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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

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ABSTRACT. The aim of the study was the evaluation of greenness and whiteness profiles of UV/Vis photo-induced cold vapor generation (UV/Vis-PVG) capacitively coupled plasma microtorch optical emission spectrometry methods for Hg determination and speciation as CH_3Hg^+ and Hg^{2+}. Sample preparation for CH_3Hg^+ determination in fish tissue consisted of an extraction in HBr–toluene–aqueous L-cysteine solution and UV-PVG in 0.6 mol L^{-1} HCOOH. Total Hg was determined in food samples following ultrasound assisted extraction in concentrated HCOOH and UV-PVG. Hg speciation was based on extraction in HCOOH and UV/Vis selective derivatization of total Hg/Hg^{2+}. The greenness profile was assessed by National Environmental Methods Index, Analytical Eco-Scale, Green Analytical Procedure Index and Analytical Greenness Metric, while the whiteness profile was evaluated using the Red–Green–Blue (RGB) 12 algorithm. The methods, based on miniaturized instrumentation, were characterized by a higher greenness and whiteness compared to the traditional $SnCl_2$ cold vapor generation inductively coupled plasma optical emission spectrometry, $SnCl_2$ cold vapor generation atomic fluorescence spectrometry, and sometimes than that of thermal decomposition atomic absorption spectrometry. This study is a novelty because, to the best of our knowledge, is the first approach of this kind for Hg determination and speciation based on optical emission spectrometry using a fully miniaturized instrumentation.

Keywords: *greenness and whiteness profile, mercury speciation, capacitively coupled plasma microtorch*

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INTRODUCTION

Several national and international organizations, such as the United States Environmental Protection Agency (1970) [1] and the World Commission on Environment and Development (1983) [2] have been established with the aim to develop an appropriate legislative framework to prevent and reduce environmental pollution. Anastas and Warner introduced for the first time in 1998 the concept of Green Chemistry or Sustainable Chemistry, and laid down its 12 principles [3]. Namiesnik and his coworkers introduced for the first time the concept of Green Analytical Chemistry (GAC) in 2012 [4]. The 12 GAC principles provide the reduction or complete elimination of reagents for sample preparation, preservation and analysis, decrease of energy consumption, efficient waste treatment and increase operator safety [4]. Numerous analytical methods have been developed, which were considered green by the authors, but unfortunately without clearly defined metrics for evaluation. Therefore, the assessment was often subjective and not properly justified [5, 6]. As a consequence, several objective procedures for the greenness profile have been developed, such as the National Environmental Methods Index (NEMI) [7], the pictogram developed by Raynie *et al*. [8], Analytical Eco-Scale (AES) [9], Green Analytical Procedure Index (GAPI) [10], Hexagon pictogram [11], Analytical Method Greenness Score (AMGS) [12] and the Analytical Greenness metric (AGREE) [13]. The greenness evaluation procedures were applied mainly for chromatographic methods, in which a significant number of reagents are used, and less for non-chromatographic methods [14–20]. These procedures are based on the establishment of the green profile, usually on some economic and sample preparation aspects and do not take into account other important aspects of the analytical methods, such as analytical performance, level of utility and applicability, which should be as wide as possible.

Due to these shortcomings, Nowak and his coworkers recently introduced the White Analytical Chemistry (WAC) concept and its 12 principles [21], based on Red (analytical performance), Green (green chemistry) and Blue (practical aspects) principles, known as the RGB 12 procedure, for the evaluation of the analytical methods in a global manner [6, 21, 22].

The aim of the study was the assessment of greenness and whiteness profiles of 3 methods based on UV/Vis photochemical vapor generation capacitively coupled microtorch optical emission spectrometry (UV/Vis-PVG-μCCP-OES) for total Hg determination and its speciation as CH_3Hg^+ and Hg^{2+} in food and water samples. The multiple metrics, based on NEMI, AES, GAPI and AGREE were applied for greenness profile, while the RGB 12 approach

was used for whiteness profile assessment. The procedure was applied for CH_3Hg^+ speciation in fish tissue samples by UV-PVG-μCCP-OES using the selective extraction in HBr–toluene–aqueous L-cysteine solution, in comparison with the thermal decomposition atomic absorption spectrometry (TD-AAS) method, recommended by the European Commission for CH_3Hg^+ determination in such foods [23, 24]. The second method, UV-PVG-μCCP-OES was evaluated for total Hg determination in water and diverse food, such as fish tissue, animal meat and organs, vegetables, fruits and food supplements, in comparison with $SnCl_2$ cold vapor generation capacitively coupled microtorch optical emission spectrometry ($SnCl_2$-CVG-μCCP-OES), $SnCl_2$ cold vapor generation inductively coupled plasma atomic emission spectrometry ($SnCl_2$-CVG-ICP-OES), $SnCl_2$ cold vapor generation atomic fluorescence spectrometry ($SnCl_2$-CVG-AFS) and TD-AAS based on direct solid sampling. The greenness and whiteness profile of UV/Vis-PVG-μCCP-OES method was assessed for Hg speciation as CH_3Hg^+ and Hg^{2+} in fish samples. Details of sample preparation, analytical performances and operating conditions of the miniaturized instrumentation with the plasma microtorch were already published [24–27]. Therefore, the novelty of this study consists in the evaluation for the first time of the greenness and whiteness profiles in an integrated approach for several non-chromatographic methods, developed in our laboratory, for Hg determination and speciation using optical emission spectrometry, based on a cost-effective and fully miniaturized instrumentation, in which the core is a low power and low Ar consumption capacitively coupled plasma microtorch interfaced with a low resolution microspectrometer.

