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Mercury determination in various environmental, food and material complex matrices using unified operating conditions for a cold vapor generation high-resolution continuum source quartz tube atomic absorption spectrometry method†

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An analytical method with broad applicability based on cold vapor generation high-resolution continuum source quartz tube atomic absorption spectrometry was developed and evaluated for the determination of total mercury in matrices with various complexities and compositions. Sample preparation for different matrices of food, environmental samples and (bio)polymeric materials and unified operating conditions for derivatization and measurement were evaluated. The method was validated according to established requirements (Eurachem Guide 2014, EC Decisions 657/2002; 333/2007; 836/2011 and Association of Official Analytical Chemists Guide – AOAC). Analytical versatility was checked on various samples of fish fillets, mushrooms, soil, water and water sediment, sludge from a wastewater treatment unit, and (bio)polymeric materials from waste recycled from food packaging, computers and garden tools. Under optimal conditions for cold vapor generation in a batch system, namely 3% (v/v) HCl as reaction medium for 5 mL aliquot samples and a volume of 3.5 mL 0.3% (m/v) $NaBH_4$ stabilized in 0.2% (m/v) NaOH as derivatization reagent, the detection limit for Hg in terms of peak height measurement (n = 7 days) was in the range 0.064 ± 0.004 μg L^{-1} in water, 0.014 ± 0.001 mg kg^{-1} in environmental samples and 0.009 ± 0.001 mg kg^{-1} in (bio)polymeric materials. Overall recovery of Hg by analysis of certified reference materials was 102 ± 20% (k = 2) in food, soil, wastewater and water sediment, and polyethylene. Precision for the measurement of various real samples ranged between 4.2 and 15.0%. A performance study highlighted that the method was sensitive, free of non-spectral interference coming from the multielemental matrix and that it complied with the requirements for Hg determination set in EC Decisions and AOAC Guidelines at least for the more common matrices analyzed for social impact.

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Introduction

As mercury is highly persistent, volatile and bioaccumulates, it is considered a priority hazardous element and very harmful to human health and the environment.[1] Details of the sources of Hg, level of environmental contamination and current legislation on protecting the environment and food against contamination are presented in ESI, Section 1.†

Given the high toxicity of mercury and its species even at extremely low concentrations, there is virtually a continuing need to develop and validate sample preparation and determination procedures.[2] Well-established spectral methods based on atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma optical emission spectrometry and mass spectrometry (ICP-OES, ICP-MS), laser-induced breakdown spectroscopy (LIBS), X-ray fluorescence spectrometry (XRF) and optical emission spectrometry in various microplasma sources have been extensively used for Hg determination and speciation.[3–18] Hyphenated techniques such as high-performance liquid chromatography in conjunction with post-column cold vapor (CV) derivatization and detection by AFS (HPLC-CV-AFS) or inductively coupled plasma mass spectrometry (HPLC-ICP-MS), gas chromatography coupled to AFS (GC-AFS) or ICP-MS (GC-ICP-MS) have been developed to provide high sensitivity, accuracy and lack of spectral and non-spectral interference for Hg speciation in food, biological and environmental samples.[19–25]

In the last 20 years the most significant advance in atomic spectrometry, high-resolution continuum source flame atomic absorption spectrometry/graphite furnace/electrothermal atomic absorption spectrometry (HR-CS-FAAS, HR-CS-GFAAS/ETAAS), has

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been developed and implemented in laboratories. It has multiple advantages over low-resolution line source atomic absorption spectrometry (LR-LS-AAS), such as: (i) its capability of fast multi-element analysis (metals and non-metals) at atomic lines and molecular bands; (ii) its use of a high-intensity Xe arc lamp in the UV-vis region for the determination of all elements; (iii) high-resolution display of spectral range around the analytical line due to its echelle monochromator; (iv) accurate correction of the continuous and fine-structured background and (v) improved stability of the signal following simultaneous measurement of analyte and background.[26–30] The HR-CS-GFAAS/ETAAS method has been successfully applied to determine total mercury in a variety of matrices, such as fertilizers, soil and sludge, water, blood and urine using direct solid/liquid sampling or cold vapor generation (CVG).[31–36] Also, Hg speciation analysis in fish by high-performance liquid chromatography and post-column ultraviolet-photochemical vapor generation high-resolution continuum source quartz tube-atomic absorption spectrometry (HPLC-UV-PVG-HR-CS-QTAAS) after extraction in tetramethylammonium hydroxide (TMAH) was investigated.[37] Compared to traditional CV-AFS and thermal decomposition atomic absorption spectrometry (TD-AAS) as dedicated methods for mercury determination, the HR-CS-GFAAS/ETAAS method with or without CVG has the advantage of fast sequential multielement determination of chemical-vapor-generating elements (Hg, As, Sb, Bi, Sn).[7,35,36,38]

