentration expressed in μg L^{-1}.

Table 5.4. Results for total Hg by UV-PVG-μCCP-OES with preconcentration in spiked water samples[79]

Sample	Total Hg in the original sample (ng L^{-1})	Hg^{2+} added in the sample (ng L^{-1})	Total Hg in spiked sample (ng L^{-1})	Recovery (%)
Tap water	< LOD	16	16.2	101
Well water	< LOD	80	75.7	95
Well water	7.3	80	87.8	100
Bottled water (still)	6.1	16	19.2	82
Bottled water (still)	15.4	10	26.2	108
Pooled recovery ± C.I.[a]				97 ± 12

[a] C.I. is the 95% confidence interval for m=5 complete analysis sequences.

5.5. Use of the UV-PVG-μCCP-OES method for the determination of total Hg in water and food samples extracted in HCOOH

The applicability of the method with/without preconcentration for the determination of total Hg was checked by the analysis of real samples of fish muscles, mushroom, food of animal and vegetable origin, and water samples, respectively. Table 5.5 presents the results obtained in fish and mushroom muscle in comparison to the reference methods $SnCl_2$-CV-ICP-OES and $SnCl_2$-CV-AFS, both without preconcentration, while Table 5.6 the results for vegetables, samples of animal origin and water, after preconcentration.

The precision of the UV-PVG-μCCP-OES method with/without preconcentration fulfills both the AOAC requirement (3–15% RSD) and that of the EC, as the RSD values ranged between 1.2–12.8%, while the HorRat Index was below 2 as limit value.

The characteristics of the Bland and Altman plot using the data for the test samples by UV-PVG-μCCP-OES and the reference methods ($SnCl_2$-CV-μCCP-OES, $SnCl_2$-CV-ICP-OES and $SnCl_2$-CV-AFS) are presented in Table 5.7. The UV-PVG-μCCP-OES method has been shown to provide results that do not differ significantly from those obtained by the reference methods, as the confidence interval of the mean deviation contains the zero value. The average results obtained in the two methods fall between the upper and lower confidence limits, and the confidence interval of these limits is enough narrow compared to the level of total Hg in the analyzed samples.

Table 5.5. Results for total Hg (mg kg^{-1}) in fish fillet and mushroom determined by UV-PVG-μCCP-OES, $SnCl_2$-CV-μCCP-OES, $SnCl_2$-CV-ICP-OES and $SnCl_2$-CV-AFS without preconcentration [79]

	UV-PVG-μCCP-OES[a]			$SnCl_2$-CV-μCCP-OES[a]			$SnCl_2$-CV-ICP-OES[a]			$SnCl_2$-CV-AFS[a]		
Sample	**Mean ± C.I.**	**RSD[b] (%)**	**HorRat Index[c]**	**Mean ± C.I.**	**RSD[b] (%)**	**HorRat Index [c]**	**Mean ± C.I.**	**RSD[b] (%)**	**HorRat Index[c]**	**Mean ± C.I.**	**RSD[b] (%)**	**HorRat Index[c]**
Tuna	0.212 ± 0.017	6.4	0.34	0.191 ± 0.013	5.5	0.29	0.226 ± 0.008	2.8	0.15	0.211 ± 0.008	3.0	0.16
Trout	0.110 ± 0.004	2.9	0.15	0.095 ± 0.010	8.5	0.45	0.094 ± 0.006	5.1	0.27	0.096 ± 0.006	5.0	0.26
Salmon	0.108 ± 0.007	5.2	0.27	0.111 ± 0.005	3.6	0.19	0.109 ± 0.014	10.3	0.54	0.099 ± 0.007	5.7	0.30
Hake	0.216 ± 0.007	2.6	0.14	0.216 ± 0.080	3.0	0.16	0.219 ± 0.008	2.9	0.15	0.218 ± 0.009	3.3	0.17
Carp	0.089 ± 0.006	5.4	0.28	0.086 ± 0.004	3.7	0.20	0.100 ± 0.003	2.4	0.13	0.086 ± 0.004	3.7	0.20
Mackerel	0.198 ± 0.025	10.2	0.53	0.211 ± 0.014	5.3	0.28	0.197 ± 0.009	3.7	0.19	0.206 ± 0.009	3.5	0.18
Cod	0.372 ± 0.014	3.0	0.16	0.368 ± 0.006	1.3	0.07	0.359 ± 0.007	1.6	0.08	0.387 ± 0.013	2.7	0.14
Cod	0.305 ± 0.048	12.7	0.67	0.317 ± 0.033	8.4	0.44	0.306 ± 0.031	8.2	0.43	0.309 ± 0.017	4.4	0.23
Mushroom	0.268 ± 0.038	11.4	0.60	0.258 ± 0.021	6.6	0.34	0.254 ± 0.028	8.9	0.47	0.286 ± 0.018	5.1	0.27
Mushroom	4.48 ± 0.25	4.5	0.24	4.26 ± 0.33	6.2	0.33	4.51 ± 0.40	7.1	0.38	4.33 ± 0.21	3.9	0.20
Minimum	0.089	2.6	0.14	0.086	1.3	0.07	0.094	1.6	0.08	0.086	2.7	0.14
Maximum	4.48	12.7	0.67	4.26	8.5	0.45	4.51	10.3	0.54	4.33	5.7	0.30
Median	0.214	5.3	0.28	0.214	5.4	0.28	0.222	4.4	0.23	0.214	3.8	0.20

[a] Results for m=5 complete analysis for 95% confidence level;

[b] RSD is percentage relative standard deviation;

[c] HorRat Index calculated for 19% PRSD

Table 5.6. Results for total Hg (mg kg^{-1}) in vegetables, food of animal origin, food supplements and water analyzed by UV-PVG-μCCP-OES with/without preconcentration[79]

UV-PVG-μCCP-OES without preconcentration				UV-PVG-μCCP-OES with preconcentration			
Sample	**Mean ± CI[a] (μg kg^{-1})**	**RSD[b] (%)**	**HorRat Index[c]**	**Sample**	**Mean ± CI[a] (ng L^{-1})**	**RSD[b] (%)**	**HorRat Index[c]**
Apple	5.2 ± 0.4	6.2	0.33	River water	10.0 ± 0.8	6.4	0.34
Cabbage	11.6 ± 0.8	5.6	0.29	River water	11.6 ± 0.8	5.6	0.29
Potatoes	64.2 ± 3.8	4.8	0.25	Tap water	9.2 ± 0.9	7.9	0.41
Carrot	54.0 ± 5.9	8.8	0.46	Tap water	5.7 ± 0.9	12.4	0.65
Onion	89.4 ± 10.0	9.0	0.47	Well water	68.7 ± 2.6	3.0	0.16
Celery root	36.7 ± 3.3	7.2	0.38	Well water	75.7 ± 4.3	4.6	0.24
Parsley	122 ± 7	4.6	0.24	Well water	40.2 ± 2.2	4.4	0.23
Rice	37.8 ± 2.8	6.0	0.31	Bottled water (still)	13.8 ± 1.2	7.0	0.37
Wheat bran	3.3 ± 0.4	9.8	0.51	Bottled water (still)	1.2 ± 0.2	12.4	0.65
Brown brad	1.0 ± 0.1	8.0	0.42	Bottled water (still)	3.1 ± 0.4	10.4	0.55
Corn	52.1 ± 3.6	5.6	0.29	Bottled water (still)	8.4 ± 0.7	6.7	0.35
Pork fillet	46.4 ± 3.6	6.2	0.33	Bottled water (still)	68.9 ± 3.9	4.6	0.24
Chicken meat	59.8 ± 5.7	7.7	0.40	Bottled water (sparkling)	24.6 ± 2.7	8.8	0.46
Chicken liver	123 ± 9	5.9	0.31	Bottled water (sparkling)	26.1 ± 2.3	7.1	0.37
Supplements for athletes	65.6 ± 5.2	6.4	0.34	Bottled water (sparkling)	33.1 ± 2.5	6.1	0.32
Multimineral supplements (tablet)	1.1 ± 0.2	12.8	0.67	Bottled water (sparkling)	61.8 ± 4.5	5.9	0.31
Minimum	1.0	4.6	0.24		1.2	3.0	0.16
Maximum	123	12.8	0.67		75.7	12.4	0.65
Median	49.25	6.3	0.33		19.2	6.6	0.35

[a] Results for m=5 complete analysis and 95%confidence interval;

[b] RSD is the percentage relative standard deviation;

[c] HorRat Index calculated for 19% PRSD.

Table 5.7. Statistics in the Bland and Altman test for the determination of total Hg (mg kg^{-1}, m=5 parallel measurements for each sample; n=8 samples of fish fillet) by UV-PVG-μCCP-OES and the reference methods ($SnCl_2$-CV-μCCP-OES, $SnCl_2$-CV-ICP-OES and $SnCl_2$-CV-AFS)

Method X	Method Y	s_X[a]	s_Y[a]	$s_{B_{X-Y}}$[b]	Bias[c]	Limits of agreement[d]
UV-PVG-μCCP-OES	$SnCl_2$-CV-μCCP-OES	0.017	0.026	0.031	0.002 ± 0.015	0.063 ± 0.015 -0.059 ± 0.015
UV-PVG-μCCP-OES	$SnCl_2$-CV-ICP-OES	0.017	0.011	0.022	0.000 ± 0.013	0.042 ± 0.011 -0.042 ± 0.011
UV-PVG-μCCP-OES	$SnCl_2$-CV-AFS	0.017	0.008	0.020	0.000 ± 0.011	0.039 ± 0.011 -0.039 ± 0.011

[a] Within-method standard deviation; [b] Between methods standard deviation; [c] Bias calculated as $\bar{\Delta} \pm t\frac{s_{B_{X-Y}}}{\sqrt{n}}$ for 95% confidence level.; [d] Limits of agreement calculated as $\bar{\Delta} \pm 1{,}96 \cdot s_{B_{X-Y}} \pm t \cdot s_{LL,UL}$ for 95% confidence level.