RESULTS AND DISCUSSION

Greenness profile of the UV/Vis-PVG-μCCP-OES methods according to the NEMI procedure

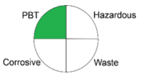
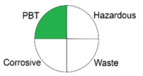
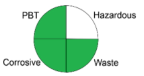
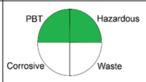
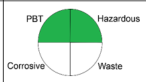
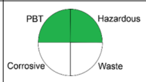
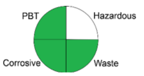
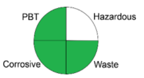
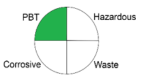
According to Table 1, the UV-PVG-μCCP-OES method, used for CH_3Hg^+ determination in fish tissue samples based on a double liquid-liquid extraction in the HBr–toluene–L-cysteine system, presents a greenness score of only 25% (1 green quadrant), similar with the TD-AAS method, due to the use of persistent, bioaccumulative and toxic (PBT) solvents (toluene), use of corrosive and hazardous reagents (HBr), and due to the generation of large amount of waste, 95 mL and 51 mL, respectively. Anyway, this sample preparation procedure was established in an inter-laboratory study and is recommended by the European Commission for the determination of CH_3Hg^+

[23], because it is easy to apply and does not require complicated instrumentation, such as the TD-AAS, specially designed for Hg determination. The original UV-PVG-μCCP-OES method for total Hg determination, developed in our laboratory, in fish tissue and food by ultrasound assisted extraction in concentrated HCOOH and UV photochemical vapor generation (UV-PVG) in 0.6 mol L^{-1} HCOOH has a greenness score of 75% (3 green quadrants), due to the fact that it does not use PBT and corrosive reagents, and does not generate waste in a large quantity. The same greenness score was obtained by the same UV-PVG-μCCP-OES method for total Hg determination in water using UV-PVG in 0.6 mol L^{-1} HCOOH. The classical methods, $SnCl_2$-CVG-ICP-OES and $SnCl_2$-CVG-AFS, have a lower greenness score (~50%), because they require a complete sample digestion in HNO_3 – H_2O_2 system, uses HCl as medium for $SnCl_2$ cold vapor generation and generate a higher amount of waste (50–60 mL). The conclusion is also valid in the case of the $SnCl_2$-CVG-μCCP-OES method, although it uses the same miniaturized instrumentation, because the sample preparation procedure is paramount to the characteristics of the instrumentation. In these circumstances, the TD-AAS method, based on direct solid sampling for Hg determination has a greenness score of 100%, because it allows the determination of Hg in fresh or dry sample, and thus the sample preparation is simple. Instead, there may be issues with sensitivity and reproducibility.

The UV/Vis-PVG-μCCP-OES specially designed for Hg speciation as CH_3Hg^+ and Hg^{2+} in fish tissue, based on ultrasound assisted sample extraction in concentrated HCOOH and selective UV/Vis-PVG of CH_3Hg^+/Hg^{2+} in 0.6 mol L^{-1} HCOOH has a greenness score of 75%. On the other hand, the use of TD-AAS method for Hg speciation presents a score of only 25%, due to the hazardous and corrosive reagents used in the double liquid-liquid extraction in HBr–toluene–L-cysteine system.

The study revealed the limitation of the NEMI approach in the evaluation of the greenness profile of a method, because this procedure considers only the sample preparation protocol (type and amount of reagents, and waste generated), and does not take into account the economic aspects and instrument miniaturization, important aspects in the state-of-the-art development of cost-effective and highly sensitive methods. However, it is clear that regardless of the instrumentation, the use of ultrasound assisted sample extraction in concentrated HCOOH and UV-PVG derivatization, in which only HCOOH is used, has a higher degree of greenness, compared to the classical HNO_3 and H_2O_2 extraction methods and the conventional $SnCl_2$ derivatization in HCl medium.

Table 1. Greenness profile of the UV/Vis-PVG-µCCP-OES methods for Hg determination and speciation in food and water using the NEMI procedure [7], in comparison with classical methods

Species determined (sample)	Method				
	UV-PVG-µCCP-OES	TD-AAS	$SnCl_2$-CVG-ICP-OES	$SnCl_2$-CVG-AFS	$SnCl_2$-CVG-µCCP-OES
CH_3Hg^+ (fish tissue)			-	-	-
Total Hg (food)					
Total Hg (water)		-	-	-	-
Hg speciation as CH_3Hg^+ and Hg^{2+} (fish tissue)*			-	-	-

* Hg speciation as CH_3Hg^+ and Hg^{2+} by µCCP-OES was achieved by UV/Vis-PVG-µCCP-OES

Greenness profile of the UV/Vis-PVG-µCCP-OES methods according to the AES procedure

The AES procedure ensures a more advanced assessment of the greenness profile of an analytical method, as it takes into account not only the reagents use and waste generated, but also economic aspects, such as energy consumption and professional risk of the operator. The results presented in Table 2, highlights that the UV-PVG-µCCP-OES method, developed for CH_3Hg^+ determination presents an AES score of 61%, similar to that of TD-AAS (62%), due to the same sample preparation protocol (double liquid-liquid extraction in HBr–toluene–L-cysteine) and similar energy consumption of around 0.2 kWh.

