Determination of methylmercury using liquid chromatography – photochemical vapour generation – atomic fluorescence spectroscopy (LC-PVG-AFS): a simple, green analytical method

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Abstract

Reliable, fast and cost efficient mercury analysis is paramount to assess Hg levels in a huge variety of matrices, from soil and water to a variety of foodstuffs. In this work, a novel concept was adopted for seafood and hair analysis, which combines a simplified methylmercury extraction and photochemical vapour generation of Hg^0 with liquid chromatography and atomic fluorescence spectrometry (LC-AFS). This concept reduces the number of reagents required to methanol and APDC for extraction and separation of Hg species, and acetic acid, which when combined with UV generates volatile Hg^0 in one single step.

Here, we compare conventional chemical vapour generation (CVG) with photochemical vapour generation (PVG) for seafood and hair matrix. Our results show that the LC-PVG-AFS method offers lower detection limits and higher precision than that of LC-CVG-AFS measurements by a factor of two. The extraction and LC-PVG-AFS technique was validated using 7 seafood and hair reference materials, yielding methylmercury recoveries of 93.2% to 105%. Further validation was carried out by comparing results from methylmercury determination in 14 tuna samples by the LC-PVG-AFS method to results obtained by GC-AFS, showing R^2 = 0.98 and a gradient of 1.0077, which indicates good correlation between the newly developed LC-PVG-AFS technique to an already validated GC-AFS method.

1. Introduction

Mercury is a ubiquitous element of global concern. Since the beginning of the industrial age, anthropogenic sources have been responsible for much greater mercury emissions than natural geological emissions.[1] The mercury released from sources, such as coal fired power plants and gold mines, can cycle around the planet and be deposited in various areas that affect human life, such as plants, water sources, and fish. Moreover, mercury exists as several species, with elemental (Hg^0), inorganic (Hg^{2+}), and organic species such as methylmercury ($MeHg^+$). The toxicity of mercury depends on its species, with methylmercury being the most toxic. Methylmercury is a known neurotoxin and particularly problematic for children in the early development stage. Studies have shown that low level methylmercury can cause severe neurological disorders in the prenatal stages as the methylmercury can be transported through the placenta.[2]

The main exposure of methylmercury in the human diet comes from seafood.[3,4] The predominant uptake pathway for fish is ingestion of food rather than from the water as methylmercury concentrations in marine water are very low: within the pg L^{-1} Hg range.[5] Once methylmercury enters the food chain, bio magnification occurs through increasing trophic levels.[6] In 2004, the

European Food Safety Authority (EFSA) advised pregnant women against the consumption of predatory fish at the top of the food chain, such as tuna and swordfish, due to containing higher concentrations of methylmercury than other fish species.[7] In 2012, the EFSA set a tolerable weekly intake (TWI) for methylmercury of 1.3 μg kg^{-1} body weight.[8] Current European regulation states a total mercury concentration limit of 1.0 mg kg^{-1} (wet weight basis) in seafood.

Several extraction techniques have been reported for methylmercury in seafood and hair samples. Most commonly, a high temperature assisted HCl extraction is employed before analysis.[9,10] A much simpler method was provided by *Jagtap et al.*[11] whereby the extraction was carried out using the mobile phase of 5% (v/v) 2-mercaptoethanol. Recently, an online pre-concentration technique was developed by *Brombach et al.*[12–15], which uses a mobile phase containing a high methanol concentration and ammonium pyrrolidinedithiocarbamate as the modifier. An extraction with this mobile phase has never been attempted before, but would potentially have good methylmercury extraction properties in samples with high lipid concentrations, such as fish.

A reliable method for the analysis of mercury species is cold vapour generation of mercury coupled with atomic fluorescence spectroscopy (CV-AFS). The most commonly used method of cold vapour generation involves oxidising all organic mercury species to inorganic mercury (Hg^{2+}), followed by a reduction to elemental mercury (Hg^0) with tin(II) chloride or sodium borohydride. However, this method uses a plethora of chemicals which is both costly and prone to error as well as instrumental issues due to the complex wet chemistry necessary. In addition, these chemicals are highly toxic, with bromide/bromate and sodium borohydride being category 2 carcinogens, requiring extra caution when handling. Another pathway is photochemical vapour generation (PVG), which has been used in the past in conjunction with a variety of detection methods such as atomic fluorescence spectroscopy (AFS) as an alternative method of cold vapour generation. The method was originally used in wastewater treatment, but was first utilized by *Hou et. al.*[16] as an alternate method of vapour generation for mercury analysis using AFS. Since then, PVG has been applied to total mercury measurements using UV with formic acid[17–21] and acetic acid[22], eg. utilizing matrix assisted photochemical vapour generation to determine total mercury in white vinegar.[23] Additionally, it has been used in the past in conjunction with LC-AFS to provide reliable speciation measurements.[24,25] PVG promises a much simpler and more cost effective approach, which uses fewer, safer and more environmentally friendly chemicals, giving high generation yields[26] and potentially lower LOD's compared to the CVG approach.