5.6. Conclusions

Following the study on the determination of total Hg by UV-PVG-μCCP-OES based on the extraction in HCOOH and photo-induced derivatization, with/without *on-line* preconcentration of Hg vapor, the following conclusions were drawn:

1. The UV-PVG-μCCP-OES method with photo-induced derivatization in 0.6 mol L^{-1} HCOOH was successfully applied for the determination of total Hg in seafood (fish muscle), food of vegetable and animal origin, food supplements, river water, tap water and bottled water (still and sparkling). In the case of fish muscle and mushroom, the analysis was performed without preconcentration, while for foods of vegetable and animal origin, food supplements and water it was necessary the *on-line* preconcentration of the Hg vapor on an Au filament to achieve detection limits at the level of μg kg^{-1} and ng L^{-1};
2. Compared to the current literature data, the ultrasonic-assisted extraction of the Hg species in concentrated HCOOH has been extended from seafood to foods of vegetable and animal origin and food supplements, for which such applications have not yet been reported;
3. The UV-PVG-μCCP-OES method proved to be general eco-scale, using only HCOOH as biodegradable reagent, both for extraction and derivatization to cold vapors, and showed very good sensitivity, precision and accuracy. The extraction procedure is milder and much more beneficial than the microwave-assisted classical digestion needing HCl, HNO_3 and H_2O_2; at the same time, the expensive, toxic and unstable derivatization reagents ($SnCl_2$, $NaBH_4$) were eliminated;
4. The UV-PVG-μCCP-OES method for total Hg, in which only HCOOH is used for extraction and derivatization, has proven to be a viable analytical alternative, with high greenness degree on the eco-scale hierarchy, to methods using the classical derivatization with $SnCl_2$,

and detection by optical emission in capacitively coupled plasma ($SnCl_2$-CV-μCCP-OES), inductively coupled plasma ($SnCl_2$-CV-ICP-OES) and atomic fluorescence spectrometry ($SnCl_2$-CV-AFS); UV-PVG-μCCP-OES has better detection limits, and similar accuracy and precision to the reference methods;

5. In addition to its outstanding analytical performance, the UV-PVG-μCCP-OES method involves much lower costs by eliminating expensive reagents, and use of a low-power microplasma with low Ar consumption, compared to laboratory instrumentation based on ICP-OES;
6. The non-spectral effects coming from alkali, earth alkali and transition elements, anions and organic matter could be overcome in the UV-PVG-μCCP-OES method used for the determination of total Hg in food by performing a simple dilution of the sample; thus, the method is a selective one;
7. The use of HCl for the preservation of water samples should be avoided because of the depressive non-spectral interference from Cl^- in the photo-induced CV generation in 0.6 mol L^{-1} HCOOH;
8. One of the weaknesses of HCOOH extraction, also reported by other authors, is the long extraction time, even in the case of microwave assistance; however, this disadvantage could be eliminated by increasing the number of samples subjected to extraction in the same batch;
9. A vulnerability of the UV-PVG-μCCP-OES method, unrelated to the instrumentation, is the presence of Hg as an impurity in the HCOOH reagent, at higher concentration than in $SnCl_2$ and $NaBH_4$ reagents, which are available in the quality required for analysis; however, this impediment was overcome by further purification through sonication and Ar bubbling in the concentrated HCOOH solution;
10. The present study highlighted, among other things, the need for high quality formic acid, of special purity, intended for the determination of Hg. In this way, this method based on photo-induced derivatization and spectral detection will become much more sensitive.

6. General eco-scale method for Hg speciation as Hg^{2+} and CH_3Hg^+ in seafood by UV/Vis photochemical vapor generation and detection by optical emission spectrometry in a capacitively coupled microplasma

6.1. Status at international level. Working hypothesis and objectives

Because of the high toxicity of Hg, its speciation in seafood is still a challenge for the scientific community and several chromatographic and non-chromatographic methods coupled with spectral detectors were developed.[65,82-87] The non-chromatographic speciation is based on either separation of species by selective extraction or selective derivatization of Hg species and spectrometric detection by AAS, ETAAS or ICP-OES.[62-64] In both cases, sophisticated and expensive instrumentation are needed, as well as high-purity, expensive reagents. In compliance with the principles of GAC [2], eco-scale methods were developed for Hg^{2+} determination in water by photo-induced derivatization under Vis light exposure[88,89], as well as for Hg speciation as total Hg (sum $CH_3Hg^+ + Hg^{2+}$)/Hg^{2+} by selective photo-induced derivatization under UV/Vis irradiation in 20% HCOOH[73]. The study on various derivatization reagents from the LMWOC group showed that HCOOH was the most effective.[42,90,91]

The ultrasound-assisted extraction of biological samples in concentrated formic acid was assessed for multielemental analysis by ETAAS, LA-ICP-MS, ICP-AES, determination of total Hg by CV-AAS with KBH_4 derivatization after additional oxidation of sample or UV-PVG-AAS with photo-induced derivatization in HCOOH.[74,77,78,92] However, the methods of sample extraction in HCOOH coupled with photo-induced derivatization using the same reagent, in the presence of UV/Vis radiation, have not yet been investigated in conjunction with microplasma technology. Thus, the aim of this study was to develop a simple method for Hg speciation as CH_3Hg^+ and Hg^{2+} in seafood using the UV/Vis-PVG-μCCP-OES miniaturized instrumentation and only HCOOH for both extraction of Hg species from solid and photo-induced derivatization to cold vapor. The novelty of the approach compared to data reported in the literature consists in the general eco-scale nature of the UV/Vis-PVG-μCCP-OES method, which uses fully miniaturized instrumentation based on a low Ar/energy consumption plasma microtorch interfaced with a low-resolution microspectrometer. In the same time, a single reagent is used for sample extraction and derivatization, namely HCOOH.

6.2. Sample preparation for Hg speciation as CH_3Hg^+ and Hg^{2+}

The principle of sample preparation for Hg speciation using the UV/Vis-PVG-μCCP-OES eco-scale method is presented in Figure 6.1.

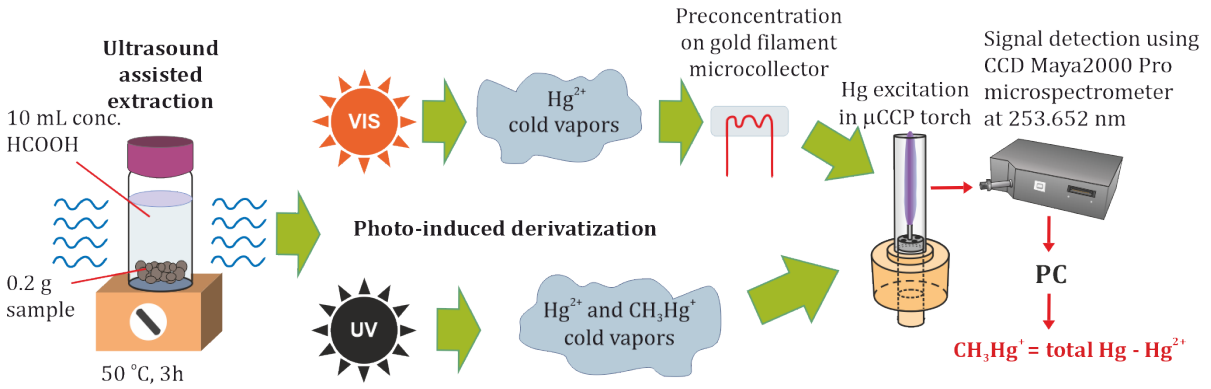


Figure 6.1. Principle of the procedure for Hg speciation as CH_3Hg^+ and Hg^{2+} in seafood using the UV/Vis-PVG-μCCP-OES eco-scale method with/without preconcentration[93]

The procedure for Hg speciation by UV/Vis-PVG-μCCP-OES involved: (1) extraction of Hg species from 0.2 g sample in 10 mL HCOOH 98–100% (w/w) by ultrasonication for 3 h at 50 °C; (2) determination of Hg^{2+} by Vis-PVG-μCCP-OES after selective derivatization under Vis light exposure (UV lamp off) in 0.6 mol L^{-1} HCOOH and preconcentration of Hg cold vapor on a gold filament using an aliquot of 25 mL sample; (3) determination of total Hg ($CH_3Hg^+ + Hg^{2+}$) by photo-induced derivatization under UV irradiation (UV lamp on) in 0.6 mol L^{-1} HCOOH without preconcentration, (4) determination of CH_3Hg^+ as difference between total Hg and Hg^{2+}.

Mercury speciation by the TD-AAS reference method involved determination of total Hg in solid sample and of CH_3Hg^+, after double liquid-liquid extraction in the HBr–toluene–1% L-cysteine system (described in Chapter 3), and calculation of the Hg^{2+} concentration by subtraction.

6.3. Instrumentation of the UV/Vis-PVG-μCCP-OES method used for the speciation of Hg as Hg^{2+} and CH_3Hg^+

The experimental set-up for the UV/Vis-PVG-μCCP-OES method used for Hg speciation as CH_3Hg^+ and Hg^{2+} in fish is provided in Figure 6.2 (a, b). The total Hg extracted in concentrated HCOOH was determined after UV photo-induced (Hg lamp, 500 W) derivatization. For the selective determination of the Hg^{2+} species, the UV lamp was turn off, Hg vapor was preconcentrated on an Au filament microcollector, then released by heating the filament by applying a voltage of 5 V and 1.8 A. The optimal working conditions for the determination of total Hg and Hg^{2+} species are given in Table 6.1.

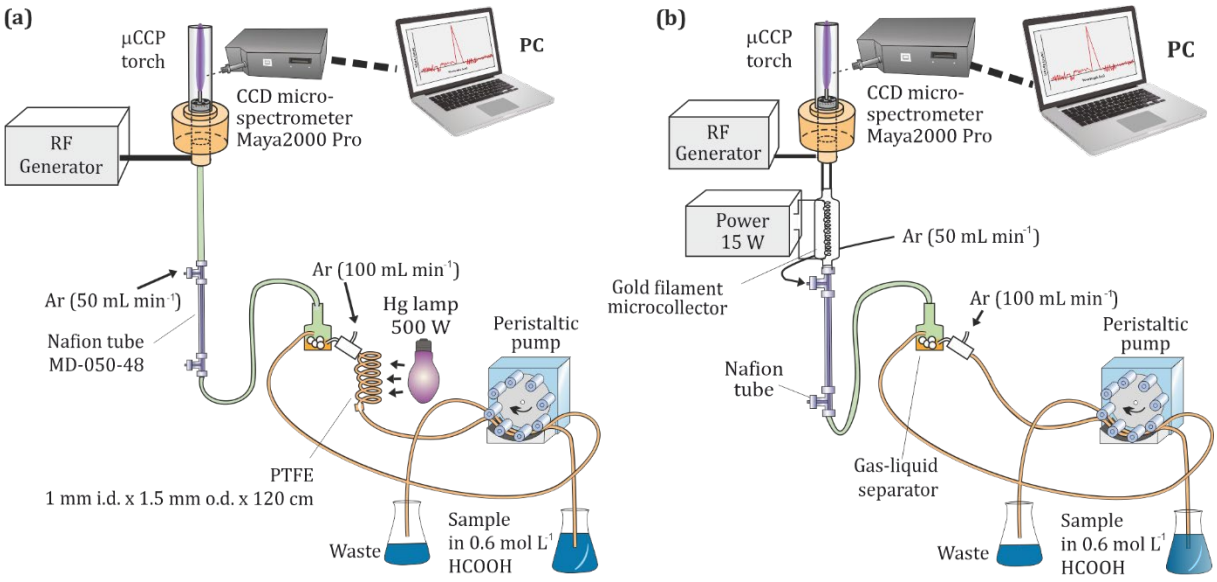


Figure 6.2. The experimental set-up of UV-PVG-μCCP-OES without preconcentration (a) for the determination of total Hg and Vis-PVG-μCCP-OES with preconcentration (b) for the determination of Hg^{2+} in fish [94]