The UV-PVG-µCCP-OES method, developed for total Hg determination in vegetables and foods of animal origin, presents an excellent greenness score of 78%, *versus* 66% in the case of the traditional method $SnCl_2$-CVG-ICP-OES. This score could be attributed to the use of a single reagent (HCOOH) for extraction and derivatization, while in the case of $SnCl_2$-CVG-ICP-OES a complete sample digestion with HNO_3 and H_2O_2 and derivatization with $SnCl_2$ is mandatory. Furthermore, additional penalty points are assigned to the ICP-OES instrument due to the high energy demand, which is about 200 times higher than that of the miniaturized UV-PVG-µCCP-OES set-up. Also, UV-PVG-µCCP-OES method has a higher degree of greenness, compared to $SnCl_2$-CVG-AFS method, in which case a complete sample digestion was necessary.

Table 2. Greenness profile of the UV/Vis-PVG-μCCP-OES methods for Hg determination and speciation in food and water using the AES procedure [9], in comparison with classical methods

Species determined (sample)	AES criteria	Methods penalty points/AES score				
		UV-PVG-μCCP-OES	TD-AAS	$SnCl_2$-CVG-ICP-OES	$SnCl_2$-CVG AFS	$SnCl_2$-CVG-μCCP-OES
CH_3Hg^+ (fish tissue)	Reagents	27	28	-	-	-
	Instrumentation, professional risk, waste	12	12	-	-	-
	AES score	**61**	**62**	-	-	-
Total Hg (food)	Reagents	13	8	23	22	21
	Instrumentation, professional risk, waste	9	1	11	10	10
	AES score	**78**	**91**	**66**	**68**	**69**
Total Hg (water)	Reagents	7	-	-	-	-
	Instrumentation, professional risk, waste	5	-	-	-	-
	AES score	85	-	-	-	-
Hg speciation as CH_3Hg^+ and Hg^{2+} (fish tissue)*	Reagents	13	28	-	-	-
	Instrumentation, professional risk, waste	9	12	-	-	-
	AES score	**78**	**62**	-	-	-

* Hg speciation as CH_3Hg^+ and Hg^{2+} by μCCP-OES was achieved by UV/Vis-PVG-μCCP-OES

The TD-AAS method has the highest greenness score (91%), according to the AES procedure, as the total Hg determination is based on the direct solid sampling without further preparation. In the case of this method, only 8 and 1 penalty points were attributed to the use of O_2 for sample decomposition and to the energy consumption for sample drying by lyophilization, respectively. Because the UV-PVG-μCCP-OES method has a slightly lower AES score, it could be considered as a viable alternative to TD-AAS.

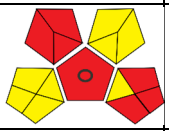
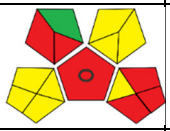
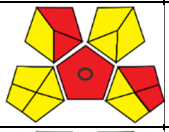
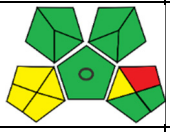
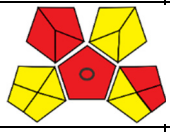
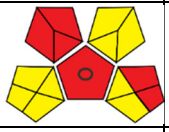
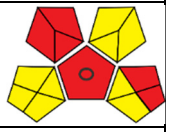
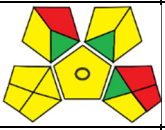
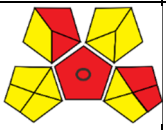
Furthermore, the UV-PVG-μCCP-OES set-up enables Hg speciation as CH_3Hg^+ and Hg^{2+} in fish tissue samples after extraction in HCOOH and UV/Vis-PVG in 0.6 mol L^{-1} HCOOH with and without *on-line* preconcentration of the Hg vapor on a gold filament microcollector. On the other hand, the $SnCl_2$-CVG-ICP-OES and $SnCl_2$-CVG-AFS methods, are not appropriate to be used for this purpose, because they do not include the *on-line* preconcentration device, necessary for Hg^{2+} determination.

Also, the UV/Vis-PVG-μCCP-OES method has a higher score of AES compared to the TD-AAS method, due to the sample preparation considerations presented in the previous procedures.

Greenness profile of the UV/Vis-PVG-μCCP-OES methods according to the GAPI procedure

According to Table 3, the UV-PVG-μCCP-OES method used for total Hg determination in water samples is the greenest one, because it could be observed 3 green triangles in different pentagons, attributed to the sample preparation (no extraction), amount of reagents and energy consumption. The TD-AAS method has the highest greenness profile for Hg determination directly in solid samples, because it has the most pentagons colored in green. All the methods based on the miniaturized μCCP-OES system with or without *on-line* Hg vapor preconcentration show a lower profile of greenness.