In this work, the newly developed extraction procedure and photochemical reduction method has been applied to mercury speciation measurements using liquid chromatography (LC) as an LC-PVG-AFS technique, providing a much simpler analysis of methylmercury in seafood and hair matrices. A comparison has been made between the photochemical and chemical vapour generation methods to observe whether PVG can provide similar accuracy, precision, and sensitivity to that of CVG. Validation of the LC-PVG-AFS technique has been carried out by analysing 7 reference materials to assess the recoveries for seafood and hair matrices. In addition, 14 tuna fish samples have been analysed using the LC-PVG-AFS method and compared with a separate analysis using GC-AFS.

2. Experimental section

2.1. Chemicals and preparation

Deionised water was produced from the Elga Purelab Option DV35 (Elga, UK). A 1000 μg mL^{-1} stock solution of methylmercury chloride was prepared by diluting the compound (Fluorochem Ltd., UK) in methanol. Subsequent dilutions were carried out in 0.12 M HCl. The mobile phase contains 80% (v/v) methanol (Chromasolv™ for HPLC, ≥99.9%; Riedel de Haën, UK) with 1.5 mM ammonium pyrrolidinedithiocarbamate (~99%; Sigma-Aldrich, UK) in deionised water. For photochemical vapour generation of Hg^0, 25% (v/v) acetic acid (Puriss grade; Fluka, UK) was prepared in deionised water as photo-reductant. For chemical vapour generation of Hg^0 from $MeHg^+$ and Hg^{2+}, 0.005 M Tritrisol® bromide/bromate solution (Merck, Darmstadt, Germany) prepared in 1.2 M hydrochloric acid (Certified AR; Fisher Chemicals, UK) was used as an oxidant and 2% (m/v) tin(II) chloride dihydrate (96%; VWR, UK) prepared in 1.2 M hydrochloric acid (Certified AR; Fisher Chemicals, UK) was used as a reductant.

Extraction of methylmercury from seafood and hair was carried out using a solution of 80% (v/v) methanol (Chromasolv™ for HPLC, ≥99.9%; Riedel de Haën, UK) with 10 mM ammonium pyrrolidinedithiocarbamate (~99%; Sigma-Aldrich, UK) in deionised water. Alkaline digestions were carried out in 6 M potassium hydroxide (AnalaR NORMAPUR®; VWR, UK). For Tuna samples, selective extraction of methylmercury was carried out using dichloromethane (DCM, Chromasolv™ for HPLC, ≥99.8%; Sigma-Aldrich, UK). A solution of 1.5 M potassium bromide (AnalaR NORMAPUR®, ≥99.5%; VWR, UK) prepared in 0.9 M sulphuric acid (AnalaR NORMAPUR®; VWR, UK) was combined with a solution of 1.5 M copper(II) sulphate (A. C. S. reagent, 98+%; Sigma-Aldrich, UK) in a 2:1 ratio to convert methylmercury species to methylmercury bromide before selective extraction into DCM. Clean-up of the DCM extract was carried out using 0.01 M sodium thiosulphate (purchased as sodium thiosulphate pentahydrate, AnalaR; BDH, UK). Drying of the final DCM extract was carried out using anhydrous sodium sulphate (A. C. S. reagent, 99+%; Sigma-Aldrich, UK).

2.2. Instrumentation

2.2.1. Sample digestion

A hot block digestor (DigiPREP; SCP Science, Canada) was used for assisted digestion in tightly capped polypropylene vials at 60 ºC for 30 min. An ultrasonic bath (Ultrasonic Cleaner, Model 010; Skymen, China) was used to assist digestion at room temperature when required. Automatic shaking of samples was carried out using an automatic shaker (Orbital Shaker SO1; Stuart Scientific, UK). Vortex mixing was carried out using a vortex mixer (Whirlmixer; Fisons, UK). Filtration of the sample extracts were carried out using 0.45 μm filter discs (Chromacol Filter PTFE 30-SF-45(T); Thermo Scientific, USA).