The main challenges for the determination and speciation of Hg by non-chromatographic methods are the need for high sensitivity and avoidance of non-spectral interference coming from multielemental matrices contained in the original samples.[15,16] These goals can be achieved by the separation/preconcentration of Hg species using continuous-flow solid-phase extraction on gold, palladium, silver, silica or magnetic nanoparticles functionalized with 1,5 bis(di-2-pyridyl) methylene thiocarbohydrate (MSPE-DPTH-MNPs), or CVG with/without vapor preconcentration.[4,5,7–9,11–17,32–37] Undoubtedly, CVG performed either classically with $SnCl_2$ or $NaBH_4$, or by green methods using low molecular weight organic reagents, considered a critical step in analysis by atomic spectrometry, ensures a substantial improvement in the detection limit (LOD) due to the high rate of vapor introduction into the spectral source, the opportunity for on-line preconcentration, as well as the elimination of non-spectral effects following the efficient separation of mercury species from the sample matrix.[39–47]

Usually, the methods presented in the literature are fit-for-purpose and they are optimized only to obtain the best performance for a certain matrix. For example, there are no studies that highlight the determination of Hg and the analytical performance in matrices with various complexities and compositions that use the same CV derivatization conditions and the same operating conditions of commercially available spectral instrumentation. Therefore, this study explored the development of a method with broad applicability that would be sensitive, robust, free of non-spectral interference, based on cold vapor generation high-resolution continuum source quartz tube atomic absorption spectrometry (CVG-HR-CS-QTAAS) using unified conditions for CVG and instrumental operating parameters that would be broadly applicable to the determination of total Hg at least in matrices of wide interest for their social impact, such as food of marine and vegetable origin, environmental samples and (bio)polymeric materials. For this purpose, a performance and analytical versatility study was carried out on samples of fish muscles, mushrooms, soil, water, water sediment, sludge from a wastewater treatment plant, (bio)polymeric materials, such as corn starch, polyethylene (PE), polyethylene terephthalate (PET), and acrylonitrile butadiene styrene (ABS) recovered from packaging waste and electronic equipment recycling. Accordingly, the working conditions for CVG and instrumental operation were optimized using an Hg standard solution, which were then applied to certified reference materials (CRMs) and real test samples in order to check whether the unified conditions are appropriate for matrices with various complexities and compositions. The figures of merit were evaluated in terms of LOD and limit of quantification (LOQ), non-spectral effects coming from the multielemental matrices, precision expressed as repeatability and reproducibility, and accuracy through an analysis of CRMs matching the test sample matrices. The study was conducted in compliance with Eurachem Guide 2014, the demands in Decisions of European Commission and recommendations of the association of official analytical chemists (AOAC) concerning the performance of analytical methods and interpretation of results for the control of contaminants.[48–52] The results indicated that the developed procedure has practically no limitations from the point of view of the studied matrices and the obtained analytical performance. The high analytical potential and versatility of the CVG-HR-CS-QTAAS method developed in this study will provide good reasons to use it in routine analysis in laboratories where the related instrumentation is available.

Materials and methods

Instrumentation

Experiments were carried out on CVG-HR-CS-QTAAS equipment (Analytik Jena AG, Jena, Germany) consisting of a high-resolution continuum source atomic absorption spectrometer Model ContrAA 300, an HS55-manual chemical vapor generation system and a quartz tube (140 mm length, 15 mm i.d. and end windows) mounted in the place of the acetylene-air burner. The experimental set-up and the operating procedure are presented in ESI (Section 2, Fig. S1).† A ContrAA 300 spectrometer equipped with an air-acetylene flame was used for determination of elements in the sample matrix. A Berghof MWS3+ system (Berghof, Germany) was used for the microwave-assisted wet digestion (MAWD) of samples. A Labconco free Zone 2.5 freeze-drying system (Kansas, USA) was used for freeze drying of samples. A Retsch RS 200 (Retsch, Haan, Germany) with a tungsten carbide grinding set was used for milling of soil and sediment samples, while an SM 100 cutting mill, also from Retsch, was used for cutting of (bio)polymeric materials.