Table 3. Greenness profile of the UV/Vis-PVG-μCCP-OES methods for Hg determination and speciation in food and water using the GAPI procedure [10], in comparison with classical methods

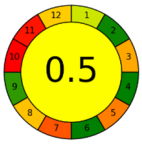
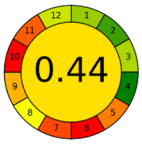
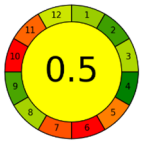
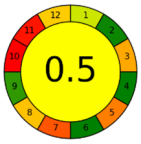
Species determined (sample)	Method				
	UV-PVG-μCCP-OES	TD-AAS	$SnCl_2$-CVG-ICP-OES	$SnCl_2$-CVG-AFS	$SnCl_2$-CVG-μCCP-OES
CH_3Hg^+ (fish tissue)			-	-	-
Total Hg (food)					
Total Hg (water)		-	-	-	-
Hg speciation as CH_3Hg^+ and Hg^{2+} (fish tissue)*			-	-	-

* Hg speciation as CH_3Hg^+ and Hg^{2+} by μCCP-OES was achieved by UV/Vis-PVG-μCCP-OES

Greenness profile of the UV/Vis-PVG-μCCP-OES methods according to the AGREE procedure

The results presented in Table 4 show that the UV-PVG-μCCP-OES method used for CH_3Hg^+ determination, has a greenness score of only 55%, similar to the TD-AAS method (50%), due to the reagents and solvents used in the sample preparation protocol for the two methods. However, the AGREE calculator sets the scores for each of the 12 criteria, and therefore it could be identified those which determines the greenness profile. Consequently, it could be observed that the sample amount, number of steps in the sample preparation protocol and energy consumption has the highest greenness score.

Table 4. Greenness profile of the UV/Vis-PVG-μCCP-OES methods for Hg determination and speciation in food and water using the AGREE procedure [13], in comparison with classical methods

Species determined (sample)	Method				
	UV-PVG-μCCP-OES	TD-AAS	$SnCl_2$-CVG-ICP-OES	$SnCl_2$-CVG-AFS	$SnCl_2$-CVG-μCCP-OES
CH_3Hg^+ (fish tissue)			-	-	-
Total Hg (food)					
Total Hg (water)		-	-	-	-
Hg speciation as CH_3Hg^+ and Hg^{2+} (fish tissue)*			-	-	-

* Hg speciation as CH_3Hg^+ and Hg^{2+} by μCCP-OES was achieved by UV/Vis-PVG-μCCP-OES

The UV-PVG-μCCP-OES method for total Hg determination in food and water with or without *on-line* cold vapor preconcentration was found to have a good greenness profile (65%), compared to $SnCl_2$-CVG-ICP-OES (44%) and $SnCl_2$-CVG-AFS (49%). The results are in agreement with the lower energy consumption of the μCCP-OES instrumentation, and simpler sample preparation (ultrasound assisted extraction in HCOOH) in comparison with total sample digestion, required in the case of the classical methods. Also, a significant improvement of the greenness degree (15%) can be observed if UV-PVG derivatization is connected to a miniaturized instrumentation with a microplasma source, compared to the classical derivatization assisted by $SnCl_2$ and HCl medium coupled with classical spectral instrumentation based on optical emission and atomic fluorescence spectrometry. Consequently, the UV-PVG-μCCP-OES method for total Hg determination following HCOOH extraction and UV-PVG approach could be considered a viable alternative in terms of sample preparation and derivatization. The TD-AAS method presented the highest degree of greenness, namely 79%. On the other hand, UV/Vis-PVG-μCCP-OES method for Hg speciation has a higher greenness profile (65%), compared to the TD-AAS method (50%), because the method developed on the miniaturized instrumentation is based only on the selective derivatization of Hg species, while the TD-AAS is based on the double selective liquid-liquid extraction.

Whiteness profile of the UV/Vis-PVG-μCCP-OES methods according to the RGB 12 procedure

As shown in Figures 1 and 2, the UV/Vis-PVG-μCCP-OES methods present similar or higher whiteness profile, compared to those of reference methods. The significant differences were found to be in the case of methods developed for total Hg determination using ultrasound assisted extraction in HCOOH and UV-PVG in 0.6 mol L^{-1} HCOOH medium. The developed methods and those of reference can be ordered in a descending manner according to their whiteness profile as follows: UV-PVG-μCCP-OES (95%) > TD-AAS (87%) ≈ $SnCl_2$-CVG-μCCP-OES (81%) > $SnCl_2$-CVG-AFS (75%) >> $SnCl_2$-CVG-ICP-OES (60%). This ordering is in accordance with the RGB levels, which include the 12 principles and consider the analytical performance, operating conditions, mode of operation, applicability, etc. Therefore, the miniaturized UV-PVG-μCCP-OES instrumentation present the highest redness score in terms of scope of application (R1) and analytical performance (R2), such as the best LOD and LOQ, compared to traditional methods.

The higher whiteness profile is also determined by the higher greenness profile due to the lower toxicity of HCOOH used for sample extraction and UV/Vis-PVG, a lower amount of reagents, a lower amount of generated waste, but mainly by the instrumentation with a lower energy and Ar consumption (G1–G3 criteria).