2.2.2. LC-AFS

An LC speciation system coupled with AFS (PSA 10.820; PSA Millennium Merlin) was used for measurements by LC-AFS. The method parameters are given in Table 1. A schematic representation of the method is provided in Figure 1. Briefly, the sample was loaded across a 6-port valve equiped with a 250 μL sample loop. The valve was switched to inject the sample with mobile phase. A C18 column was used to separate the mercury species. The reagents added online to reduce all mercury

species to Hg^0 were different depending on whether CVG or PVG was used. The specific reagents for both vapour generation methods are shown in Table 1. Reagent 1 was added online before the sample was passed through a UV coil, which consisted of Teflon tubing (6 m, 1.6 mm OD, 0.8 mm ID) wrapped around a UV lamp (212 × 15 mm, 10 W, 253.7 nm wavelength). Reagent 2 was added online after the UV step. The mercury vapour was separated from the solution by bubbling argon through a gas-liquid separator (GLS), where it was carried to the atomic fluorescence spectrometer for quantification.

Table 1. Instrumental parameters for LC-AFS with CVG or PVG.

LC	PSA 10.820 (P S Analytical, Orpington, UK)
Column	Phenomenex SphereClone ODS2 C18 (250 × 4.6 mm, 5 µm)
Mobile phase	80% (v/v) methanol with 1.5 mM ammonium pyrrolidinedithiocarbamate, 1 mL min^{-1}
CVG	
Reagent 1 (Oxidant)	0.05 M bromide/bromate solution in 1.2 M hydrochloric acid, 2 mL min^{-1}, UV coil used
Reagent 2 (Reductant)	2% (m/v) tin(II) chloride dihydrate in 1.2 M hydrochloric acid, 5 mL min^{-1}
PVG	
Reagent 1 (Photo-reductant)	25% (v/v) acetic acid, 2 mL min^{-1}, UV coil used
Reagent 2 (DIW)	15 MΩ cm de-ionised water, 5 mL min^{-1}
AFS	PSA Millennium Merlin (P S Analytical, Orpington, UK)
Carrier gas	Argon, 250 mL min^{-1}

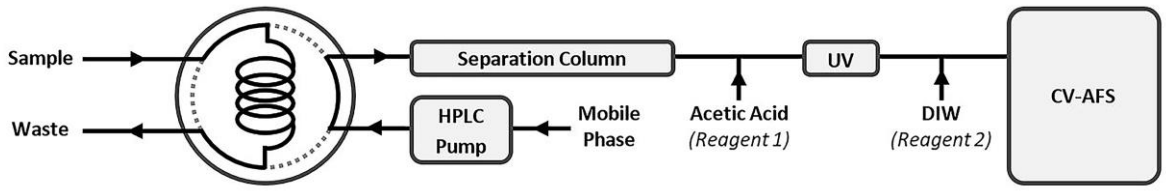

Figure 1. Schematic representation of the LC-PVG-AFS method. For LC-CVG-AFS, the added reagents are changed to oxidant (reagent 1) and reductant (reagent 2).

2.2.3. GC-AFS

Gas chromatography coupled to AFS (Agilent 6890N GC; PSA 10.750 AFS coupled with an unheated teflon transfer line) was used for GC-AFS measurements. The paramaters for GC-AFS are shown in Table 2.

Table 2. Instrumental parameters for GC-AFS

GC	Agilent 6890N GC (Agilent Technologies, USA)
Column	100% dimethylpolysiloxane (J&W Technologies, USA) 15 m × 0.53 mm × 1.50 µm
Injector type, volume and pressure	Capillary column inlet, splitless, 2 µL, 250 ^{o}C
Temperature programme	Hold at 30 ^{o}C for 2 min, ramp from 30 to 80 ^{o}C at 20 ^{o}C min^{-1}, ramp from 80 to 120 ^{o}C at 50 ^{o}C min^{-1}, hold for 1 min, ramp from 120 to 300 ^{o}C at 100 ^{o}C min^{-1}, hold for 4 min.
Carrier gas	Argon, 25 mL min^{-1}
AFS	PSA 10.750 (P S Analytical, Orpington, UK)
Pyrolyser temperature	800 ^{o}C
Make-up gas	Argon, 60 mL min^{-1}
Sheath gas	Argon, 150 mL min^{-1}

2.3. Reference materials and samples

A total of 7 reference materials were purchased for analysis in seafood (SRM 1566b, TORT-2, ERM-CE464, DOLT-4, and DORM-2) and human hair (IAEA-085 and IAEA-086). The reference materials were prepared and measured by the LC-AFS method. Both CVG and PVG were utilized when analysing the extracts.