Reagents and CRMs

Hydrochloric acid 37% (m/m) for Hg determination ($\leq 10^{-8}$% Hg), HNO_3 65% (10^{-9}% Hg), H_2SO_4 96% (m/m), $NaBH_4$ for

analysis ($\leq 10^{-4}$% Hg), NaOH microselect (>98%) and H_2O_2 30% (m/m) for analysis, ICP standard 1000 mg L^{-1} Hg in 10% HNO_3 and chromic acid cleaning solution, all purchased from Merck (Darmstadt, Germany), were used in this study. A solution containing 5 μg L^{-1} Hg in 0.5–6% (v/v) HCl and solutions of 0.05–0.5% (m/v) $NaBH_4$ stabilized in 0.2% (m/v) NaOH and 0.3% (m/v) $NaBH_4$ stabilized in 0.05–0.4% (m/v) NaOH were used for the optimization of CVG conditions. Eight standards in the range 0.1–10 μg L^{-1} Hg stabilized in 3% (v/v) HCl were prepared to generate the calibration curve. A solution of 3% (v/v) HCl as blank was used for background correction. Solutions of real test samples and CRMs were stabilized in 3% (v/v) HCl. All solutions were prepared daily. Ultra-pure water (18 MΩ cm) prepared with a Milli-Q water purification system (Millipore, Bedford, USA) was used for the preparation of samples and all standard solutions. A solution containing 1.50% (m/v) KBr and 1.08% (m/v) $KBrO_3$ in concentrated HCl was prepared to be used for the decontamination of glassware, the PTFE reaction cell of the HS55 CVG system and PTFE digestion vessels of the samples.[38]

The accuracy of the method was checked by analysing several certified materials (CRMs): BCR-463 Tuna fish, ERM-CE464 Tuna fish, ERM-BB422 Fish muscle, ERM-CE278k Mussel tissue, ERM-CA713 Wastewater, BCR-280R Lake sediment, ERM-CC580 Estuarine Sediment, ERM-EC680k and ERM-EC681k of PE as granules from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium), SRM 2976 Mussel Tissue (National Institute of Standards and Technology, Gaithersburg, USA), CSM-3 Mushroom Powder (Institute of Nuclear Chemistry and Technology, Warsaw, Poland), Tort-2 Lobster Hepatopancreas Reference Material for Trace Metals (National Research Council Canada, Ottawa, Ontario, Canada), LGC 6141 Soil Contaminated with Clinker Ash (Department of Trade and Industry, Teddington Middlesex, UK), Metranal-34 Loam metals, from Analytika Spol (Vysocany, Czech Republic) and CRM025050 Metals in soil (Resource Technology Corporation, Laramie, USA).

Description and preparation of test samples

To assess the applicability of the CVG-HR-CS-QTAAS method, several samples with various matrices were analysed: fish fillet such as hake, Atlantic cod, Nile perch, herring, tuna, tilapia, carp and trout (8), mushrooms (1), soils collected in the vicinity of a former chlor-alkali plant (5), river sediment (4), sludge from a water treatment unit (2), plastics based on PE from shopping bags (1), ABS from recyclable electronic equipment and garden tools (5), PET from bottles for still mineral water (1) and biopolymeric material based on corn starch from shopping bags for food packaging (1).

Fish samples were prepared after washing the fillet with ultrapure water, and removing the skin and bone debris. The mushrooms were washed and the skin was removed. Then both samples were chopped into small pieces with a knife. Food samples were lyophilized at −50 °C for 48 h in half-filled 50 mL freeze flasks, and then sieved (<100 μm). Soil and sediment samples were sieved (<2 mm) to remove roots and stones. The sludge collected from the water treatment unit, soil and sediment samples were dried in an oven at 105 ± 5 °C for 24 h, ground in a ball mill and sieved (<100 μm). (Bio)polymeric test samples were washed with ultrapure water, dried and cut (<2 mm) using a cutting mill. All samples were kept in brown flasks in a refrigerator at 4 °C until sample preparation for analysis. Details of the pre-treatment procedure of the samples have already been published by Frentiu *et al.*[7,38,53] Amounts of 0.5 g CRM or lyophilized test sample of fish fillet and mushroom were subjected to MAWD in a mixture of 9 mL of HNO_3 and 3 mL of H_2O_2, while the soil, sediment and sludge was in 12 mL of *aqua regia*, following the thermal program used by Frentiu *et al.*[7,38] Amounts of 0.3 g of (bio)polymeric materials were digested in a mixture of 3 mL of HNO_3 and 3 mL of H_2SO_4, running the forementioned MAWD program.[53] After cooling, the digests were diluted to 25 mL with ultrapure water, filtered (0.45 μm) and stored in polyethylene flasks in the refrigerator (4 °C) for at most one week. Dilution ratios to 25 or 50 mL were: 1 : 5–1 : 20 for CRM samples of fish, mushroom, and water; 1 : 10–1 : 1000 for CRMs of sediment and soil; 1 : 10–1 : 100 for CRMs of PE; and 1 : 5–1 : 10 for all test samples after the addition of 1.5 mL of 37% HCl. Five parallel measurements were performed using 5 mL sample aliquots in which 3.5 mL of 0.3% (m/v) $NaBH_4$ solution stabilized in 0.2% (m/v) NaOH were added to the reaction cell. All test samples and CRMs were analysed by HR-CS-FAAS for determination of metals forming the multielemental matrix, under the operating conditions related to air–acetylene flow rate ratio and observation height for each element at the principal line recommended by the manufacturer of the instrument, and accessible in Aspects CS 2.2.1. software. The optimization of measurement order of elements was selected in order to reduce acetylene consumption and analysis time.