Table 6.1. Optimal working conditions for Hg speciation using the UV/Vis-PVG-μCCP-OES eco-scale method [94]

	Total Hg	**Hg^{2+}**
Analytical system	**UV-PVG-μCCP-OES**	**Vis-PVG-μCCP-OES**
Calibration range (μg L^{-1}) using Hg^{2+} standards (n=7)	0-10	0-1
Preconcentration	No	Yes (25 mL sample)
Derivatization reagent	0.6 mol L^{-1} HCOOH	0.6 mol L^{-1} HCOOH
UV irradiation	On	Off
Sample flow rate (mL min^{-1})	10	10
UV irradiation time (s)	5	Signal does not vary significantly over time
Plasma power (W)	15	15
Ar flow rate (mL min^{-1})	100	100
Plasma observation height (mm)	1.6	1.6
Signal measurement	Peak height for 1 s integration time; linear two-point model background correction	High Speed Acquisition; 20 episodic spectra for 1 s integration time; linear two-point model background correction for each episodic emission spectrum

6.4. Characterization and validation of the UV/Vis-PVG-μCCP-OES method

The parameters of the calibration curves, LODs and LOQs in the speciation analysis by UV/Vis-PVG-μCCP-OES are presented in Table 6.2. LODs for total Hg/CH_3Hg^+/Hg^{2+} were 56/104/250 times lower than the maximum admitted level of total Hg in fish, while LOQs

18/36/83 times lower, respectively. Thus, the UV/Vis-PVG-μCCP-OES method fulfils the demands in the Decision 2007/333/EC[52] and is suitable for Hg speciation in such samples.[52,95]

Table 6.2. Limits of detection and quantification for total Hg, Hg^{2+} and CH_3Hg^+ in fish by UV/Vis-PVG-μCCP-OES[94]

Species	Method	Calibration sensitivity ± C.I.[a]	$s_{y/x}$	LOD		LOQ	
				ng L^{-1}	μg kg^{-1}	ng L^{-1}	μg kg^{-1}
Total Hg	UV-PVG-μCCP-OES without preconcentration	5905 ± 9	7	3.5	9	10.5	27
Hg^{2+}	Vis-PVG-μCCP-OES with preconcentration	17.430 ± 14	11	1.9	4.8	5.7	14
CH_3Hg^+	UV-PVG-μCCP-OES without preconcentration	2150 ± 10	6.2	7	2	21	6

[a] C.I. is the confidence interval for n=7 calibration standards of Hg^{2+} and 95% confidence level.

The accuracy of Hg speciation by UV/Vis-PVG-μCCP-OES was assessed using five matrix-matched CRMs. The analysis provided recovery of 99 ± 6% for total Hg, 99 ± 9% for Hg^{2+} and 99 ± 10% for CH_3Hg^+ (Table 6.3), similar values to those achieved in the reference method TD-AAS (Table 6.4). Thus, the UV/Vis-PVG-μCCP-OES method complies with the requirements in the Decision 2007/333/EC[52] and AOAC[54].

6.5. Use of the speciation method in the analysis of real samples of fish

The results obtained for Hg speciation in samples of fish fillet or fish trunk of different varieties using the UV/Vis-PVG-μCCP-OES eco-scale method compared to the reference method TD-AAS are shown in Tables 6.5 and 6.6, respectively. The precision of the UV/Vis-PVG-μCCP-OES method for the determination of total Hg, CH_3Hg^+ and Hg^{2+} in fish expressed as RSD was in the range 2.0–13.4%, below 20% as recommended by AOAC[54]. At the same, the UV/Vis-PVG-μCCP-OES speciation method meets the demand of the European Commission in the Decision 333/2007/EC[52] in terms of HorRat index to be lower than 2, for a predicted relative standard deviation (PRSD) of 19%.

The Bland and Altman analysis using the results for total Hg, CH_3Hg^+ and Hg^{2+} in fish samples found by UV/Vis-PVG-μCCP-OES and TD-AAS is summarized in Table 6.7. The test showed that the methods do not differ significantly as the differences between the corresponding results for Hg, CH_3Hg^+ and Hg^{2+} fell within the confidence limits. Also, the positive bias of 0.004 for Hg^{2+}, and negative bias of (-0,004) for CH_3Hg^+ were random, since the confidence interval contained the zero value, while the standard deviation was low compared to the determined concentrations.

Table 6.3. Accuracy of the UV/Vis-PVG-μCCP-OES method for Hg speciation as CH_3Hg^+ and Hg^{2+} in seafood[94]

CRM	Certified value ± U[a] (mg kg^{-1})			Found value ± C.I.[b] (mg kg^{-1})		
	Total Hg	Hg^{2+}	CH_3Hg^+	Total Hg	Hg^{2+}	CH_3Hg^+
DOLT-4	2.58 ± 0.22	1.25 ± 0.16	1.33 ± 0.12	2.59 ± 0.24	1.23 ± 0.13 [c]	1.36 ± 0.18 [c]
BCR-463	2.85 ± 0.16	0.02 ± 0.002	3.04 ± 0.16	2.84 ± 0.18	0.02 ± 0.002 [d]	3.00 ± 0.33 [d]
ERM-CE-464	5.24 ± 0.10	0.12 ± 0.004	5.50 ± 0.17	5.25 ± 0.30	0.12 ± 0.02 [d]	5.46 ± 0.91 [d]
TORT-2	0.27 ± 0.06	0.118 ± 0.028	0.152 ± 0.013	0.25 ± 0.02	0.117 ± 0.011 [c]	0.133 ± 0.016 [c]
SRM 2976	0.0610 ± 0.0036	0.0329 ± 0.0020	0.0281 ± 0.0024	0.0627 ± 0.0046	0.0325 ± 0.0028 [c]	0.0302 ± 0.0033 [c]
Pooled recovery ± C.I. (%)				99 ± 6	99 ± 9	99 ± 10

[a] U is expanded uncertainty for 95% confidence level; [b] m = 5 extractions/complete analysis for each sample; [c] Content expressed as Hg; [d] Content expressed as CH_3Hg^+.

Table 6.4. Accuracy of the TD-AAS method for Hg speciation as CH_3Hg^+ and Hg^{2+} in seafood[94]

CRM	Certified value ± U[a] (mg kg^{-1})			Found value ± C.I.[b] (mg kg^{-1})		
	Total Hg	Hg^{2+}	CH_3Hg^+	Total Hg	Total Hg	Hg^{2+}
DOLT-4	2.58 ± 0.22	1.25 ± 0.16	1.33 ± 0.12	2.37 ± 0.29	1.03 ± 0.13	1.34 ± 0.14 [c]
BCR-463	2.85 ± 0.16	0.02 ± 0.002	3.04 ± 0.16	2.81 ± 0.18	0.02 ± 0.002	2.97 ± 0.26 [d]
ERM-CE-464	5.24 ± 0.10	0.12 ± 0.004	5.50 ± 0.17	5.11 ± 0.32	0.08 ± 0.01	5.36 ± 0.33 [d]
TORT-2	0.27 ± 0.06	0.118 ± 0.028	0.152 ± 0.013	0.28 ± 0.07	0.128 ± 0.015	0.152 ± 0.015 [c]
SRM 2976	0.0610 ± 0.0036	0.0329 ± 0.0020	0.0281 ± 0.0024	0.0630 ± 0.0060	0.0330 ± 0.0044	0.0300 ± 0.0041 [c]
Pooled recovery ± C.I. (%)				99 ± 7	92 ± 10	96 ± 8

[a] U is expanded uncertainty for 95% confidence level; [b] m = 5 extractions/complete analysis for each sample; [c] Content expressed as Hg; [d] Content expressed as CH_3Hg^+.

Table 6.5. Results for Hg speciation in fish by UV/Vis-PVG-μCCP-OES[94]

Sample	Total Hg			Hg^{2+}			CH_3Hg^+		
	Mean ± C.I.[a] (mg kg^{-1})	RSD (%)	HorRat Index	Mean ± C.I. [a] (mg kg^{-1})	RSD (%)	HorRat Index	Mean ± C.I. [a] (mg kg^{-1})	RSD (%)	HorRat Index
Tuna	0.212 ± 0.017	6.4	0.34	0.022 ± 0.002	7.3	0.38	0.190 ± 0.023	9.7	0.51
Trout	0.110 ± 0.004	2.9	0.15	0.079 ± 0.007	7.1	0.38	0.031 ± 0.003	7.8	0.41
Salmon	0.126 ± 0.007	4.5	0.23	0.018 ± 0.003	13.4	0.71	0.108 ± 0.019	14.2	0.74
Hake	0.216 ± 0.007	2.6	0.14	0.035 ± 0.002	4.6	0.24	0.181 ± 0.012	5.3	0.28
Carp	0.089 ± 0.006	5.4	0.28	0.019 ± 0.002	8.5	0.45	0.070 ± 0.009	10.4	0.54
Mackerel	0.198 ± 0.025	10.2	0.54	0.031 ± 0.004	10.4	0.55	0.167 ± 0.030	14.5	0.76
Cod	0.305 ± 0.032	8.4	0.44	0.120 ± 0.003	2.0	0.10	0.185 ± 0.020	8.7	0.46
Minimum	0.089	2.6	0.14	0.018	2.0	0.10	0.031	5.3	0.28
Maximum	0.305	10.2	0.54	0.120	13.4	0.71	0.190	14.5	0.76
Mean	0.198	5.4	0.28	0.031	7.3	0.38	0.167	9.7	0.51
Median	0.179	5.8	0.30	0.046	7.6	0.40	0.133	10.1	0.53

[a] m = 5 complete extractions for each sample and 95% confidence interval

Table 6.6. Results for Hg speciation in fish by TD-AAS[94]