Tuna fish samples were obtained from 14 yellowfin tunas taken from the eastern equatorial Pacific Ocean. The tuna was shipped in aluminium cans. The tuna was blended using a household blender (Moulinex, France). A portion of the blended sample was dried in open vessels in a hot block digester at 70 ^{o}C for 48 hours. The dried sample was ground into a powder with a mortar and pestle. The powdered tuna samples were stored in a dry, dark cupboard before analysis. The samples were prepared and measured by LC-PVG-AFS and GC-AFS methods.

2.4. Sample preparation

2.4.1. LC-AFS

50–200 mg of reference material was extracted with 5 mL of a solution containing 10 mM ammonium pyrrolidinedithiocarbamate in 80% methanol. The sample was extracted at 60 ^{o}C for 30 min in a hot block digester, followed by sonication for 15 min in an ultrasonic bath. The extract was filtered through 0.45 µm filter discs and diluted as appropriate before analysis with LC-AFS.

2.4.2. GC-AFS

50–100 mg of dry sample was added to 4 mL of deionised water. An alkaline digestion was carried out by adding 4 mL of 6 M KOH and shaken for 4 hours. The digest was acidified with 4.4 mL of HCl before adding 8 mL of acidified KBr/$CuSO_4$ mixture. The methylmercury in the digest was selectively extracted into 10 mL DCM with constant shaking overnight. The samples were centrifuged at 2000 rpm. A known volume of the DCM extract was removed and extracted with 1.5 mL of 0.01 M

sodium thiosulphate solution with 30 min of constant shaking followed by 30 s of mixing with a vortex mixer. The thiosulphate layer was removed and the DCM layer was extracted with another 1.5 mL of thiosulphate solution. The two thiosulphate extracts were combined and 1.2 mL of the acidified KBr/$CuSO_4$ solution was added. The methylmercury was extracted into 1 mL DCM with constant shaking for 30 min followed by 30 s of mixing with a vortex mixer. The DCM extract was removed and dried with anhydrous sodium sulphate before analysis by GC-AFS.

3. Results and discussion

3.1. Optimisation of LC-AFS extraction method

The extraction method was initially developed by analysing seafood and hair reference materials using CVG to generate the volatile mercury species. Initially, the extractions were carried out using APDC concentrations of 1.5 mM and 10 mM in the extraction solution. A comparison of the recoveries and error between replicates for SRM-1566b and TORT-2 has been given in Table 3. Low recoveries and high RSD's were obtained using the extraction solution containing 1.5 mM APDC, whereas the 10 mM APDC extraction solution yielded much higher recoveries and lower RSD's. Therefore, an APDC concentration of 10 mM was chosen for extraction of the samples. Using higher APDC concentrations beyond 10 mM APDC caused the baseline to dip when the APDC in the sample eluted through the system. Thus, an APDC concentration of 10 mM was chosen for the extraction because the dip in the baseline was minimised and did not interfere with the measurements.

Table 3. Comparison of methylmercury extracted for reference materials TORT-2 (lobster hepatopancreas) and SRM-1566b (oyster tissue) using 1.5 mM and 10 mM APDC in the extraction solution.