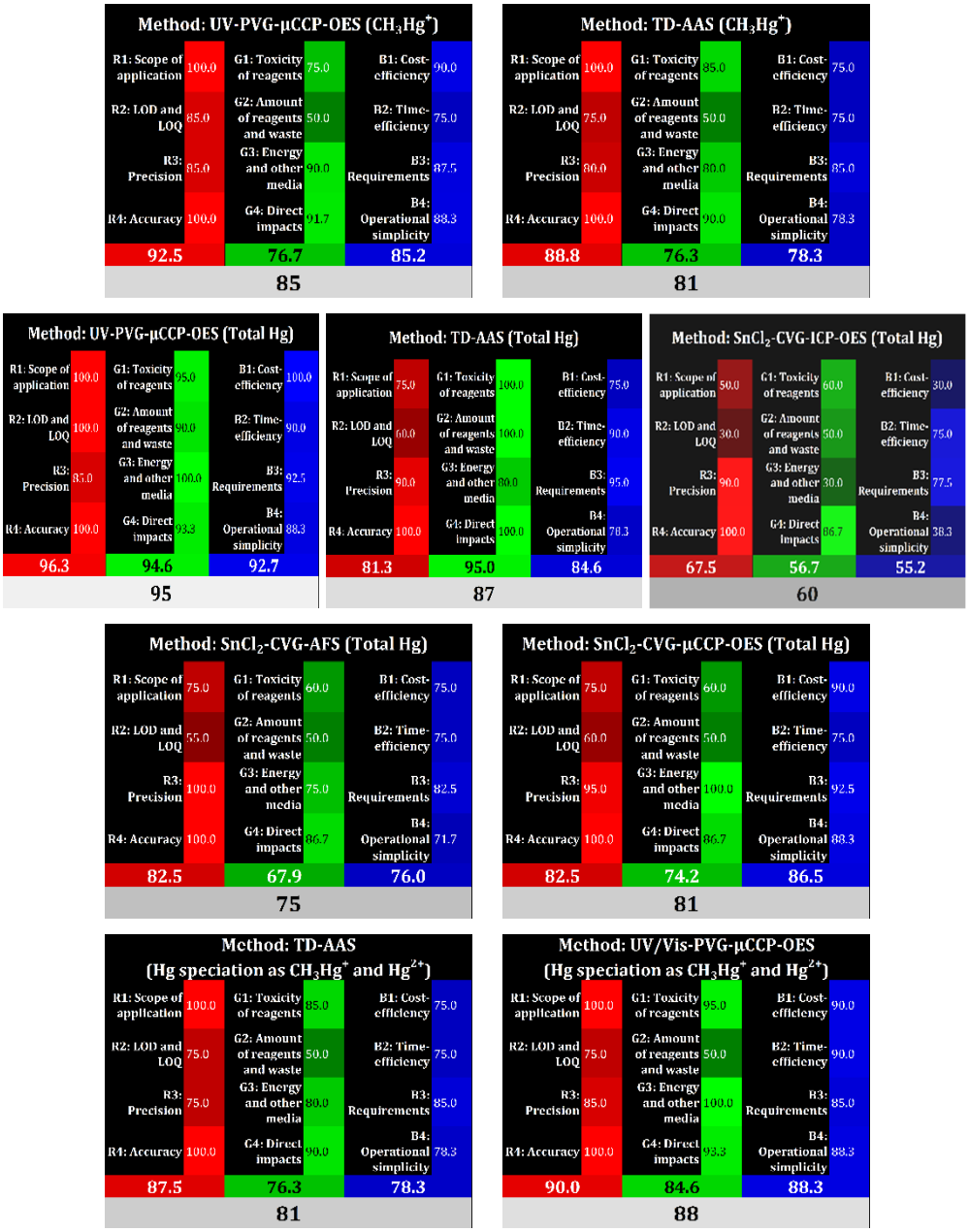


Figure 1. Visualization of the redness, greenness, blueness and whiteness profiles of the UV/Vis-PVG-µCCP-OES methods, in comparison with traditional methods for Hg determination and speciation, according to the RGB 12 algorithm [21]

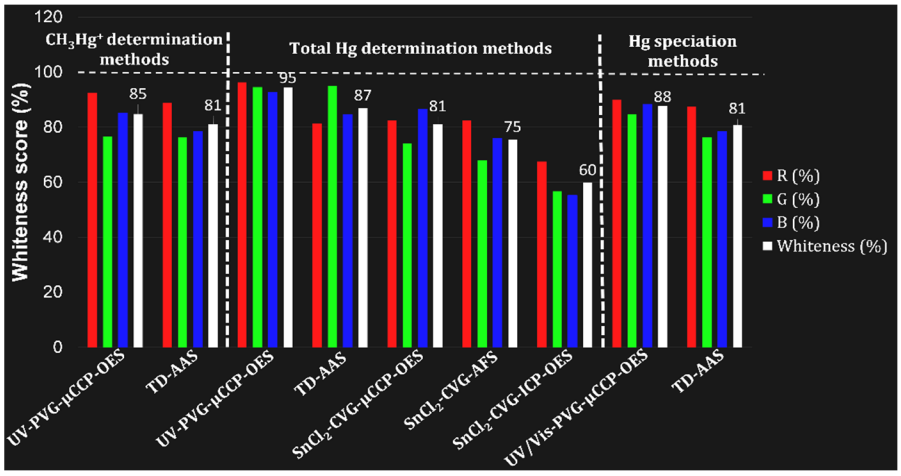


Figure 2. Comparison of the redness, greenness, blueness and whiteness profiles of the methods, obtained by the RGB 12 algorithm [21]