Sample	Total Hg			Hg^{2+}			CH_3Hg^+		
	Mean ± C.I.[a] (mg kg^{-1})	RSD (%)	HorRat Index	Mean ± C.I.[a] (mg kg^{-1})	RSD (%)	HorRat Index	Mean ± C.I.[a] (mg kg^{-1})	RSD (%)	HorRat Index
Tuna	0.215 ± 0.012	4.5	0.24	0.013 ± 0.002	8.9	0.47	0.202 ± 0.022	8.8	0.46
Trout	0.104 ± 0.006	4.6	0.24	0.063 ± 0.008	9.5	0.50	0.041 ± 0.005	9.8	0.52
Salmon	0.125 ± 0.004	2.6	0.14	0.028 ± 0.003	7.1	0.37	0.097 ± 0.008	6.6	0.35
Hake	0.216 ± 0.010	3.7	0.20	0.047 ± 0.007	11.2	0.59	0.169 ± 0.025	11.9	0.63
Carp	0.099 ± 0.008	6.5	0.34	0.010 ± 0.002	18.4	0.97	0.089 ± 0.019	17.0	0.89
Mackerel	0.190 ± 0.019	8.1	0.43	0.025 ± 0.005	14.2	0.75	0.165 ± 0.025	12.2	0.64
Cod	0.308 ± 0.023	6.0	0.32	0.110 ± 0.014	9.4	0.50	0.198 ± 0.021	8.5	0.45
Minimum	0.099	2.6	0.14	0.010	7.1	0.37	0.041	6.6	0.35
Maximum	0.308	8.1	0.43	0.115	18.4	0.97	0.202	17	0.89
Mean	0.190	4.6	0.24	0.028	9.5	0.50	0.165	9.8	0.52
Median	0.180	5.2	0.27	0.043	11.3	0.59	0.137	10.7	0.56

[a] m = 5 complete extractions for each sample and 95% confidence interval

Table 6.7. Results (mg kg^{-1}) of the Bland and Altman test for the determination of total Hg, Hg^{2+} and CH_3Hg^+ (m = 5 parallel measurements) by UV/VIS-PVG-µCCP-OES and TD-AAS in 7 samples of fish for 95% confidence level

Species	**s_X[a]**	**s_Y[a]**	**$s_{B_{X-Y}}$[b]**	**Bias[c]**	**Limits of agreement[d]**
Total Hg	0.014	0.011	0.017	0.000 ± 0.007	0.033 ± 0.008 -0.034 ± 0.008
Hg^{2+}	0.003	0.006	0.012	0.004 ± 0.013	0.028 ± 0.009 -0.020 ± 0.009
CH_3Hg^+	0.015	0.016	0.012	-0.004 ± 0.015	0.042 ± 0.012 -0.050±0.012

[a] Within-method standard deviation in UV/Vis-PVG-µCCP-OES (X) and TD-AAS (Y); [b] Between methods standard deviation, UV/Vis-PVG-µCCP-OES and TD-AAS; [c] Bias calculated as $\bar{\Delta} \pm t\frac{s_{B_{X-Y}}}{\sqrt{n}}$, for 95% confidence level; [d] Limits of agreement calculated as $\bar{\Delta} \pm 1.96 \cdot s_{B_{X-Y}} \pm t \cdot s_{LL,UL}$ for 95% confidence level.

6.6. Conclusions

The outcomes of the study were as follows:

1. Mercury speciation as CH_3Hg^+ and Hg^{2+} in fish is achievable by the UV/Vis-PVG-µCCP-OES eco-scale method, which uses fully miniaturized instrumentation with low consumption of Ar and energy; the method is simple and economic in terms of sample preparation and photo-induced derivatization using a single reagent (HCOOH), and has green profile by using UV/Vis exposure. In this way, harmful reagents (HBr, toluene) necessary for the double liquid-liquid extraction of the CH_3Hg^+ species are avoided.
2. The UV / Vis-PVG-µCCP-OES method for Hg speciation meets the requirements of the European Commission and the AOAC recommendations in terms of analytical performance and is, therefore, a viable alternative to sophisticated coupled methods, or even to non-chromatographic methods based on ICP-OES or ICP-MS detection;
3. The selective determination of Hg^{2+} species using the Vis-PVG-µCCP-OES eco-scale method is possible by conducting the photo-induced derivatization in the absence of UV irradiation. The low derivatization efficiency of Hg^{2+} species when the UV-lamp is turned off is overcome by coupling photo-induced derivatization with *on-line* preconcentration on a gold filament microcollector. This results in an enhancement of sensitivity necessary in the determination of the very low Hg^{2+} concentration in fish;
4. According to the Bland and Altman test, the UV/Vis-PVG-µCCP-OES eco-scale method is comparable to the reference method TD-AAS, recommended by the European Commission.

7. Greenness and whiteness degree assessment of the UV/Vis-PVG-µCCP-OES methods for the determination and speciation of Hg

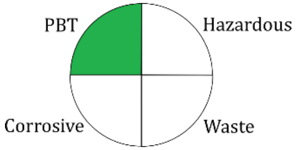
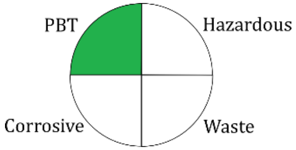
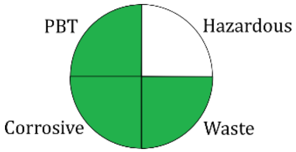
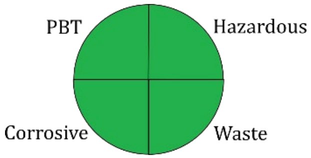
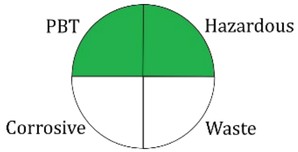
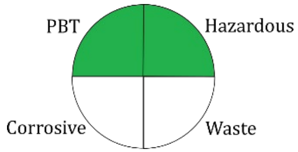
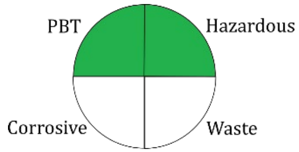
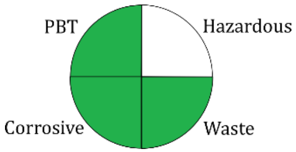
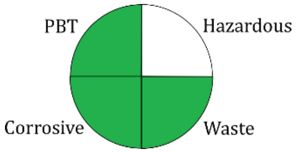
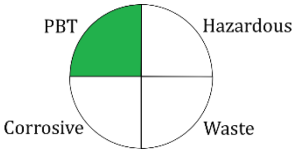
7.1. Status at international level. Working hypothesis and objectives.

The assessment of the greenness and whiteness of the new developed analytical methods in terms of sample preparation and analysis, compared to the traditional methods, is necessary in order to identify which step is mainly responsible for the decrease of the green/white profile to be addressed in further research for improvement. As shown in Chapter 1, unfortunately the greenness assessment until recently was subjective, so that the operator is tempted by an overestimation unless objective criteria are taken into account.[96,97] Instead, the assessment of whiteness is based on much more versatile and flexible criteria in which, in addition to greenness, other two key characteristics are considered, namely the red score associated to analytical efficiency and blue respectively, for practical and economic aspects.[7] Thus the aim of this study was a comprehensive assessment of greenness and whiteness of the new methods for total Hg, CH_3Hg^+ and $Hg^{2+}$by UV/Vis-PVG-µCCP-OES, in comparison with the traditional methods TD-AAS, $SnCl_2$-CV-AFS and $SnCl_2$-CV-ICP-OES, based on 4 approaches presented in Chapter 1, namely NEMI[3], AES[4], GAPI[5] and AGREE[6] procedure. Whiteness was evaluated using the RGB-12 algorithm[7], which proved to be a useful tool by including all analytical aspects and those related to method applicability, while greenness assessment took into consideration only the 12 GAC principles. In this way a much more comprehensive comparison between the new methods and those commonly used in laboratory is provided.

7.2. Results obtained for the greenness and whiteness degree assessment of the UV/Vis-PVG-µCCP-OES methods for the determination and speciation of Hg

Tables 7.1–7.5 contain data related to the greenness profile (NEMI, AES, GAPI and AGREE methods) of the new UV/Vis-PVG-µCCP-OES methods for Hg determination and speciation as CH_3Hg^+ and Hg^{2+}, while Figure 7.1 summarizes the whiteness assessment of the new methods using the RGB-12 model based on WAC, in comparison with the traditional methods, TD-AAS, $SnCl_2$-CV-ICP-OES and $SnCl_2$-CV-AFS.[98]

Table 7.1. Greenness of the new UV/Vis-PVG-µCCP-OES methods for Hg determination and speciation compared to traditional methods using the NEMI tool[3,98]

Procedure for Hg determination/ speciation	**UV-PVG-µCCP-OES**	**TD-AAS**	**$SnCl_2$-CV-ICP-OES**	**$SnCl_2$-CV-AFS**	**$SnCl_2$-CV-µCCP-OES**
CH_3Hg^+			-	-	-
Total Hg -food					
Total Hg - water		-	-	-	-
Hg speciation as CH_3Hg^+ and Hg^{2+} *			-	-	-

* Speciation of Hg as CH_3Hg^+ and Hg^{2+} using UV/Vis-PVG-µCCP-OES

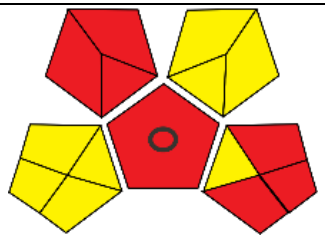
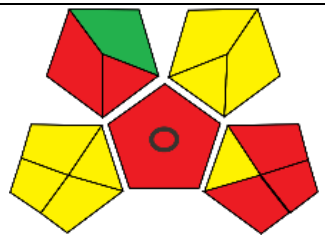
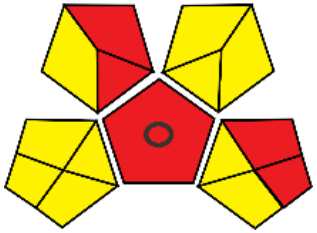
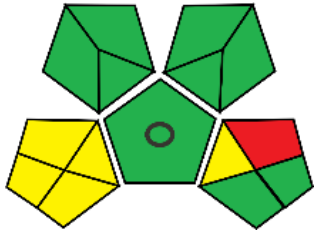
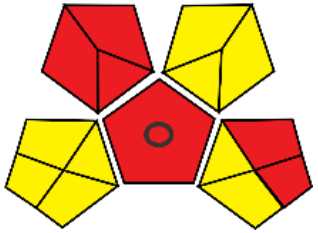
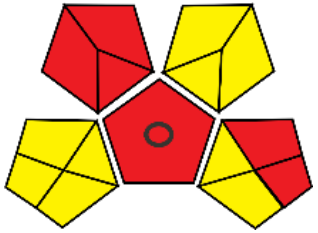
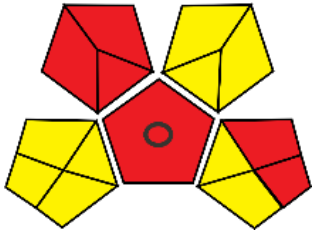
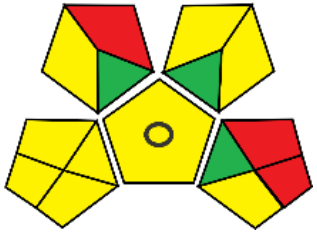
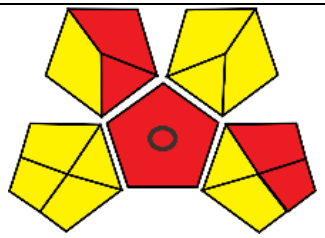
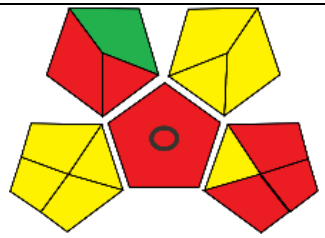
Table 7.2. Greenness of the new UV/Vis-PVG-μCCP-OES methods for Hg determination and speciation compared to traditional methods using the AES tool[4,98]