Reference material	Matrix	Certified concentration (mg kg^{-1})	Measured concentration using 1.5 mM APDC (mg kg^{-1})	Recovery for $MeHg^+$ (%)	Measured concentration using 10 mM APDC (mg kg^{-1})	Recovery for $MeHg^+$ (%)
SRM-1566b	Oyster tissue	0.0132 ± 0.0013	0.0085 ± 0.0035	64.6 ± 26.3	0.0135 ± 0.0003	102 ± 2.4
TORT-2	Lobster hepatopancreas	0.152 ± 0.013	0.143 ± 0.009	94.2 ± 6.12	0.155 ± 0.002	102 ± 1.5

3.2. Optimisation of vapour generation using different concentrations of acetic acid.

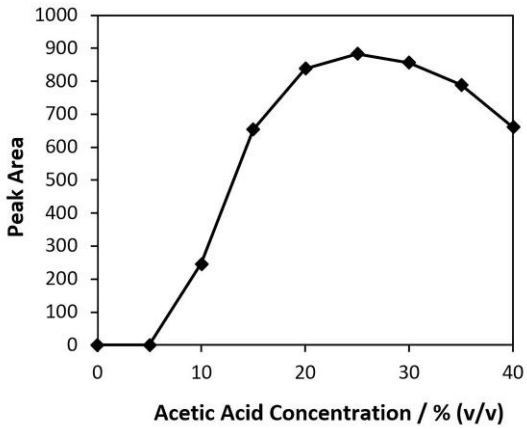

Figure 2. Optimisation of acetic acid concentration for online photo-reduction using 5 µg L^{-1} methylmercury.

Acetic acid was tested as a photo-reductant. A 5 µg L^{-1} methylmercury standard was analysed using acetic acid concentrations varying from 0% to 40% (v/v). A plot of the peak area for the methylmercury signal with varying acetic acid concentration is shown in Figure 2. The optimum acetic acid concentration for photo-reduction of methylmercury was found to be 25% (v/v) based on the peak area obtained.

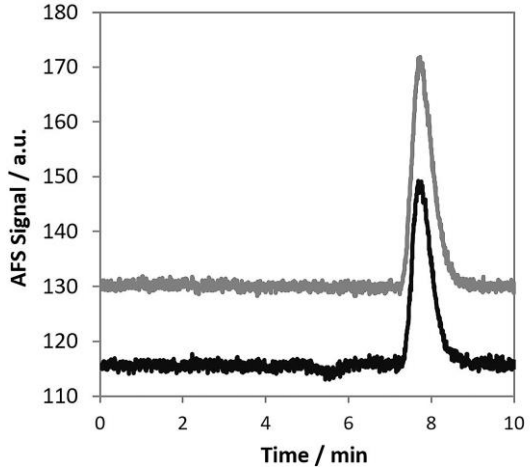

Figure 3. Overlaid chromatograms obtained from the analysis of 5 µg L^{-1} methylmercury using LC-PVG-AFS (grey) and LC-CVG-AFS (black) methods. The signal obtained by the acetic acid PVG method has been shifted by +15 a.u. for clarity.

A comparison of the signals obtained by the LC-PVG-AFS method and the LC-CVG-AFS method has been given in Figure 3. The PVG method provided a more sensitive signal with similar peak shape when compared to that obtained using the CVG method. PVG gave a peak height of 40.5 a.u., whereas CVG gave a peak height of 33.1 a.u.. It should also be noted that the dip in the baseline of the CVG chromatogram at 5.5 min is not present in the PVG chromatogram. This is the injection peak, in which the APDC in the extraction matrix binds with the mercury impurity in the acidified tin(II) chloride solution therefore reducing the baseline concentration. The acetic acid is of better purity, which can be shown as there is no dip in the baseline for the PVG method.

3.3. Method validation

The newly developed extraction and LC-PVG-AFS method was applied to seafood and hair matrixes. A comparison of the method LOD's and reproducibility for CVG and PVG has been provided in Table 4. The acetic acid PVG of mercury provided much higher reproducibility compared to that of the CVG technique. The measurement LOD in the extract was also much lower for PVG than that of CVG. This was likely due to the presence of trace levels of mercury in the tin(II) chloride reductant, causing CVG to give larger baseline noise than PVG. Therefore, PVG provides a clear advantage over CVG in terms of measurement precision and detection limits.

Table 4. Method LOD's for LC-CVG-AFS and LC-PVG-AFS and the equivalent LOD concentration in the extract. Reproducibility was calculated as the RSD for 18 measurements of a 5 μg L^{-1} standard.

Vapour generation method	Reproducibility (%)	LOD in extract (ng L^{-1})	Method LOD (μg kg^{-1})
CVG	5.1	78	2.77
PVG	2.2	30	1.06

Table 5. Measured concentrations and recoveries for methylmercury in seafood and hair reference materials. Standard deviations presented were calculated for a set of 3 measurements.

