Validation of the eco-scale excellent green methods by UV-PVG-μCCP-OES by analyzing certified reference materials, comparison to traditional methods and related European legislation

(CO-UBB, P1-ICIA)

Two analytical eco-scale methods were validated for the determination of total Hg in food and water, and speciation as Hg^{2+} and CH_3Hg^+ in seafood respectively, using the UV-PVG-μCCP-OES system as TRL4. Certified reference materials (CRMs) were analyzed and the results were compared to those found by $SnCl_2$-CV-ICP-OES, $SnCl_2$-CV-AFS and TD-AAS, and the requirements of the European Commission in Decisions 2002/657/EC, 2007/333/EC and 2011/836/EC concerning the determination of methylmercury in seafood.

Validation of the eco-scale method for the determination of total Hg in food and water

The results obtained for total Hg in CRMs of fish tissue, food of animal and vegetal origin and water by UV-PVG-μCCP-OES and the comparison to traditional methods are presented in Tables 1 and 2.

The method was validated using the following certified materials: ERM-BB422 Fish Muscle, BCR-463 Tuna Fish, ERM-CE464 Tuna Fish, ERM-CA713 waste water, ERM-CA615 groundwater (Institute for Reference Materials and Measurements – IRMM Geel, Belgium), CS-M-3 Mushroom powder *Boletus Edulis*, CS-CR-2 Carrot Root Powder (Institute of Nuclear Chemistry and Technology, Warsaw, Poland), NIM-GBW-10018 Chicken (Institute of Geophysical and Geochemical Exploration, Langfand, China), TORT-2 Lobster Hepatopancreas, SRM 2976 Mussel Tissue (National Research Council of Canada – NRC Ottawa, Ontario, Canada) and IAEA-359 Cabbage (International Atomic Energy Agency Vienna, Austria). Amounts of 200 mg CRM were subjected to ultrasound assisted extraction in 10 ml de acid formic 98-100% on a water bath at 50 ^oC for 3 h according to the procedure provided in the reference Covaci et al., JAAS, 2018.

The results indicated good agreement between certified contents and those found bz the proposed method. For 95% confidence level mean recovery for total Hg in food and water samples without/with preconcentration was 101±7%/101±12%. The average recovery for 10-80 ng l^{-1} Hg spikes in water was 97±12%. The recovery in UV-PVG-μCCP-OES was similar to those obtained in $SnCl_2$-CV-μCCP-OES, $SnCl_2$-CV-ICP-OES and $SnCl_2$-CV-AFS, so that the proposed method could be considered a viable alternative to reference methods.

Table 1. Results for total Hg in certified reference materials of fish and water by UV–PVG-μCCP-OES without preconcentration using external calibration and Hg^{2+} working standards compared to other spectrometric methods (Covaci et al., JAAS, 2018)

CRM	Certified value±U (mg kg^{-1})	Method							
		UV-PVG-μCCP-OES		$SnCl_2$-CV-μCCP-OES		$SnCl_2$-CV-ICP-OES		$SnCl_2$-CV-AFS	
		Found value±CI[a] (mg kg^{-1})	Recovery± CI[a] (%)	Found value±CI[a] (mg kg^{-1})	Recovery± CI[a] (%)	Found value±CI[a] (mg kg^{-1})	Recovery± CI[a] (%)	Found value±CI[a] (mg kg^{-1})	Recovery± CI[a] (%)
Tort-2 Hepatopancreas	0.27±0.06	0.25±0.02	93±8	0.25±0.02	93±8	0.28±0.02	104±7	0.28±0.02	104±7
BCR-463 Tuna fish	2.85±0.16	2.84±0.18	100±6	2.86±0.25	101±9	3.11±0.25	109±9	3.09±0.25	108±9
ERM-CE464	5.24±0.10	5.25±0.30	100±6	5.00±0.30	95±6	5.10±0.20	97±4	5.31±0.28	101±5
ERM-BB422 Fish muscle	0.601±0.030	0.602±0.011	100±2	0.609±0.052	101±9	0.610±0.012	101±2	0.590±0.030	98±5
SRM 2976 Mussel Tissue	0.0610±0.0036	0.0627±0.0046	103±7	0.0620±0.0052	102±8	0.0580±0.0060	95±10	0.0638±0.0030	105±5
CS-M-3 mushroom powder *Boletus edulis*	2.849±0.104	2.871±0.290	101±10	2.782±0.082	98±3	2.880±0.062	101±2	2.799±0.084	98±3
ERM-CA713 Waste water[b]	1.84±0.11	1.84±0.18	100±10	1.86±0.12	101±7			1.88±0.12	102±7
Pooled recovery±CI[a]			101±7		100±7		100±7		102±6

Table 2. Results for total Hg in certified reference materials of food and water obtained by UV–PVG--μCCP-OES with preconcentration using external calibration and Hg^{2+} working standards (Covaci, et al., JAAS, 2018)

CRM	Certified value±U (μg kg^{-1})	Found value±CI[a] (μg kg^{-1})	Recovery±CI[a] (%)
CS-CR-2 Carrot Root Powder	4.3±0.6	4.3±0.3	100±7
IAEA-359 Cabbage	13±2	12±2	93±17
NIM-GBW-10018 Chicken	3.6±1.5	4.0±0.6	111±15
ERM-CA615[b]	0.037±0.004	0.037±0.002	100±6
Pooled recovery±CI[a]			101±12