The digestion PTFE vessels were decontaminated by filling with 10% (v/v) KBr–$KBrO_3$ solution for 24 h, followed by running the same program on the microwave digestion system as for the samples using 5 mL of 1 : 1 HNO_3 solution. Finally, the digestion vessels were rinsed with ultrapure water. The glassware, flasks for sample storage and the reaction cell of the HS55 CVG system were cleaned by keeping them filled with 10% (v/v) KBr–$KBrO_3$ solution overnight and rinsing several times with ultrapure water. The reaction cell of the HS55 system was washed between measurements with ultrapure water (2 × 10 mL). Memory effects between samples were avoided by cleaning the reaction cell with 1.4 mol L^{-1} HNO_3. The quartz tube and quartz windows were decontaminated by soaking overnight in chromic acid cleaning solution, rinsing with ultrapure water and drying.

Results and discussion

Optimization of CVG and measurement conditions

Cold vapor generation and measurement conditions of the analytical signal affect the sensitivity, LOD, non-spectral effects, accuracy, and precision of Hg determination. The CVG conditions were optimized on 5 μg L^{-1} standard Hg^{2+} solutions using as criteria the best analytical signal and LOD. Then, the respective conditions were used for the analysis of all the CRMs

and test samples to check for possible systematic errors in the determination of Hg in matrices different in both complexity and composition. The size of the analytical signal in the CVG technique is dependent on the efficiency of generating CV, their purging from the solution and transport in the quartz tube. Thus, the concentrations of HCl, $NaBH_4$, NaOH and the sample:$NaBH_4$ solution ratio were optimized. The effects of 0.5–6% (v/v) HCl, 0.02–0.4% (m/v) $NaBH_4$ and 0.05–0.5% (m/v) NaOH on CVG from 5 mL aliquot volumes of 5 μg L^{-1} Hg^{2+} standard solution in terms of peak height and peak area measurement of the absorption signal are presented in ESI (Section 3, Fig. S2–S5).† The peak height measurement mode provided advantages in terms of lower consumption of borohydride solution by 40% and a lower amount of residue compared with the peak area mode. Moreover, the maximum signal in peak height mode was reached at 20 s after mixing the sample with the $NaBH_4$ solution, compared with the 60 s necessary for recording of peak area. The total running time, including the analysis sequence in the peak height mode, the manual introduction of the sample into the reaction cell, the mixing of aliquot sample with $NaBH_4$, the purging time of the reaction cell after measurement, and rinsing with ultrapure water between measurements was 120 s, *versus* 200 s in the peak area mode, which meant high-throughput chemical analysis by CVG-HR-CS-QTAAS in an HS55-manual system. By using the HS60-flow injection system the analysis time could be shortened to 60 s. Besides the increased analysis speed, reductions in concentration and reagent consumption can be achieved. An optimization study is necessary, which we are considering carrying out in our laboratory in a future study. The optimum working conditions for Hg determination by CVG-HR-CS-QTAAS in peak height mode using the HS55-manual system are presented in Table 1. The influence of the number of selected pixels associated with the analytical line (1–7 pixels) on analytical performance was studied under optimum CVG conditions and the results are presented in ESI (Section 4, Table S1).† As shown in Table S1,† the best calibration and analytical sensitivities, determination coefficient, accuracy and precision were obtained for 5 pixels (CP ± 2) associated with the analytical signal for the Hg 253.652 nm line.

Figures of merit and method validation by LOD and accuracy

The LODs and characteristics of the calibration plot under the optimal working conditions of the CVG-HR-CS-QTAAS method using the HS55-manual system are provided in Table 2.