Reference material	Matrix	Certified concentration (mg kg^{-1})	Measured con-centration using LC-CVG-AFS (mg kg^{-1})	Recovery for $MeHg^+$ (%)	Measured con-centration using LC-PVG-AFS (mg kg^{-1})	Recovery for $MeHg^+$ (%)
Seafood						
SRM-1566b	Oyster tissue	0.0132 ± 0.0013	0.0136 ± 0.0007	104 ± 5.2	0.0123 ± 0.0003	93.2 ± 2.13
TORT-2	Lobster hepatopancreas	0.152 ± 0.013	0.157 ± 0.004	103 ± 2.7	0.160 ± 0.002	105 ± 1.3
ERM-CE464	Tuna	5.50 ± 0.17	5.09 ± 0.07	92.6 ± 1.38	5.25 ± 0.08	95.5 ± 1.40
DOLT-4	Dogfish liver	1.33 ± 0.12	1.33 ± 0.01	100 ± 0.3	1.33 ± 0.02	100 ± 1.7
DORM-2	Dogfish muscle	4.47 ± 0.32	4.58 ± 0.11	102 ± 2.5	4.68 ± 0.05	105 ± 1.1
Hair						
IAEA-085	Human hair (spiked)	22.9 ± 1.0	22.5 ± 0.7	98.1 ± 2.94	23.4 ± 0.27	102 ± 1.2
IAEA-086	Human hair	0.258 ± 0.021	0.251 ± 0.001	97.3 ± 0.30	0.252 ± 0.013	97.7 ± 5.10

3.3.1. Reference materials

The concentrations and recoveries of methylmercury in the seafood and hair samples using LC-CVG-AFS and LC-PVG-AFS are provided in Table 5. For both vapour generation methods, good recoveries were observed for the reference materials: 92.6% to 104% for CVG, and from 93.2% to 105% using PVG. All concentrations obtained lie within the accepted range for the certified reference materials with the exception of ERM-CE464, which was found to be significantly different from the certified concentration within a 95% confidence interval using both CVG and PVG. When comparing the two vapour generation techniques to each other, no significant difference was found within a 95% confidence interval in the recoveries obtained. This could suggest that either the extraction is not accurate for tuna matrix, or that the tuna reference material has been poorly maintained. However, both vapour generation techniques provided recoveries within 90–110%. The method detection limits for all sample measurements, shown in Table 4, were below 2.77 μg kg^{-1} for CVG and 1.06 μg kg^{-1} for PVG based on the smallest dilution factor used. The mass of sample used in the extraction procedure did not give a noticeable effect on the recovery of methylmercury, as 50 mg of sample was extracted for ERM-CE464 and 200 mg of sample was extracted for SRM-1566b.

The newly developed extraction method is therefore suitable for extraction of methylmercury in seafood and hair matrices, with good recoveries for reference materials. The LC-PVG-AFS technique provides similar recoveries to that of its CVG predecessor, therefore assuring accuracy of the PVG method. PVG offers a much greener approach to analysis compared to CVG in accordance with green

analytical chemistry, namely “elimination” and “reduced labour”.[27] Tin(II) chloride, which is toxic to humans and very toxic to the marine environment, and bromide/bromate solution, which is a suspected carcinogen, are eliminated and replaced with acetic acid, which is much less toxic in comparison. Waste concentrations of acetic acid are approximately 6–7% (v/v), which is equivalent to the concentration observed in household vinegar. Additionally, acidified bromide/bromate solution and tin(II) chloride are not stable over multiple days and are usually prepared fresh or one day in advance, whereas acetic acid is stable for much longer. Therefore, using PVG can also reduce overall preparation time, leading to reduced labour. The high stability of reagents could also potentially benefit online instrumentation in industry for applications such as waste water monitoring, as long reagent lifetime is required to allow the instrument to run unattended for long periods of time. Cost is also reduced using PVG, as the expensive tin(II) chloride, in addition to the other chemicals used, is replaced with much cheaper acetic acid and deionised water. This makes the overall cost per analysis cheaper and could potentially appeal to remote labs in low and mid-income countries as the overall cost per measurement is considerably reduced.