[a] CI is the confidence interval for n=5 complete extraction/analysis sequences for each sample and 95% confidence level

[b] Concentration expressed in μg l^{-1}

Validation of the eco-scale method by UV-Vis-PVG-μCCP-OES for Hg speciation as Hg^{2+} and CH_3Hg^+ in seafood and comparison with TD-AAS

The characteristic of the method is the use of a single reagent, formic acid, both for the extraction of Hg species from the sample and as reaction medium for the photo-induced cold vapor generation. Under UV exposure in 0.6 mol l^{-1} HCOOH both Hg^{2+} and CH_3Hg^+ species are derivatized with same efficiency to cold vapor, while under Vis exposure only the Hg^{2+}species is converted to cold vapor. Concentration of CH_3Hg^+ is calculated by subtracting Hg^{2+} content from total. Working conditions are presented in Table 3 (Covaci et al., Microchem. J., 2018).

Table 3. Working conditions for Hg speciation by UV-VisCV-μCCP-OES eco-scale method (Covaci et al., Microchem. J., 2018)

	Total Hg	Hg^{2+}
Analytical system	UV-PVG-μCCP-OES	Vis-PVG-μCCP-OES
Derivatization reagent	0.6 mol l^{-1} HCOOH	0.6 mol l^{-1} HCOOH
UV Lamp	On	Off
Calibration range	10	1
Sample flow rate (ml min^{-1})	10	10
UV irradiation time of sample (s)	5	Signal does not vary significantly over time
Preconcentration of Hg vapor	-	From 25 ml sample
Plasma power (W)	15	15
Argon flow rate (ml min^{-1})	100	100
Observation height in plasma (mm)	1.6	1.6

The proposed provides limits of detection/determination of 9/36 μg kg^{-1} total Hg and 4.8/14.4 μg kg^{-1} Hg^{2+} species. The lower sensitivity to Hg^{2+} is compensated by the use of preconcentration of Hg vapor on a gold filament. The proposed method fulfils the demands of the European legislation for analytical methods used in food control to provide detection/quantification limit 10/5 times lower than the maximum admitted Hg concentration in fish (500 μg kg^{-1}).

The results obtained in the speciation analysis by UV–Vis-PVG-μCCP-OES and TD-AAS in certified reference materials are presented in Table 4. The speciation method provides good accuracy with recovery in the range of 99±6% total Hg, 99±9% Hg^{2+} and 97±10% CH_3Hg^+ consistent with provisions of European Commission. The proposed speciation method provides a greener profile in terms of sample preparation and derivatization as a single regent, formic acid, is used. Moreover, the method is easy to use involving a simple dilution to adjust the concentration of formic acid, necessitates a simple manifold and miniaturized instrumentation of low energy and gas consumption.

Table 4. Accuracy in Hg speciation by UV-Vis-PVG-μCCP-OES and TD-AAS (Covaci et al., Microchem. J., 2018)

CRM	Certified value±U[a] (mg kg^{-1})			Found value±CI[b] (mg kg^{-1})			Found value±CI[b] (mg kg^{-1})		
				UV-Vis-PVG-μCCP-OES			TD-AAS		
	Total Hg	Hg^{2+}	CH_3Hg^+	Total Hg	Hg^{2+}	CH_3Hg^+	Total Hg	Hg^{2+}	CH_3Hg^+
DOLT-4	2.58±0.22	1.25±0.16	1.33±0.12[c]	2.59±0.24	1.23±0.13	1.36±0.18[c]	2.37±0.29	1.03±0.13	1.34±0.14[c]
BCR-463	2.85±0.16	0.02±0.002	3.04±0.16[d]	2.84±0.18	0.02±0.002	3.00±0.33[d]	2.81±0.18	0.02±0.002	2.„97±0.26[d]
ERM-CE-464	5.24±0.10	0.12±0.004	5.50±0.17[d]	5.25±0.30	0.12±0.02	5.46±0.91[d]	5.11±0.32	0.08±0.01	5.36±0.33[d]
TORT-2	0.27±0.06	0.118±0.028	0.152±0.013[c]	0.25±0.02	0.117±0.011	0.133±0.016[c]	0.28±0.07	0.128±0.015	0.152±0.015[c]
SRM 2976	0.0610±0.0036	0.03291±0.00197	0.02809±0.00031'	0.0627±0.0046	0.03250±0.00280	0.03020±0.00330[c]	0.0630±0.0060	0.03302±0.00443	0.02998±0.00410[c]
Pooled recovery ±CI (%)				99±6	99±9	97±10	99±7	92±10	96±8

[a] U is expanded uncertainty for 95% confidence level (k=2)
[b] n = 5 complete extractions/analysis for each sample
[c] Expressed as Hg
[d] Expressed as CH_3Hg^+

Results:

- Validated methods for the determination of total Hg and speciation as Hg^{2+} and CH_3Hg^+
- 3 papers published in ISI journals (Covaci et. al., JAAS, 2018, Covaci et al., Microchem. J., 2018 and Senilă et al., Chem. Pap., 2018)
- 1 participation in international conference *(The 45nd International Conference of the Slovak Society of Chemical Engineering, Tatranske Matliare, May 2018)*
- 2 methods validated for the determination of total Hg and speciation as CH_3Hg^+ and Hg^{2+} respectively, in fish filet by UV-Vis-PVG-μCCP-OES