The data in Table 2 show good calibration linearity with a 95% determination coefficient of 0.9996 ± 0.0003 over 7 days when using peak height measurement and a 5 mL sample aliquot. Reproducibility of the calibration plot and LOD was also good with an RSD of 6.3% and an instrumental LOD (3σ criterion) of 0.051 ± 0.003 μg L^{-1}. CVG-HR-CS-QTAAS provided a method LOD of 0.064 ± 0.004 μg L^{-1} Hg in water, 0.014 ± 0.001 mg kg^{-1} in food/environmental samples, and 0.009 ± 0.001 mg kg^{-1} in (bio)polymeric materials. Limits of quantifications were 0.211 ± 0.013 μg L^{-1} in water, 0.046 ± 0.003 mg kg^{-1} in food/environmental samples, and 0.030 ± 0.003 mg kg^{-1} in (bio)polymeric materials. The CVG-HR-CS-QTAAS method fulfilled the demands for Hg quantification in seafood, since the LOD/LOQ were 20–350/6–110-times lower than the maximum admitted concentration in fish (1 mg kg^{-1}) set in Decision 1881/2006/EC, or soil (normal value 1 mg kg^{-1}), water sediment (maximum admitted 0.3 mg kg^{-1}) and wastewater sludge (maximum admitted 5 mg kg^{-1}).[54] Quantification of Hg is possible in surface water as LOD/LOQ assessed for 5 mL sample aliquots were 780/235-times lower than the maximum admitted value of 50 μg L^{-1}.[55] CVG-HR-CS-QTAAS fulfils the demands of EC Decisions relative to a method used for Hg control to provide a detection/quantification limit 10/5-times lower than the maximum admitted values.[49–51]

Data in Table S2 (ESI, Section 5)† offers a comparison of LOD in the CVG-HR-CS-QTAAS and other methods in identical or similar matrices used for Hg determination, such as HR-CS-GFAAS/ETAAS, TD-AAS, CV-AFS, or ICP-OES and ICP-MS with/without CVG, as well as chromatography coupled with spectral detection for speciation analysis. The nature of the matrix and sample preparation procedures are also indicated.

The data in Table S2† show that the CVG-HR-CS-QTAAS method developed in this study provides a better LOD than HR-CS-GFAAS for the direct determination of total Hg in soil and sludge after *aqua regia* microwave digestion, *aqua regia*

Table 1 Working conditions for the determination of Hg by the CVG-HR-CS-QTAAS method using the HS55-manual system

Parameter	Setting
Analytical wavelength (nm)	253.652
Number of pixels associated to analytical line	5 (CP ± 2)
Measurement of the transient signal	Peak height
Time period of recording transient absorption spectrum (s)	20
Air flow rate (L h^{-1})	6
Heating temperature of the quartz tube (°C)	150 ± 10
Auto-zero time (s)	20
Volume of $NaBH_4$ solution (mL)/pumping time (s)	3.5/13
Purging time of reaction chamber (s)	20
Sample volume (mL)	5
Calibration	External standards
Concentration of Hg in standard solutions (μg L^{-1})	0.1; 0.2; 0.5; 1; 2; 5; 7; 10
Number of repeated measurements of standards and samples	5

Table 2 Characteristics of the calibration curve, LODs for Hg in water, seafood, environmental samples and (bio)polymeric materials for 5 mL aliquot samples obtained by CVG-HR-CS-QTAAS in a reproducibility study for 7 days

Calibration curve parameters				LOD		
Intercept	Slope (L μg^{-1})	R^2	Blank standard deviation ($n = 11$)	Water[a] ($\mu g\ L^{-1}$)	Food and environmental samples[b] (mg kg^{-1})	Materials[c] (mg kg^{-1})
0.0004 ± 0.0002	0.0175 ± 0.0011	0.9996 ± 0.0003	0.00030	0.064 ± 0.004	0.014 ± 0.001	0.009 ± 0.001

[a] LOD obtained for 20 mL water sample made up to 25 mL and instrumental LOD for 3σ criterion (LOD = $3s_b/m$, where s_b is the standard deviation of the blank signal for a 3% (v/v) HCl solution and m is the slope of the calibration curve). [b] LOD in dry mass (fish, soil, water sediment and sludge) calculated based on instrumental LOD and 0.5 g sample digested and made up to 25 mL, and 5-times dilution. [c] LOD in (bio)polymeric materials calculated based on instrumental LOD and 0.3 g sample digested and made up to 25 mL, and 2-times dilution.