Procedure for Hg determination/ speciation	Methods penalty points/AES score				
	UV-PVG-μCCP-OES	**TD-AAS**	**$SnCl_2$-CV-ICP-OES**	**$SnCl_2$-CV-AFS**	**$SnCl_2$-CV-μCCP-OES**
CH_3Hg^+					
Reagents	27 [1]	28 [1]	-	-	-
Instrumentation, occupational hazard, waste	12	12	-	-	-
AES score	**61**	**62**	-	-	-
Total Hg - food					
Reagents	13 [2]	8 [3]	23 [4]	22 [4]	21 [4]
Instrumentation, occupational hazard, waste	9	1	11	10	10
AES score	**78**	**91**	**66**	**68**	**69**
Total Hg - water					
Reagents	7 [5]	-	-	-	-
Instrumentation, occupational hazard, waste	8	-	-	-	-
AES score	**85**	-	-	-	-
Hg speciation as CH_3Hg^+ and Hg^{2+}					
Reagents	13 [6]	28 [7]	-	-	-
Instrumentation, occupational hazard, waste	9	12	-	-	-
AES score	**78**	**62**	-	-	-

[1] double liquid-liquid extraction in HBr–toluene–L-cysteine system and photo-induced derivatization in HCOOH;

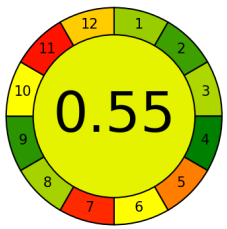
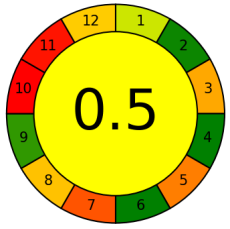
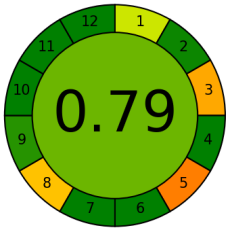
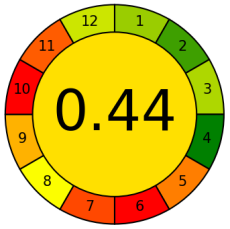
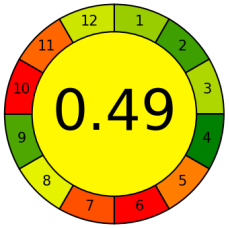
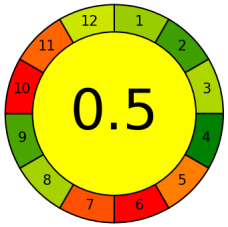
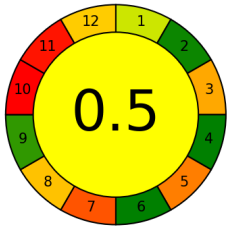
[2] total Hg extraction in HCOOH and photo-induced derivatization in HCOOH; [3] direct determination from solid sample; [4] digestion with HNO_3 and H_2O_2 and cold vapor generation using $SnCl_2$ in HCl; [5] acidulation to 0.6 mol L^{-1} HCOOH and photo-induced derivatization in HCOOH; [6] extraction in HCOOH and UV photo-induced derivatization for total Hg determination and Vis derivatization for Hg^{2+} determination; [7] double liquid-liquid extraction in HBr–toluene–L-cysteine system for CH_3Hg^+ determination and direct solid sampling for total Hg determination.

Table 7.3. Greenness of the new UV/Vis-PVG-μCCP-OES methods for Hg determination and speciation compared to traditional methods using the GAPI tool[5,98]

Procedure for Hg determination/speciation	UV-PVG-μCCP-OES	TD-AAS	$SnCl_2$-CV-ICP-OES	$SnCl_2$-CV-AFS	$SnCl_2$-CV-μCCP-OES
CH_3Hg^+			-	-	-
Total Hg -food					
Total Hg - water		-	-	-	-
Hg speciation as CH_3Hg^+ and Hg^{2+} *			-	-	-

* Speciation of Hg as CH_3Hg^+ and Hg^{2+} using UV/Vis-PVG-μCCP-OES

Table 7.4. Greenness of the new UV/Vis-PVG-μCCP-OES methods for Hg determination and speciation compared to traditional methods the AGREE tool[6,98]

Procedure for Hg determination/speciation	UV-PVG-μCCP-OES	TD-AAS	$SnCl_2$-CV-ICP-OES	$SnCl_2$-CV-AFS	$SnCl_2$-CV-μCCP-OES
CH_3Hg^+			-	-	-
Total Hg – food					
Total Hg - water		-	-	-	-
Hg speciation as CH_3Hg^+ and Hg^{2+} *			-	-	-

* Speciation of Hg as CH_3Hg^+ and Hg^{2+} using UV/Vis-PVG-μCCP-OES

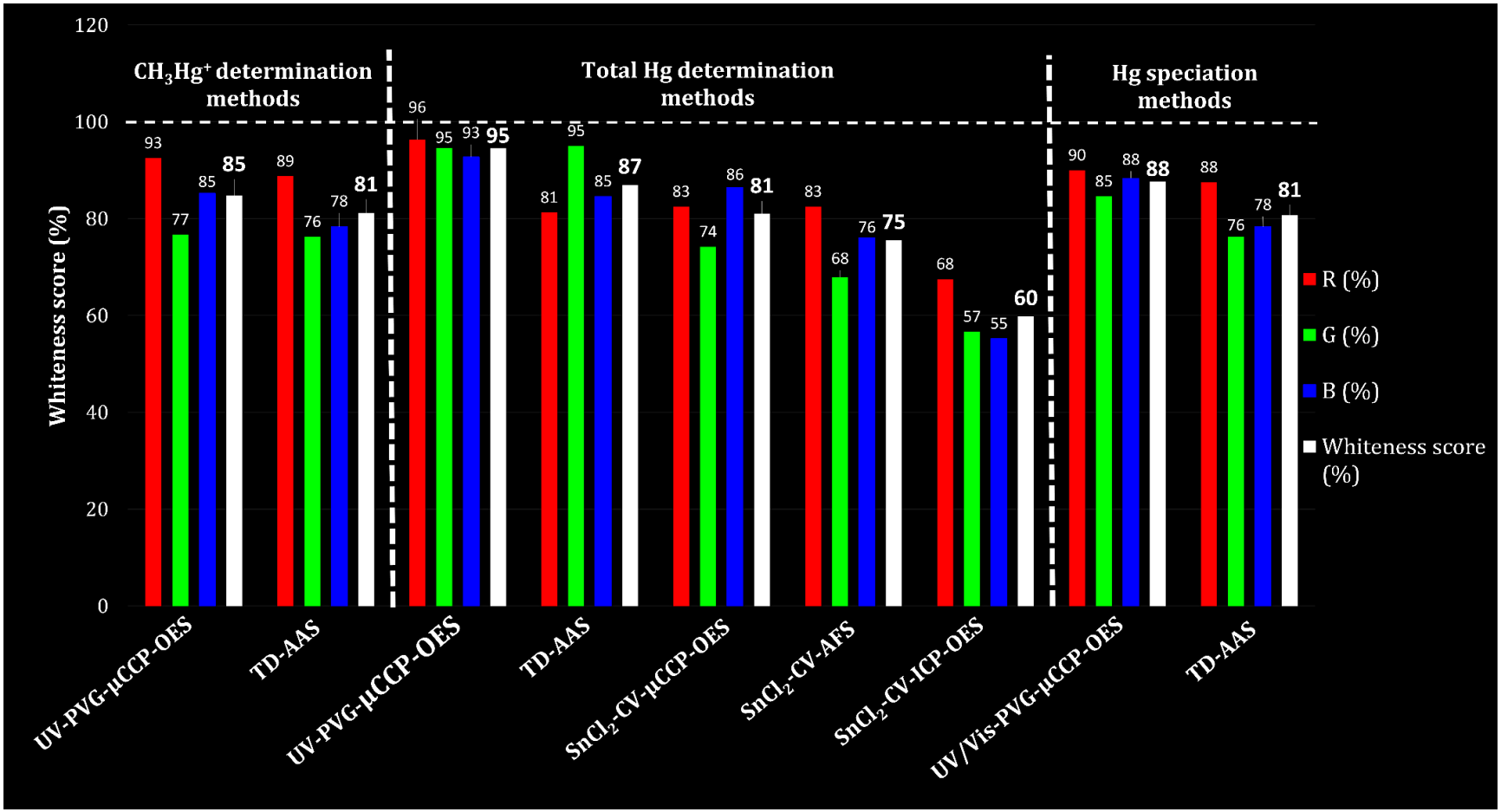


Figure 7.1. Comparison of the overall whiteness character of the methods using the RGB-12 model based on WAC[7,98]

7.3. Conclusions

The outcomes of the study were as follows:

1. The greenness of the UV–PVG-μCCP-OES method for Hg determination and speciation assessed using different tools is in the range 25–75% (NEMI), 61–85% (AES) and 55–65% (AGREE), compared to 25–50% in $SnCl_2$-CV-AFS and $SnCl_2$-CV-ICP-OES methods (NEMI), 66–68% in $SnCl_2$-CV-ICP-OES and $SnCl_2$-CV-AFS (AES), and 44% in $SnCl_2$-CV-ICP-OES and 49% in $SnCl_2$-CV-AFS (AGREE). Thus, compared to $SnCl_2$-CV-AFS and $SnCl_2$-CV-ICP-OES as traditional methods, the UV/Vis-PVG-μCCP-OES method exhibits very good/excellent greenness compared to the poor value, below 50% in ICP-OES.
2. Among the developed methods, that for CH_3Hg^+ quantification by UV-PVG-μCCP-OES shows the lowest greenness profile as a result of the use of corrosive and hazardous reagents in the double liquid-liquid extraction with HBr–toluene–L-cysteine solution.
3. The greenness of the UV-PVG-μCCP-OES method for total Hg in seafood and foods of vegetable and animal origin is substantially improved due to the use of HCOOH for both extraction and photo-induced derivatization instead of conventional mineralization with corrosive reagents (HNO_3–H_2O_2 mixture) and derivatization with $SnCl_2$ in HCl medium. Compared to $SnCl_2$-CV-ICP-OES, the UV-PVG-μCCP-OES method is much greener due to a much lower consumption of energy and Ar, as well as the use of miniaturized instrumentation.
4. The UV/Vis–PVG-μCCP-OES method for Hg speciation exhibits excellent greenness, namely 75% by NEMI tool, 78% by AES, and very good greenness degree of 65% according to AGREE procedure.
5. The UV-PVG-μCCP-OES method for the determination of CH_3Hg^+ shows very low greenness of 25% (NEMI) but similar to TD-AAS method, explained by the use of the same extraction procedure involving the system HBr–toluene–L–cysteine. The TD-AAS method has higher scores for Hg determination in seafood (direct analysis on solid without using reagents), namely 100% (NEMI), 91% (AES), 79% (AGREE). On the other hand, the method is less sensitive than UV-PVG-μCCP-OES using extraction and derivatization in HCOOH, and thus could not be taken as reference for the analysis of foods of vegetable and animal origin and water.
6. Of the 4 approaches used for greenness assessment, the GAPI tool seems to be the most problematic and subjective, as it provides only pictograms without numerical data. Thus, an objective greenness assessment of an analytical method in a decreasing order of tools is: AES > AGREE > GAPI > NEMI.