3.3.2. Comparison of LC-PVG-AFS with GC-AFS in a range of tuna samples

GC-AFS is a well-established method for mercury speciation analysis in literature, which makes it a good method to use for further validation of the LC-PVG-AFS method. The extraction method used is a variation on a method by *Cai et al.*[28], in which the methylmercury is converted to methylmercury bromide and analysed by GC-AFS without any derivatisation by propylation or phenylation. Since fish contain fats and oils, which can be extracted into the DCM layer, a clean-up step was employed to remove these organics by extracting the methylmercury from the first DCM extract into 0.01 M sodium thiosulphate and then back extracting into DCM. Here, we compared the methylmercury concentrations obtained by LC-PVG-AFS to the concentrations obtained by GC-AFS using a scatter plot with linear regression shown in Figure 4. The methylmercury concentration in the tuna samples varied from 1.06–6.60 mg kg^{-1} using LC-PVG-AFS and from 1.07–6.73 mg kg^{-1} using GC-AFS. Converting the methylmercury concentration obtained by both methods from dry weight to wet weight basis using the moisture content determined after drying showed that 7 of the 14 tuna samples were found to exceed the limit of 1 mg kg^{-1} (wet weight basis) for total mercury in seafood, as set in current EU regulation[29], from methylmercury alone.

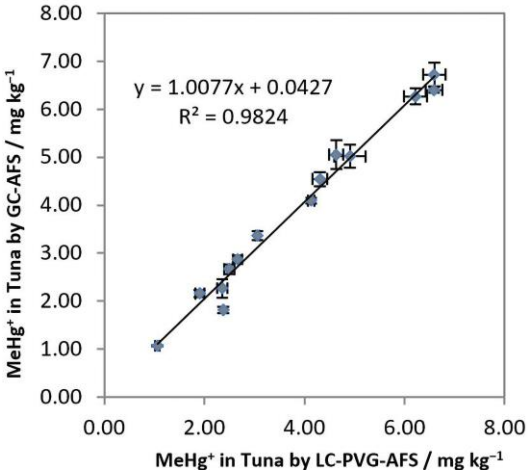

Figure 4. Methylmercury concentrations for 14 yellowfin tuna samples determined by LC-PVG-AFS and GC-AFS. Error bars for both methods are one standard deviation of three replicates.

The methylmercury results from measurements of the tuna samples using both methods show great correlation with a slope of 1.0077 and an R^2 value of 0.9824. The value of the slope is very close to 1, which shows negligible bias on the slope. The intercept of +0.0427 is close to 0, but shows a small bias, which is not significant when considering the samples with higher concentrations, but can become significant at lower concentrations. For the tuna sample with the lowest methylmercury concentration, the intercept gives a bias of 4.02% on the concentration of the sample which, at a 95% confidence interval, is not significant for the samples measured. Despite the analysis of tuna reference material ERM-CE464 giving a low recovery for methylmercury, the comparison of concentrations obtained for real samples with an already well established technique has shown that the new extraction procedure is a valid approach for the determination of methylmercury in tuna matrix. Although the measured samples contain methylmercury concentrations >1 mg kg^{-1}, the method still provided accurate and precise measurements of seafood certified reference material, which can be confidently reported down to 0.01 mg kg^{-1} dry weight of sample, as presented in Table 5.

The simplified extraction and LC-PVG-AFS method provides a clear advantage over the validated GC-AFS in terms of green analytical chemistry, while providing good similarity of results. Highly volatile and suspected carcinogen dichloromethane used for extraction, is replaced with methanol. The reduced number of chemicals required also reduces the overall cost. The new method also provides a much faster sample preparation of approximately 1 hour per batch of samples, as opposed to the 2 day sample preparation per batch required for GC-AFS measurements, which reduces the overall labour.

4. Conclusions

A new, simpler extraction and analysis technique utilising photochemical vapour generation has been developed for mercury speciation measurements using LC-PVG-AFS. The methylmercury measurements obtained using PVG yielded higher precision and lower detection limits, while maintaining similar sharp peak shapes to those obtained by CVG. Satisfactory recoveries were obtained for 7 certified reference materials with seafood and hair matrixes. The extraction and analysis method were further validated against GC-AFS, which showed excellent correlation of the results for 14 tuna fish samples between the two methods. Utilising a simple extraction with LC-PVG-AFS analysis technique can compete with GC-AFS and CVG methods by providing a much simpler, faster, and more cost effective analysis while maintaining good accuracy, precision, and sensitivity.

Conflicts of interest

There are no conflicts to declare.

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