leaching, slurry sampling and preconcentration on Au/Pd nanoparticles (AuPNs/PdNPs) (0.2–1.3/0.3–0.7 mg kg^{-1}), and CV-HR-CS-ETAAS in blood and urine (2.3 $\mu g\ L^{-1}$) using AuNPs as a chemical modifier, and water after preconcentration by on-line MSPE-DPTH-MNPs (0.22 $\mu g\ L^{-1}$) or silica modification (0.17 $\mu g\ L^{-1}$).[32,34–36] Also, our LOD was better than those reported for the speciation of inorganic and organic Hg in fish by HPLC-UV-PVG-HR-CS-QTAAS after extraction in TMAH or HCl (0.47 $\mu g\ L^{-1}$).[37] The detection capability of CV-HR-CS-QTAAS was found to be similar to that of HPLC-UV-CV-AFS used for Hg speciation in seafood, yeast and garlic (0.11 $\mu g\ L^{-1}$), and determination of total Hg by ultraviolet photo-induced vapor generation inductively coupled plasma optical emission spectrometry (UV-PVG-ICP-OES) in wastewater and estuarine sediment (0.090 $\mu g\ L^{-1}$).[8,19] On the other hand, our LOD was similar to that reported on dedicated Hg instrumentation based on direct solid sampling TD-AAS and on-line trapping on an Au amalgamator (0.010 mg kg^{-1}), but poorer than that in CV-AFS (0.012 $\mu g\ L^{-1}$) after classical derivatization with $SnCl_2$, applied for total Hg determination in fish.[7,38] The LOD in CVG-HR-CS-QTAAS was also poorer than those reported for Hg determination in fertilizer by HR-CS-GFAAS (0.0048 mg kg^{-1}) using direct solid sampling, in water by a combination of ultrasound-assisted dispersive micro solid-phase extraction (USA-DMSPE) on AgNPs and leaching in 7 mol L^{-1} HNO_3 and detection by the HR-CS-GFAAS method (0.005 $\mu g\ L^{-1}$), or in marine sediment, marine biota and seawater by cold vapor inductively coupled plasma mass spectrometry (CV-ICP-MS) (0.00072 $\mu g\ L^{-1}$), all recognized for their high sensitivity.[11,31,33] In terms of LODs in polymeric materials (plastics) our method ensures a better LOD compared to direct solid sampling by laser ablation and measurement by inductively coupled plasma mass spectrometry (LA-ICP-MS) (1 mg kg^{-1}), CV-ICP-OES using microwave-assisted wet digestion (MAWD) and microwave-assisted wet digestion in a single reaction chamber (MAWD-SRC) in an HNO_3 + HCl mixture and CV generation (0.054 mg kg^{-1} and 0.029 mg kg^{-1}), and was similar to microwave-induced combustion (MIC) and detection by CV-ICP-MS (0.011 mg kg^{-1}).[56–58] Obviously, the LOD for Hg depends not only on the determination technique itself but also on the sample preparation and procedure for Hg preconcentration, which is required for Hg determination at ultratrace level. In our study, LOD improvement by

Table 3 Results for total Hg obtained by the CVG-HR-CS-QTAAS method in CRMs of seafood, mushrooms, water sediment, soil, polyethylene and wastewater

CRM sample	Certified value ± U_{CRM}[a] (mg kg^{-1})	Found value ± U_{lab}[b] (mg kg^{-1})	Recovery ± U_{lab}[b] (%)
BCR-463 Tuna fish	2.85 ± 0.16	3.00 ± 0.52	105 ± 17
ERM-CE464 Tuna fish	5.24 ± 0.10	5.30 ± 0.81	101 ± 15
ERM-BB422 Fish muscle	0.601 ± 0.030	0.627 ± 0.090	104 ± 14
Tort-2 Lobster Hepatopancreas	0.27 ± 0.06	0.29 ± 0.08	107 ± 27
ERM-CE278k Mussel tissue	0.071 ± 0.007	0.074 ± 0.021	104 ± 28
SRM 2976 Mussel tissue	0.0610 ± 0.0036	0.0640 ± 0.0140	105 ± 22
CSM-3 Mushroom Powder	2.849 ± 0.104	2.945 ± 0.313	103 ± 11
BCR-280R Lake sediment	1.46 ± 0.20	1.36 ± 0.45	93 ± 33
ERM-CC580 Estuarine sediment	132 ± 3	130 ± 30	99 ± 23
CRM 025050 Metals in soil	99.8 ± 18.2	97.6 ± 18.7	98 ± 19
Metranal-34 Loam metals	0.223 ± 0.016	0.238 ± 0.042	107 ± 18
LGC 6141 Soil contaminated with clicker ash	1.2 (indicative value)	1.25 ± 0.17	104 ± 14
ERM-CE681k Polyethylene (high level)	23.7 ± 0.8	23.1 ± 3.1	98 ± 13
ERM-EC680k Polyethylene (low level)	4.64 ± 0.20	4.58 ± 0.89	99 ± 19
ERM-CA713 Wastewater	1.84 ± 0.11[c]	1.89 ± 0.30[c]	103 ± 16
Pooled recovery (%)	—	—	102 ± 20