7. Some shortcomings were observed in greenness assessment tools, namely: NEMI tool takes into account only the reagents used for sample preparation and ignores the steps of the analysis (instrumentation). The AGREE procedure, although very recent, uses criteria from the 12 principles of GAC, mainly related to chromatographic methods and less for the spectrometric ones, and thus, some aspects are not considered in the AGREE software. It is of interest whether the instrumentation is automated or not and penalty points are assigned only for total energy consumption, not on distinct steps of analysis. The AES procedure proved to be the most suitable as it is based on assigning well-established penalty points to parameters of the analytical process, such as sample preparation, instrumentation, waste, working conditions, etc.
8. The whiteness of the new methods was assessed for the first time based on the RGB-12 model, which assigns a color resulting from the share of individual primary colors, red (analytical efficiency), green (environmental friendliness and safety) and blue (practical and economic aspects). The UV/Vis-PVG-μCCP-OES methods show a whiteness score of 81–95%, far superior to conventional $SnCl_2$-CV-AFS method (75%) and especially compared to $SnCl_2$-CV-ICP-OES (60%), but similar to TD-AAS (81–88%). It was emphasized the important role of the miniaturized instrumentation by the economic benefits associated to low energy and Ar consumption and greener methods for extraction and photo-induced derivatization using only HCOOH.
9. The main contribution of the present study consists in assessing for the first time greenness and whiteness degree of methods used for Hg determination and speciation in comparison to conventional methods through an integrated approach. This provides an objective positioning in terms of individual inputs corresponding to red, green and blue scores attributed to analytical performance, reagents, sample preparation, economic benefits, energy and Ar consumption, waste, instrumentation (miniaturization, automation, portability) and applicability. It was highlighted the advantage of greener methods used for extraction and ultrasonic-assisted/photo-induced derivatization, as well as the economic benefits related to fully miniaturized instrumentation with a microplasma source with much lower Ar and energy consumption than the widely used laboratory instrumentation (ICP-OES).

8. Originality and innovative contributions of the thesis. General conclusions.

The following original results and innovative contributions were obtained within the doctoral program:

1. Four methods have been developed for the determination of CH_3Hg^+, total Hg and speciation as CH_3Hg^+ and Hg^{2+} in seafood, foods of vegetable and animal origin, environmental samples, based on optical emission spectrometry in a capacitively coupled plasma microtorch (μCCP-OES), interfaced with low-resolution microspectrometers; 1 procedure for CH_3Hg^+ determination exhibits medium greenness, while 3 procedures for total Hg and its speciation as CH_3Hg^+ and Hg^{2+} are excellent green.
2. A simple home-made photoreactor for photo-induced cold vapor generation from CH_3Hg^+, total Hg species and Hg^{2+} in the presence/absence of UV light from a 500 W Hg lamp was interfaced for the first time with a low energy and Ar consumption capacitively coupled microplasma. The photoreactor was operated in continuous mode, with/without *on-line* preconcentration of Hg vapor on a gold filament microcollector for enhancing method sensitivity. The preconcentration step did not complicate the procedure, as there was an *on-line* coupling between the photoreactor and gold microcollector, with the recording of the episode emission spectra.
3. It has been shown that the UV photo-induced derivatization occurs instantly with the same efficiency and rate for both CH_3Hg^+ and Hg^{2+}, which made it possible to use external calibration for their quantification, thus simplifying the procedure for Hg determination and speciation.
4. A method for Hg speciation as CH_3Hg^+ and Hg^{2+} was developed using only HCOOH, both for sample extraction and UV/Vis photo-induced derivatization, thus eliminating the toxic and unstable reagents. The low rate of derivatization of the Hg^{2+} species in the absence of UV-light and the low sensitivity were compensated by the *on-line* preconcentration of Hg vapor on the Au filament microcollector.
5. A method for the determination of CH_3Hg^+ and total Hg in seafood has been proposed using only HCOOH for extraction and cold vapor generation, as an alternative to the procedure recommended by the European Commission for the determination of CH_3Hg^+, based on double extraction in 47% HBr–toluene–1% L-cysteine solution and determination by TD-AAS.
6. The methods for determining Hg in seafood have been extended to foods of animal and vegetable origin; even though the concentration of Hg in such foods is much lower, they are consumed in greater quantities than seafood, and thereby may contribute to Hg exposure.

7. All the methods for total Hg and its speciation based on classical derivatization or photo-induced in HCOOH medium have been validated in compliance with the European legislation in Decisions 2007/333/CE and 2002/657/CE on the determination of Hg in seafood. Also, statistical comparisons were made with the traditional methods for Hg determination (TD-AAS, $SnCl_2$-CV-AFS and $SnCl_2$-CV-ICP-OES) using the Bland and Altman test.
8. The Bland and Altman statistics together with the achieved analytical performance showed that the methods for the determination of CH_3Hg^+, total Hg and its speciation as CH_3Hg^+ and Hg^{2+} in food are viable alternative to TD-AAS, $SnCl_2$-CV-AFS and $SnCl_2$-CV-ICP-OES methods.
9. By using three integrated procedures (2 recommended by EFSA and 1 by USEPA) it was concluded that there is no health risk by fish consumption; thus, 2-3 servings of 150 g of ocean fish (weekly) and practically an unlimited amount of farmed fish (daily) consumed by an adult of 60 kg body weight do not represent any risk; there is no risk for children of 15 kg body weight when consuming 20 g servings of the analyzed fish varieties.
10. The studies have highlighted the significance of Hg speciation as CH_3Hg^+ and Hg^{2+} in fish for a realistic assessment of risk exposure, given the much higher toxicity of CH_3Hg^+ (PTWI 1.3 μg kg^{-1}/body weight), compared to Hg^{2+} (PTWI 4 μg kg^{-1}/body weight).
11. The results of the assessment of green degree of UV/Vis-PVG-μCCP-OES methods using the four tools NEMI, AES, GAPI and AGREE, and white degree using the RGB-12 model, respectively show that the new developed methods have higher scores when using only HCOOH for extraction and derivatization, then CV-AFS, and especially CV-ICP-OES based on microwave-assisted mineralization with HNO_3 and H_2O_2 followed by classical derivatization with $SnCl_2$ in HCl medium. The UV/Vis-PVG-μCCP-OES methods are greener due to the use of miniaturized instrumentation, lower operation cost and better LODs when using Hg vapor preconcentration.
12. The study carried out within the doctoral thesis has clearly highlighted the viability and fiability of the UV/Vis-PVG-μCCP-OES methods for the determination and speciation of Hg in food as alternatives to the traditional methods based on CV-ICP-OES, CV-AFS and TD-AAS.
13. The results obtained during in this doctoral thesis were published in 5 ISI papers, with impact factor sum of 20,896, of which 4 as first author in Q1 journals. The results were also presented at 9 international conferences, of which 2 as scientific communications in proceedings, 2 as oral presentations and 5 as posters.

Bibliography

1. P. T. Anastas şi J. C. Warner, *Green chemistry: Theory and Practice*, Oxford University Press, New York, 1998.
2. A. Gałuszka, Z. Migaszewski şi J. Namieśnik, *TrAC, Trends Anal. Chem.*, 2013, **50**, 78-84.
3. L. H. Keith, L. U. Gron şi J. L. Young, *Chem. Rev.*, 2007, **107**, 2695-2708.
4. A. Gałuszka, Z. M. Migaszewski, P. Konieczka şi J. Namieśnik, *TrAC, Trends Anal. Chem.*, 2012, **37**, 61-72.
5. J. Płotka-Wasylka, *Talanta*, 2018, **181**, 204-209.
6. F. Pena-Pereira, W. Wojnowski şi M. Tobiszewski, *Anal. Chem.*, 2020, **92**, 10076-10082.
7. P. M. Nowak, R. Wietecha-Posłuszny şi J. Pawliszyn, *TrAC, Trends Anal. Chem.*, 2021, **138**, article number 116223.
8. P. M. Nowak şi P. Kościelniak, *Anal. Chem.*, 2019, **91**, 10343-10352.
9. C. Bendicho, I. Lavilla, F. Pena-Pereira şi V. Romero, *J. Anal. At. Spectrom.*, 2012, **27**, 1831-1857.
10. X. Yuan, J. Tang şi Y. Duan, *Appl. Spectrosc. Rev.*, 2011, **46**, 581-605.
11. R. Foest, M. Schmidt şi K. Becker, *Int. J. Mass Spectrom.*, 2006, **248**, 87-102.
12. F. Meng, X. Yuan, X. Li, Y. Liu şi Y. Duan, *Appl. Spectrosc. Rev.*, 2014, **49**, 533-549.
13. P. Pohl, K. Greda, A. Dzimitrowicz, M. Welna, A. Szymczycha-Madeja, A. Lesniewicz şi P. Jamroz, *TrAC, Trends Anal. Chem.*, 2019, **113**, 234-245.
14. European Food Safety Authority (EFSA), Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA J.* 2012, **10**(12):2985, 1-241, https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2012.2985, (accessed 15.11.2016).
15. N. Pirrone, S. Cinnirella, X. Feng, R. B. Finkelman, H. R. Friedli, J. Leaner, R. Mason, A. B. Mukherjee, G. Stracher, D. G. Streets şi K. Telmer, in *Mercury Fate and Transport in the Global Atmosphere: Emissions, Measurements and Models*, eds. R. Mason and N. Pirrone, Springer US, Boston, MA, 2009, DOI: 10.1007/978-0-387-93958-2_1, pp. 1-47.
16. D. Mergler, H. A. Anderson, L. H. Chan, K. R. Mahaffey, M. Murray, M. Sakamoto şi A. H. Stern, *Ambio*, 2007, **36**, 3-11.
17. *National Research Council Committee on the Toxicological Effects of Methylmercury. Toxicological Effects of Methylmercury*, National Academies Press (US), Washington D.C., USA, 2000.
18. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, Official Journal of the European Union, 2006, L1364/1885.
19. Commission Regulation (EC) No 629/2008 of 2 July 2008 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs, Official Journal of the European Union, 2008, L173/6.
20. EFSA, *EFSA J.*, 2015, **13**, article number 3982.
21. K. B. S. Perelonia, K. C. D. Benitez, R. J. S. Banicod, G. C. Tadifa, F. D. Cambia şi U. M. Montojo, *Food Control*, 2021, **130**, article number 108363.
22. D. Kozaki, M. Mori, S. Hamasaki, T. Doi, S. Tanihata, A. Yamamoto, T. Takahashi, K. Sakamoto şi S. Funado, *Anal. Methods*, 2021, **13**, 1106-1109.
23. Y. C. Liu, J. Zou, B. Luo, H. R. Yu, Z. G. Zhao şi H. Xia, *Microchem. J.*, 2021, **169**, article number 106547.
24. M. T. Shi, X. A. Yang şi W. B. Zhang, *Anal Chim Acta*, 2019, **1074**, 33-42.