The better whiteness profile of the method based on miniaturized instrumentation is in agreement with the better blueness score, mainly due to the cost-effective fully miniaturized instrumentation (B1 criteria), which is almost ten times cheaper compared to ICP-OES, and the simplicity of its operation (B4 criteria). It could be observed that the UV/Vis-PVG-μCCP-OES method for Hg speciation have a higher whiteness profile (88%), compared to that of TD-AAS (81%). This feature can be explained by the ultrasound assisted extraction in HCOOH and UV/Vis-PVG selective derivatization of total Hg/Hg^{2+} species, compared to the procedure based on double liquid-liquid extraction in the HBr–toluene–L-cysteine system, used for selective extraction of CH_3Hg^+ for the determination of TD-AAS. Consequently, the greenness and blueness profiles of the methods based on microplasma source have higher scores (85% and 88%, respectively), than that for the TD-AAS method (76% and 78%, respectively). The redness profile of the UV/Vis-PVG-μCCP-OES and TD-AAS methods have similar scores (90% and 88%, respectively) as a result of the similar analytical performances of the two methods.

CONCLUSIONS

The greenness profile of the UV/Vis-PVG-μCCP-OES methods for Hg determination and speciation as CH_3Hg^+ and Hg^{2+} were evaluated by four procedures, namely NEMI, AES, GAPI and AGREE. Each of these unique

approaches have proven to be limited, but by combining them it has been possible to highlight which method has the greenest profile and which steps of the analytical method are responsible for improving its greenness degree. The greenness profiles of the new methods were characterized by scores in the range of 25–75% according to NEMI procedure, 61–85% according to AES procedure and 55–65% according to AGREE procedure, compared to scores of 25–68% obtained for $SnCl_2$-CVG-ICP-OES and $SnCl_2$-CVG-AFS as reference methods. On the other hand, the UV-PVG-μCCP-OES usually exhibits very high greenness scores. However, the lowest greenness score was obtained for CH_3Hg^+ determination, based on a double liquid-liquid extraction in the HBr–toluene–L-cysteine system and determination by UV-PVG-μCCP-OES and TD-AAS. It was also highlighted a higher greenness of the methods based on ultrasound assisted extraction in HCOOH combined with UV/Vis-PVG for total Hg determination and its speciation. The evaluation of whiteness profile by the RGB 12 procedure of the UV/Vis-PVG-μCCP-OES methods highlighted their advantage in terms of analytical performance and economic aspects due to the miniaturized and cost-effective instrumentation, low Ar and energy consumption.

EXPERIMENTAL SECTION

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

The sample preparation for determination of methylmercury in fish tissue by UV-PVG-μCCP-OES and TD-AAS methods was based on a double liquid-liquid selective extraction, developed by the European Commission [23, 24]. Amounts of 0.2 g fish tissue sample was extracted with 10 mL HBr 47% and 0.5 mL distilled water, then CH_3Hg^+ species were selectively extracted twice in 20 mL and 15 mL toluene, respectively, and finally re-extracted in 6 wL 1% (w/v) aqueous L-cysteine solution. For quantification by UV-PVG-μCCP-OES, aliquot volumes of 1–5 mL were diluted to 50 mL and brought in 0.6 mol L^{-1} HCOOH medium for the UV-PVG of CH_3Hg^+. In the case of TD-AAS method, CH_3Hg^+ was determined in 1% (w/v) L-cysteine solution, without derivatization.

Sample preparation for total Hg determination in food samples (fish tissue, vegetables, fruits, food supplements, meat and organs) by UV-PVG-μCCP-OES was based on an ultrasound assisted extraction of 0.2 g sample in 98-100% HCOOH for 3 h at 50 °C, followed by UV-PVG in 0.6 mol L^{-1} HCOOH medium. The Hg determination in fish tissue samples was carried out without preconcentration, while in other foods the *on-line* preconcentration of

Hg cold vapor on a gold filament microcollector was applied. In water samples the total Hg was determined by derivatization in 0.6 mol L^{-1} HCOOH, without any further sample preparation [26].

Sample preparation for Hg, CH_3Hg^+ and Hg^{2+} determination by UV/Vis-PVG-μCCP-OES was also based on ultrasound assisted extraction in HCOOH and selective UV/Vis-PVG in 0.6 mol L^{-1} HCOOH [27].

Sample preparation for total Hg determination in fish tissue by $SnCl_2$-CVG-ICP-OES, $SnCl_2$-CVG-AFS and $SnCl_2$-CVG-μCCP-OES consisted of a microwave assisted digestion in 8 mL 60% (w/w) HNO_3 and 2 mL 30% (w/w) H_2O_2, and dilution to 50 mL in 5% (v/v) HCl. A solution of $SnCl_2$ in HCl was used as derivatization reagent [25, 26].

Instrumentation

The UV/Vis-PVG-μCCP-OES instrumentation (Babes-Bolyai University, Cluj-Napoca, Romania) consist of a fully miniaturized set-up, containing a continuous flow photochemical vapor generator equipped with a high-power UV lamp (500 W), a gas-liquid separator, a Nafion tube for Hg cold vapor drying, a low power (10–15 W) and low Ar consumption (100 mL min^{-1}) capacitively coupled plasma microtorch, a low-resolution CCD microspectrometer (QE65 Pro or Maya2000 Pro, Ocean Optics, Dunedin, USA) for signal registration and data processing [24, 26, 27]. A preconcentration step on a gold filament microcollector, mounted between the gas-liquid separator and the Nafion tube was used for the determination of total Hg in food samples with low concentration (vegetables, fruits, water, etc.). The operating mode consisted of Hg cold vapor trapping on the gold filament at room temperature from 25 mL aliquot sample, thermal desorption after electrical heating (5 V, 1.8 A) and recording several episodic spectra at 253.652 nm [26]. For the CH_3Hg^+ and Hg^{2+} speciation the UV/Vis-PVG-μCCP-OES instrumentation with and without cold vapor preconcentration and UV-PVG for total Hg (UV lamp turned on) and Vis-PVG for Hg^{2+} species (UV lamp turned off) [27].