[a] U_{CRM} is expanded uncertainty for certified concentration ($k = 2$; 95% confidence level). [b] U_{lab} is expanded uncertainty in laboratory ($k = 2$, $n = 5$ parallel measurements and 95% confidence level). [c] Concentration expressed in $\mu g\ L^{-1}$.

a preconcentration procedure was not an objective, because the CVG-HR-CS-QTAAS method provides good LODs for Hg without a preconcentration step, sufficient for the determination of Hg in various matrix samples.

Table 3 presents the results for the determination of total Hg in several CRMs of seafood, mushrooms, sediment, soil, PE and wastewater.

The results presented in Table 3 indicate that the values found by the CVG-HR-CS-QTAAS method do not differ significantly from the certified values, because the bias (Δm), which is the difference between the found and certified mean values is lower than the U_{CRM} from the certificate and U_{lab} ($\Delta m < U_{CRM}$ and $\Delta m < U_{lab}$) for $k = 2$ (95% confidence level). Also, Dunnett's statistical test indicated the absence of systematic errors between the found and certified values for $p > 0.05$ (0.174–0.774).[59] Therefore, the overall recovery of Hg in CRMs of fish, mushroom, water sediment, soil, PE and wastewater was in the range 102 ± 20% ($k = 2$). These values were consistent with EC Decisions setting a combined uncertainty limit for recovery of ± 10% when quantifying contaminants, as well as AOAC Guidelines to provide recovery in the range 80–120%.[49–52] The accuracy of the CVG-HR-CS-QTAAS method is similar to that of fit-for-purpose conventional and non-conventional spectrometric methods (82–110%) based on HR-CS-GFAAS/ETAAS, CV-HR-CS-ETAAS, SPE-CVG-HR-ETAAS, CV-AFS, TDAAS, CV-ICP-MS and LA-ICP-MS for the determination of Hg in matrices, as presented in ESI (Section 5, Table S2).†

The composition of the multielement matrix determined in CRMs by HR-CS-FAAS and subjected to determination of Hg by the CVG-HR-CS-QTAAS method for which no matrix effects were found is presented in ESI (Section 6, Table S3).† Transitional elements that could cause interference are Fe, Cr, Mn, Co, Ni and Cu. Anyway, it can be stated that the CVG-HR-CS-QTAAS method for Hg determination is free from non-spectral interference generated by the multielement matrix of samples. Thus, the same conditions for CVG can be used for samples with various matrices analyzed in this study, which simplified the analytical method.

Precision of the CVG-HR-CS-QTAAS method by analysis of real samples

Table 4 presents the results obtained for Hg determination and precision in fish fillets of different varieties, mushrooms, soil collected in the vicinity of a former chlor-alkali plant, river sediment, sludge from a wastewater treatment unit and various (bio)polymeric materials. The compositions of the multielement matrices in test samples subjected to analysis for Hg determination are presented in ESI (Section 6, Table S3).† Relative uncertainties (%) of calibration standards, sample preparation, calibration curve fitting and measurement by aliquots analysis are presented in ESI (Section 7, Fig. S6).† According to Fig. S6,† as expected, the highest weight of uncertainty for Hg determination by CVG-HR-CS-QTAAS using the HS55-manual system, regardless of sample matrix, is due to aliquots analysis, which includes uncertainties of sample digestion and destruction of the organic matrix, and the manual introduction of sample aliquots into the HS55 system. It can be observed that the weight of uncertainty of the aliquots analysis is at least twice that of the uncertainty of prior measurement steps, including uncertainties of Hg concentration in stock solution, standards preparation, sample preparation and calibration curve fitting. Also, a slight worsening in method precision in the order foodstuffs < environmental samples < (bio)polymeric materials can be observed in Fig. S6.† This trend is in accordance with the complexity and difficulty of sample digestion. It is known that the digestion of (bio)polymeric materials is difficult and requires high-pressure systems and acid mixtures.