25. X. Zhu şi S. D. Alexandratos, *Microchem. J.*, 2007, **86**, 37-41.
26. S. Kulomäki, S. Perämäki şi A. Väisänen, *Talanta*, 2020, **218**, article number 121125.
27. M. Senila, O. Cadar, A. Drolc, A. Pintar, L. Senila şi T. Frentiu, *Stud. Univ. Babes-Bolyai, Chem.*, 2016, **61**, 321-332.
28. P. Pohl, *TrAC, Trends Anal. Chem.*, 2004, **23**, 87-101.
29. Y. Gao, R. Liu şi L. Yang, *Chin. Sci. Bull.*, 2013, **58**, 1980-1991.
30. P. Hu, X. Wang, Z. Wang, R. Dai, W. Deng, H. Yu şi K. Huang, *TrAC, Trends Anal. Chem.*, 2019, **119**, article number 115617.
31. K. Anderson, *Mercury Analysis in Environmental Samples by Cold Vapor Techniques, in Encyclopedia of analytical chemistry*, John Wiley & Sons Ltd, Chichester, United Kingdom, 2006.
32. P. J. Craig, R. O. Jenkins şi G. H. Stojak, *Chemosphere*, 1999, **39**, 1181-1197.
33. E. Kenduzler, M. Ates, Z. Arslan, M. McHenry şi P. B. Tchounwou, *Talanta*, 2012, **93**, 404-410.
34. R. E. Sturgeon, *J. Anal. At. Spectrom.*, 2017, **32**, 2319-2340.
35. Y. Yin, J. Liu şi G. Jiang, *TrAC, Trends Anal. Chem.*, 2011, **30**, 1672-1684.
36. M. Ślachciński, *J. Anal. At. Spectrom.*, 2019, **34**, 257-273.
37. R. Cornelis, J. Caruso, H. Crews şi K. Heumann, *Handbook of Elemental Speciation II: Species in the Environment, Food, Medicine and Occupational Health*, John Wiley & Sons Ltd., Chichester, United Kingdom, 2005.
38. CODEX STAN 193-1995, Codex General Standard for Contaminants and Toxins in Food and Feed, 2009, pp. 1991-1944.
39. A. S. Ribeiro, M. A. Vieira, S. Willie şi R. E. Sturgeon, *Anal Bioanal Chem*, 2007, **388**, 849-857.
40. G. Chen, B. Lai, N. Mei, J. Liu şi X. Mao, *Spectrochim. Acta, Part B*, 2017, **137**, 1-7.
41. L. Wu, Z. Long, L. Liu, Q. Zhou, Y.-I. Lee şi C. Zheng, *Talanta*, 2012, **94**, 146-151.
42. C. Han, C. Zheng, J. Wang, G. Cheng, Y. Lv şi X. Hou, *Anal. Bioanal. Chem*, 2007, **388**, 825-830.
43. S. Zhang, H. Luo, M. Peng, Y. Tian, X. Hou, X. Jiang şi C. Zheng, *Anal Chem*, 2015, **87**, 10712-10718.
44. H. Matusiewicz şi M. Ślachciński, *Spectrochim. Acta, Part B*, 2017, **133**, 52-59.
45. I. Baer, M. Baxter, V. Devesa, D. Vélez, G. Raber, R. Rubio, T. Llorente-Mirandes, J. J. Sloth, P. Robouch şi B. de la Calle, *Food Control*, 2011, **22**, 1928-1934.
46. S. G. J. Calderon, F. Cordeiro, B. de la Calle, Determination of methylmercury in seafood by direct mercury analysis: Standard operating procedure. JRC Technical Reports, European Comission, Institute for Reference Materials and Measurements, Geel, Belgium, https://ec.europa.eu/jrc/sites/jrcsh/files/Full%20JRC%20Tecnical%20report%20SOP.pdf, (accessed 15.11.2016).
47. E. Covaci, M. Senila, M. Ponta, E. Darvasi, D. Petreus, M. Frentiu şi T. Frentiu, *Talanta*, 2017, **170**, 464-472.
48. R. E. Sturgeon şi P. Grinberg, *J. Anal. At. Spectrom.*, 2012, **27**, 222-231.
49. D. Leonori şi R. E. Sturgeon, *J. Anal. At. Spectrom.*, 2019, **34**, 636-654.
50. P. Blanc, A. Burnol, N. Marty, J. Hellal, V. Guérin şi V. Laperche, *Sci. Total Environ.*, 2018, **621**, 368-375.
51. J. N Miller şi J. Charlotte Miller, *Statistics and Chemometrics for Analytical Chemistry*, Pearson Education Ltd, Edinburgh Gate, England, 2005.

52. Commission Regulation (EC) no. 333/2007 of 28 march 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs, Official Journal of the European Union, 2007, L88/29.
53. Commission Decision of 12 august 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Official Journal of the European Communities, 2002, L221/8.
54. Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals, Association of Official Agricultural Chemists (AOAC), AOAC Official Methods of Analysis, http://www.eoma.aoac.org/app_k.pdf, (accessed 16.12.2019).
55. SR ISO 1443:2008 Meat and meat products - determination of total fat content.
56. E. Covaci, M. Senila, M. Ponta, E. Darvasi, M. Frentiu şi T. Frentiu, *Food Control*, 2017, **82**, 266-273.
57. S. Díez şi J. M. Bayona, *Talanta*, 2008, **77**, 21-27.
58. A. Gonzalvez, M. L. Cervera, S. Armenta şi M. de la Guardia, *Anal Chim Acta*, 2009, **636**, 129-157.
59. K. Leopold, M. Foulkes şi P. Worsfold, *Anal. Chim. Acta*, 2010, **663**, 127-138.
60. Y. Gao, Z. Shi, Z. Long, P. Wu, C. Zheng şi X. Hou, *Microchem. J.*, 2012, **103**, 1-14.
61. Z. H. Fernández, L. A. Valcárcel Rojas, A. M. Álvarez, J. R. Estevez Álvarez, J. Araújo dos Santos, I. P. González, M. R. González, N. A. Macias, D. L. Sánchez şi D. H. Torres, *Food Control*, 2015, **48**, 37-42.
62. C. Galimberti, I. Corti, M. Cressoni, V. Moretti, S. Menotta, U. Galli şi D. Cambiaghi, *Food Control*, 2016, **60**, 329-337.
63. F. A. Duarte, C. A. Bizzi, F. G. Antes, V. L. Dressler şi E. M. M. de Flores, 2009, **64**, 513-519.
64. I. Serafimovski, I. Karadjova, T. Stafilov şi J. Cvetković, *Microchem. J.*, 2008, **89**, 42-47.
65. T. Frentiu, S. Butaciu, M. Ponta, M. Senila, E. Darvasi, M. Frentiu şi D. Petreus, *Food Anal. Methods*, 2015, **8**, 643-648.
66. L. E. Kaercher, F. Goldschmidt, J. N. G. Paniz, É. M. de Moraes Flores şi V. L. Dressler, *Spectrochim. Acta, Part B*, 2005, **60**, 705-710.
67. S. Molaei, S. Seidi, A. Saleh şi V. Ghoulipour, *Anal. Methods*, 2017, **9**, 5189-5197.
68. S. Seidi şi M. Fotouhi, *Anal. Methods*, 2017, **9**, 803-813.
69. H. Shirkhanloo, A. Khaligh, H. Z. Mousavi şi A. Rashidi, *Microchem. J.*, 2017, **130**, 245-254.
70. I. Lavilla, V. Romero, I. Costas şi C. Bendicho, *TrAC, Trends Anal. Chem.*, 2014, **61**, 1-10.
71. D. Qin, F. Gao, Z. Zhang, L. Zhao, J. Liu, J. Ye, J. Li şi F. Zheng, *Spectrochim. Acta, Part B*, 2013, **88**, 10-14.
72. R. Liu, M. Xu, Z. Shi, J. Zhang, Y. Gao şi L. Yang, *Talanta*, 2013, **117**, 371-375.
73. R. Zhang, M. Peng, C. Zheng, K. Xu şi X. Hou, *Microchem. J.*, 2016, **127**, 62-67.
74. M. A. Vieira, A. S. Ribeiro, A. J. Curtius şi R. E. Sturgeon, *Anal. Bioanal. Chem*, 2007, **388**, 837-847.
75. A. V. Zmozinski, S. Carneado, C. Ibáñez-Palomino, À. Sahuquillo, J. F. López-Sánchez şi M. M. da Silva, *Food Control*, 2014, **46**, 351-359.
76. H. Li, Z. Xu, L. Yang şi Q. Wang, *J. Anal. At. Spectrom.*, 2015, **30**, 916-921.
77. C. Scriver, M. Kan, S. Willie, C. Soo şi H. Birnboim, *Anal. Bioanal. Chem*, 2005, **381**, 1460-1466.
78. M. Kan, S. N. Willie, C. Scriver şi R. E. Sturgeon, *Talanta*, 2006, **68**, 1259-1263.
79. E. Covaci, M. Senila, C. Tanaselia, S. B. Angyus, M. Ponta, E. Darvasi, M. Frentiu şi T. Frentiu, *J. Anal. At. Spectrom.*, 2018, **33**, 799-808.