For total Hg determination by $SnCl_2$-CVG-μCCP-OES, the experimental set-up was similar, but in this case the plasma microtorch was interfaced with a HGX-200 cold vapor generator (Teledyne CETAC, Nebraska, USA), instead of the photochemical vapor generator [26].

For comparative determinations by TD-AAS an Automated Direct HG Analyzer Hydra-C Teledyne Leeman Instruments (Hudson, USA) was used [24]. A Spectro CIROS^{CCD} spectrometer (Spectro, Kleve, Germany) interfaced with a HGX-200 cold vapor generator was used for $SnCl_2$-CVG-ICP-OES determination. A Hydra-AF Mercury Analyzer (Teledyne Leeman Instruments, USA) was used for $SnCl_2$-CVG-AFS determinations [26].

The working conditions of the UV/Vis-PVG-μCCP-OES and reference methods are presented in Table 5, while figures of merit in Table 6 [24–27].

Table 5. Working conditions for Hg determination and speciation as CH_3Hg^+ and Hg^{2+} by UV/Vis-PVG-μCCP-OES and reference methods

Method (species determined)	Working conditions
UV/Vis-PVG-μCCP-OES with or without preconcentration (total Hg and CH_3Hg^+ speciation) [24, 26, 27]	Derivatization reagent: 0.6 mol L^{-1} HCOOH at 10 mL min^{-1} Sample throughput: 20 analyses/hour Plasma power: 15 W Argon flow rate: 100 mL min^{-1} Amount of waste: 95 mL for CH_3Hg^+ determination and 35 mL for total Hg and Hg^{2+} determination
$SnCl_2$-CVG-ICP-OES (total Hg) [26]	Sample flow rate: 5 mL min^{-1} Derivatization reagent: 20% (w/v) $SnCl_2$ stabilized in 15% (v/v) HCl at 2 mL min^{-1} Sample throughput: 10 analyses/hour Plasma power: 1400 W Argon flow rate: 1500 mL min^{-1} Amount of generated waste: 62 mL
$SnCl_2$-CVG-AFS (total Hg) [26]	Sample flow rate: 5 mL min^{-1} Derivatization reagent: 2% (w/v) $SnCl_2$ stabilized in 10% (v/v) HCl at 1 mL min^{-1} Sample throughput: 12 analyses/hour Argon flow rate: 150 mL min^{-1} Amount of generated waste: 55 mL
$SnCl_2$-CVG-μCCP-OES (total Hg) [26, 26]	Sample flow rate: 3.5 mL min^{-1} Derivatization reagent: 20% (w/v) $SnCl_2$ stabilized in 15% (v/v) HCl in 1 mL min^{-1} Sample throughput: 20 analyses/hour Plasma power: 15 W Argon flow rate: 150 mL min^{-1} Amount of generated waste: 53 mL
TD-AAS (CH_3Hg^+ and total Hg) [24]	Amount of sample: 0.2 g or 0.2 mL Sample throughput: 6 analyses/hour Oxygen flow rate: 300 mL min^{-1} Amount of generated waste: 51 mL for CH_3Hg^+ determination and insignificant for total Hg determination by direct solid sampling

Table 6. Figures of merit for total Hg determination and speciation as CH_3Hg^+ and Hg^{2+} by UV/Vis-PVG-μCCP-OES and reference methods

Determined Hg species /Method	Calibration range (μg L^{-1})	Precision[1]	Accuracy[2]	LOD		LOQ		Ref.
				(ng L^{-1})	(μg kg^{-1})	(ng L^{-1})	(μg kg^{-1})	
CH_3Hg^+								
UV-PVG-μCCP-OES	0–5 (n=7)	2.7–9.4	99 ± 8	7	2	21	6	[24]
TD-AAS	0–25 ng	3.5–10.4	99 ± 9	-	-	5	15	[24]
Total Hg								
UV-PVG-μCCP-OES without preconcentration	0–1 (n=6)	2.6–12.7	101 ± 7	3.5	9	10.5	36	[26]
UV-PVG-μCCP-OES with preconcentration	0–0.1 (n=6)	3.0–12.8	101 ± 12	0.1	0.3	0.25	0.75	[26]
TD-AAS	0–25 ng	2.6–8.1	99 ± 7	-	0.2	-	0.6	[27]
$SnCl_2$-CVG-ICP-OES	0–10 (n=6)	1.6–10.3	100 ± 7	19	57	5	15	[26]
$SnCl_2$-CVG-AFS	0–1 (n=6)	2.7–5.7	102 ± 6	5	15	12.5	38	[26]
$SnCl_2$-CVG-μCCP-OES	0–1 (n=6)	1.3–8.5	100 ± 7	3.5	10.5	9	27	[26]
Hg speciation as CH_3Hg^+ and Hg^{2+}								
UV-PVG-μCCP-OES (Total Hg)	0–1 (n=7)	2.6–10.2	99 ± 6	3.5	9	10.5	27	[27]
Vis-PVG-μCCP-OES (Hg^{2+})	0–10 (n=7)	2.0–13.4	99 ± 9	1.9	4.8	5.7	14	[27]