Table 4 Results for total Hg determination and precision by the CVG-HR-CS-QTAAS method in samples with various matrices

Sample	Mean concentration ± U_{lab}[a] (mg kg^{-1})	RSD[b] (%)
Fish (dry mass)		
Tuna	0.45 ± 0.05	5.6
Tilapia	0.24 ± 0.02	4.2
Carp	0.20 ± 0.06	15.0
Hake	0.43 ± 0.08	9.3
Cod	0.43 ± 0.04	4.7
Nile perch	0.37 ± 0.07	9.5
Trout	0.34 ± 0.06	8.8
Herring	0.49 ± 0.10	10.2
Mushroom (dry mass)		
Sample 1	1.66 ± 0.50	15.0
Soil (dry mass)		
Sample 1	0.23 ± 0.06	13.0
Sample 2	0.92 ± 0.15	8.2
Sample 3	0.34 ± 0.05	7.4
Sample 4	0.34 ± 0.05	7.4
Sample 5	0.19 ± 0.03	7.9
River sediment (dry mass)		
Sample 1	0.15 ± 0.02	6.7
Sample 2	0.09 ± 0.02	11.1
Sample 3	0.18 ± 0.03	8.3
Sample 4	0.18 ± 0.03	8.3
Sludge		
Sample 1	2.79 ± 0.46	8.2
Sample 2	0.56 ± 0.15	14.3
(Bio)polymeric materials		
Corn starch bag	0.28 ± 0.05	8.9
PET[c] (water bottle)	0.17 ± 0.04	11.8
PE[c] shopping bag	0.11 ± 0.03	13.6
ABS 1[c]	0.23 ± 0.03	6.5
ABS 2	0.10 ± 0.03	15.0
ABS 3	0.056 ± 0.008	7.1
ABS 4	0.09 ± 0.02	11.1
ABS 5	0.12 ± 0.03	12.5

[a] U_{lab} is expanded uncertainty in laboratory ($k = 2$, $n = 5$ parallel measurements and 95% confidence level). [b] RSD(%) is relative standard deviation (RSD = U_{lab} × 100/mean concentration). [c] PET – polyethylene terephthalate; PE – polyethylene; ABS – acrylonitrile butadiene styrene (plastic wastes from computer components and garden tools).

Our results indicated that adequate digestion of the (bio) polymeric materials using the high-pressure (100 atm) MAWD system and HNO_3 + H_2SO_4 mixture was obtained. Under these conditions, the precision of the CVG-HR-CS-QTAAS method using the HS55-manual system for Hg determination based on combined uncertainty was 4.2–15.0% for contents of 0.20–0.49 mg kg^{-1} in fish fillet, 15.0% for 1.66 mg kg^{-1} in mushrooms, 6.7–14.3% for measurements on soil, water sediment and sludge containing 0.09–2.79 mg kg^{-1} Hg and 6.5–15.0% for (bio)polymeric materials with contents of 0.056–0.28 mg kg^{-1} Hg. The comparative data presented in ESI (Section 5, Table S2)† indicates that other analytical systems characterized by a high degree of automation, such as (CV)HR-CS-GFAAS(ETAAS), CV-AFS, CV-ICP-MS and TDAAS have a precision only slightly better than CVG-HR-CS-QTAAS equipped with the HS55-manual system. In all methods, an improvement in precision can be observed when classical CVG or non-conventional UV-PVG are used. The precision of the CVG-HR-CS-QTAAS method could be improved by automation using the HS60 flow-injection system. However, in all cases the CVG-HR-CS-QTAAS method using the HS55-manual system fulfilled the requirements of EU legislation in terms of precision expressed as RSD% to be better than 20%, and AOAC Guidelines not to be higher than 15%.[49–52]

Conclusions

The study highlighted the fact that a mercury determination method based on CVG-HR-CS-QTAAS has broad applicability in at least common complex matrices of concern to scientific and human society. This was demonstrated through an analysis of CRMs and real matrices of food, environmental and (bio)polymeric materials using the same CVG conditions in an HS55-manual system. The analytical performance study demonstrated the lack of non-spectral effects from multi-elemental matrices and a good LOD, similar to or better than those in other spectrometric systems known for their high sensibility, such as (CV)HR-CS-GFAAS, CV-AFS and CV-ICP-MS. The precision (repeatability and reproducibility), accuracy and LOD complied with the requirements of EC Decisions and AOAC Guidelines relating to methods used for the control of contaminants. The results of the present study could be the starting point for the development of a standard laboratory method for users of such instrumentation. A validation study of the CVG-HR-CS-QTAAS method would be necessary using the HS60 flow-injection system for the CVG step.

Author contributions

Lucia Chirita: investigation, methodology, data curation, formal analysis, writing – original draft. Eniko Covaci: formal analysis, visualization, data curation, software. Michaela Ponta: validation, writing – original draft. Tiberiu Frentiu: conceptualization, supervision, funding acquisition, project administration, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

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