80. T. Frentiu, S. Butaciu, E. Darvasi, M. Ponta, M. Senila, D. Petreus şi M. Frentiu, *Anal. Methods*, 2015, **7**, 747-752.
81. T. Frenţiu, E. Darvasi, S. Butaciu, S. Cadar, M. Şenilă, A. Mathe, M. Frenţiu, D. Petreuş, R. Etz, F. Puskas, D. Şulea, Automated analyzer for the simultaneous determination of elements in liquid microsamples by optical emission spectrometry, registration number RO131066 B1 30.07.2020.
82. A. Kabata-Pendias, *Trace elements in soils and plants*, CRC Press, Taylor & Francisc Group, Boca Raton, USA, 4th edn., 2011.
83. Y. C. Guangliang Liu, Nelson O'Driscoll, *Environmental chemistry and toxicology of mercury*, John Wiley & Sons, Inc., Hoboken, NJ, 2012.
84. Mercury Statistics and Information. United States Geological Survey (USGS), 2020, https://pubs.usgs.gov/periodicals/mcs2020/mcs2020-mercury.pdf, (accessed 27.08.2020).
85. E. Garcia-Ordiales, S. Covelli, J. M. Rico, N. Roqueñí, G. Fontolan, G. Flor-Blanco, P. Cienfuegos şi J. Loredo, *Chemosphere*, 2018, **198**, 281-289.
86. L. Zhao, G. Qiu, C. W. N. Anderson, B. Meng, D. Wang, L. Shang, H. Yan şi X. Feng, *Environ. Pollut.*, 2016, **215**, 1-9.
87. Hydra II$_C$ Mercury Analyzer Brochure, Teledyne Leeman Labs, Hudson, USA, http://www.teledyneleemanlabs.com/resource/Documents/Hydra%20II%20C%20Brochure.pdf, (accessed 15.01.2019).
88. H. Xia, X. Liu, K. Huang, Y. Gao, L. Gan, C. He şi X. Hou, *Spectrosc. Lett.*, 2010, **43**, 550-554.
89. L. Liu, H. Zheng, C. Yang, L. Xiao, Y. Zhangluo şi J. Ma, *Spectrosc. Lett.*, 2014, **47**, 604-610.
90. R. Zhang, B. Shen, C. Li, C. Zheng şi X. Hou, *Microchem. J.*, 2016, **129**, 98-103.
91. J. Hu, E. Pagliano, X. Hou, C. Zheng, L. Yang şi Z. Mester, *J. Anal. At. Spectrom.*, 2017, **32**, 2447-2454.
92. T. Narukawa şi S. Willie, *J. Anal. At. Spectrom.*, 2010, **25**, 1145-1148.
93. Research project - Analytical eco-scale for the determination and speciation of Hg by UV photo-induced derivatization and detection using a fully miniaturized experimental spectrometric system (ECOSPEC), project no. PN-III-P2-2.1-PED-2016-0135, contract no. 93PED/2017 http://www.chem.ubbcluj.ro/~edarvasi/Proiect2012/documente/Romana/Proceduri%2020analitice%2020de%2020determinare%2020si%2020speciere%2020a%2020mercurului.pdf, (accessed 20.06.2020).
94. E. Covaci, S. B. Angyus, M. Senila, M. Ponta, E. Darvasi, M. Frentiu şi T. Frentiu, *Microchem. J.*, 2018, **141**, 155-162.
95. Commission Regulation (EU) No 836/2011 of 19 August 2011 amending Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs, Official Journal of the Europan Union, 2011, L 215/9.
96. M. de la Guardia şi S. Garrigues, *Challenges in Green Analytical Chemistry* The Royal Society of Chemistry, Cambridge England, 2nd edn., 2020.
97. J. A. Tickner şi M. Becker, *Curr. Opin. Green Sustain. Chem.*, 2016, **1**, 1-4.
98. E. Covaci şi T. Frentiu, *Stud. Univ. Babes-Bolyai, Chem.*, 2022, **67**, DOI: 10.24193/subbchem.22022.24191.24101.

List of published papers during the Ph.D. programme

Nr.	Article	Impact factor	Influence factor
1	**E. Covaci**, M. Şenilă, M. Ponta, E. Darvasi, D. Petreuş, M. Frenţiu, T. Frenţiu. Methylmercury determination in seafood by photochemical vapor generation capacitively coupled plasma microtorch optical emission spectrometry *Talanta*, **2017**, 170, 464–472	6.057 (Q1)	1.581
2	**E. Covaci**, M. Şenilă, M. Ponta, E. Darvasi, M. Frenţiu, T. Frenţiu. Mercury speciation using non-chromatographic chemical vapor generation capacitively coupled plasma microtorch optical emission spectrometry method – Evaluation of methylmercury exposure *Food Control*, 2017, **82**, 266–273	5.548 (Q1)	1.889
3	**E. Covaci**, M. Şenilă, C. Tănăselia, S.B. Angyus, M. Ponta, E. Darvasi, M. Frenţiu, T. Frenţiu. Highly sensitive eco-scale method for mercury determination in water and food using photochemical vapor generation and miniaturized instrumentation for capacitively coupled plasma microtorch optical emission spectrometry *Journal of Analytical Atomic Spectrometry,* 2018, **33**, 799–808	4.023 (Q1)	1.572
4	**E. Covaci**, S.B. Angyus, M. Şenilă, M. Ponta, E. Darvasi, M. Frenţiu, T. Frenţiu. Eco-scale non-chromatographic method for mercury speciation in fish using formic acid extraction and UV/Vis photochemical vapor generation capacitively coupled plasma microtorch optical emission spectrometry *Microchemical Journal,* 2018, **141**, 155-162	4.821 (Q1)	1.170
5	**E. Covaci**, T. Frenţiu Greenness and whiteness profiles of UV/Vis photochemical vapor generation capacitively coupled plasma microtorch optical emission spectrometry method for mercury determination and speciation in food and water *Studia Universitatis Babes-Bolyai Chemia*, 2022, **67**, DOI: 10.24193/subbchem.2022.1.01 (accepted for publication)	0.447 (Q4)	0.097
	Sum of impact and influence factors	**20.896**	**6.309**

List of scientific communications published in proceedings

Nr.	Article
1	M. Şenilă, **E. Covaci**, O. Cadar, M. Ponta, M. Frenţiu, T. Frenţiu. Mercury speciation in fish tissue by thermal decomposition atomic absorption spectrometry: method validation and risk assessment to methylmercury exposure. Proceedings 44th International Conference of the Slovak Society of Chemical Engineering, Demanovska Dolina, 22-26 May **2017**, Pe-We-4, 006.pdf, pp. 520-528
2	**E. Covaci,** M. Şenilă, E. Darvasi, M. Ponta, M. Frenţiu, C. Tănăselia, T. Frenţiu. A non-chromatographic method for the determination of methylmercury in fish fillet using optical emission spectrometry in a capacitively coupled plasma microtorch after UV photo-induced derivatization. Proceedings 44th International Conference of the Slovak Society of Chemical Engineering, Demanovska Dolina, Slovakia, 22-26 May **2017**, Pe-We-4, 007.pdf, pp. 529-536

List of scientific communications in which the results of the doctoral thesis were disseminated

Nr.	**Conferences**	**Dissemination methods**
1	**E. Covaci**, T. Frenţiu, M. Şenilă, M. Ponta, E. Darvasi Green analytical methods for mercury determination and speciation using capacitively coupled microplasma optical emission spectrometry. Greenness assessment using the AGREE calculator. Young Researchers International Conference on Chemistry and Chemical Engineering (YRICCCE III), Cluj-Napoca, Romania, 4–5 June **2021**	**Oral presentation**
2	**E. Covaci**, M. Şenilă, M. Ponta, E. Darvasi, M. Frenţiu, T. Frenţiu. Eco-scale speciation of Hg^{2+} and CH_3Hg^+ by capacitively coupled plasma optical emission spectrometry and UV/Vis photo-induced derivatization (UV-Vis-PVG-µCCP-OES) National Conference of Doctoral Schools from the Universitaria Consortium, II edition, Timişoara, Romania, 11-14 November **2019**	**Oral presentation**

3 **E. Covaci** **Oral**

Determination and speciation of Hg in seafood using eco-scale methods: principle of the methods and dedicated miniaturized spectral instrumentation. **presentation**

XXIII International Conference of Chemistry, Deva, Romania, October **2017**

4 T. Frenţiu, **E. Covaci**, E. Darvasi, M. Ponta. **Poster**

Total mercury determination in water using on-line UV photochemical vapor generation capacitively coupled plasma microtorch optical emission spectrometry

The 45th International Conference of the Slovak Society of Chemical Engineering, Tatranske Matliare, Slovakia, 21-25 May **2018**

5 E. Darvasi, **E. Covaci**, M. Şenilă, M. Ponta, L.D. Pop, M. Frenţiu, T. Frenţiu. **Poster**

Determination of total Hg in fish using photo-induced cold vapor generation capacitively coupled plasma microtorch optical emission spectrometry. Comparison to classical approaches.

XXIII International Conference of Chemistry, Deva, Romania, October **2017**

6 **E. Covaci**, E. Darvasi, M. Şenilă, M. Ponta, I. Băbuţan, M. Frenţiu, T. Frenţiu. **Poster**

Evaluation of human risk exposure to methylmercury via fish consumption

XXIII International Conference of Chemistry, Deva, Romania, October **2017**

7 **E. Covaci**, M. Şenilă, E. Darvasi, M. Ponta, M. Frenţiu, C. Tănăselia, T. Frenţiu. **Poster**

A non-chromatographic method for the determination of methylmercury in fish fillet using optical emission spectrometry in a capacitively coupled plasma microtorch after UV photo-induced derivatization

The 44th International Conference of the Slovak Society of Chemical Engineering, Demanovska Dolina, Slovakia, 22-26 May **2017**

8 M. Şenilă, **E. Covaci**, O. Cadar, M. Frenţiu, T. Frenţiu. **Poster**

Mercury speciation in fish tissue by thermal decomposition atomic absorption spectrometry: method validation and risk assessment to methylmercury exposure

The 44th International Conference of the Slovak Society of Chemical Engineering, Demanovska Dolina, Slovakia, 22-26 May **2017**

Other articles published during the doctoral program, which were not included in this doctoral thesis

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1	M. Şenilă, **E. Covaci**, O. Cadar, M. Ponta, M. Frenţiu, T. Frenţiu. Mercury speciation in fish tissue by eco-scale thermal decomposition atomic absorption spectrometry: method validation and risk exposure to methylmercury *Chemical Papers,* **2018**, 72, 441–448	2.097	0.460