[1] Precision is expressed as Relative Standard Deviation (%)
[2] Accuracy is expressed as Pooled Recovery ± Confidence Interval (%) for 95% confidence level and n=5 complete analysis

Procedures used for evaluation of greenness and whiteness profiles

In the NEMI procedure, developed by the Methods and Data Comparability Board (MDBC) the greenness profile is shown by the aid of a four-quadrant circle, in which every quadrant represents different aspects of the analytical method, corresponding to the type and amount of reagents (3 quadrants) and amount of generated waste (1 quadrant) [7]. Each quadrant is colored in green if the corresponding criteria is met, otherwise it is left blank. The greenness criteria for reagents are not met if they are persistent, bioaccumulative and toxic, according to the Toxics Release Inventory (TRI) list (criterium 1, PBT), are found on the Recovery Conservation Act (RCA)'s D, F, P and U hazardous waste lists (criterium 2), reagents used are corrosive (pH <2 and >12) (criteria 3), and the fourth quadrant is associated with the amount of generated waste (> 50 g or >50 mL) (criterium 4) [7].

The AES procedure, developed by Galuszka *et al.* [9], is based on the assignment of 100 points to a completely green profile for a method, from which different penalty points (PP) are deducted according to the amount of reagents, their hazard to human health and environment (according to their pictograms and signal words), energy consumption, occupational hazard and the amount of generated waste. Methods with an AES score > 75 are considered excellent green, between 75 and 50 acceptable green and < 50 inadequately green.

The GAPI procedure, which is a combined method between the NEMI and AES procedures, consists of a pictogram with 5 pentagons, describing the following aspects: (1) collection, preservation, transportation and storage of samples; (2) sample preparation in terms of scale of extraction, solvents/ reagents used in the protocol and additional treatment; (3) amount of reagents and solvents, health hazard and safety hazard; (4) instrumentation, in terms of energy consumption, occupational hazard, amount of waste and waste treatment; and (5) method type, such as direct or indirect measurement. Each section of the five pentagons is colored in red, yellow and green according to its greenness profile. In the central pentagon a circle is drawn if the procedure is suitable for quantification, or if this circle is not present the procedure is appropriate only for qualitative identification. The GAPI procedure was described by Plotka-Wasylka [10].

The AGREE procedure, developed by Pena-Pereira *et al*. [13], is a calculator written in python, which takes into account the criteria from the 12 principles of GAC, presented as different color, red, yellow, green in geometric sections around of a central circle, in which the general greenness score of the method is presented as value and color, respectively. Also, in each of the 12 segments a value between 0 and 1 and a color is assigned, according to its greenness score. The general greenness score of a method is calculated as average of the 12 principles scores. A method is completely green if the overall score is equal to 1. The following 12 principles are considered in the evaluation of the green profile of a method: (1) type of sampling (remote sensing, *in-field* sampling, *at-line*, *on-line*, *off-line*, etc.); (2) amount of sample required for analysis; (3) positioning of the analytical device (*off-line*, *at-line*, *on-line* or *in-line*); (4) number of major, distinct steps in the sample preparation procedure; (5) degree of method automation and miniaturization of the sample preparation procedure; (6) derivatization reagents; (7) amount of generated waste; (8) number of analytes determined during one analysis and number of samples analyzed per hour; (9) energy consumption; (10) type of reagents used (no reagents, all/some/none of the reagents are from bio-based sources); (11) amount of toxic reagents and solvents used in the analysis and (12) threats to which the operator and the environment is

exposed (toxic to aquatic life, bioaccumulative, persistent, highly flammable, highly oxidizable, explosive and corrosive reagents). The AGREE calculator was described by Pena-Pereira *et al*. [13].

The whiteness profile was assessed using the RGB 12 algorithm, developed by Nowak *et al*. [21], based on the 12 principles of WAC, which are divided into 3 parts, each corresponding to a different aspect of the analytical method. The first group contains the “red” principles, which includes aspects of the analytical performance (R1: scope of application; R2: LOD and LOQ; R3: precision and R4: accuracy). The second group contains the “green” principles, and includes reagents toxicity, generated waste and energy consumption (G1: toxicity of reagents; G2: number and amount of reagents and waste; G3: energy and other media; and G4: direct impacts to human health and environment). Finally, the third group contains the “blue” principles, such as aspects related to analytical efficiency and practical/economic criteria (B1: cost-efficiency; B2: time-efficiency; B3: requirements of the method, such as sample amount, advanced equipment, personal qualification, etc.; and B4: operational simplicity). The RGB 12 algorithm consists in the calculation of a score between 0 and 100 for the RGB profile by the analyst in an objective manner. The 0 score for RGB procedure represents the worst results, while a score of 100 is associated to the best redness, greenness and blueness profiles. The profile of the RGB procedure is appreciated depending on the red, green and blue colors shade. An excel worksheet was developed by Nowak *et al.* [21], in which the parameters for the red, green and blue profiles are entered, which do not have to be linearly dependent. The shade of each color is set automatically in the program according to the red, green and blue score of the method.

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