Determination of Ultra-Trace Mercury in Water Samples Based on Cold Vapor Atomic Fluorescence Spectrometry Using a Gold Trap

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This paper presents a method for mercury determination in water samples, at trace level using Cold Vapor Atomic Fluorescence Spectrometry (CVAFS). Mono, divalent mercury and organo-mercury compounds are converted to divalent mercury by oxidation with $KbrO_3$-KBr in hydrochloric acid, as a result of Br and BrCl reaction. Before the analyses, the excess of Br is removed by ascorbic acid. The divalent mercury is then reduced to the elemental form using 20 g/L tin chloride in acid medium, purged with argon in a hygroscopic membrane in order to retain humidity and then withhold on a gold trap. Gold trap is used to concentrate Hg vapor prior to detection. After heating the trap, mercury is detected using an ozone-free Hg lamp. The analytical performance parameters of the method have been investigated (detection limit, quatification limit, working range, precision, recovery). The method is suitable for determination of ultra-trace mercury content from drinking, surface and wastewater samples.

Keywords: mercury, surface water, waste water, drinking water, CVAFS, gold trap

Mercury is one of the most toxic elements for human health and ecosystem and, therefore, is one of the most studied environmental pollutant. Mercury has no beneficial biological function, and its presence in living organisms is associated with cancer, birth defects.

Organomercury compounds are more dangerous than inorganic Hg compounds due to higher capacity of being permeable to biomembrane. An organomercury species that is of special environmental importance is the monomethylmercury cation (H_3C-Hg). The pathway for human's exposure to this neurotoxin is dominated by consuming contaminated fish product, polluted drinking water. In the biogeochemical cycling of Hg in aquatic ecosystems, H_3C-Hg^+ is predominantly produced through the action of aquatic microorganisms (sulfate-reducing bacteria) on Hg^{2+} [1, 2].

Directive 2013/39 / EU [3] is a restrictive legislation and refers to the approval of the measures against pollution caused by chemical substances. European Directive lays down the environmental quality standards for surface water, provides for updating the list of priority substances. The regulated data give the maximum allowable concentrations of mercury, set up to avoid irreversible consequences of acute short-term exposure for an ecosystem.

The European Drinking Water Directive (98/83/EC), transposed in Romanian Legislation as Low 458(r1)/2002, imposes limit of Hg concentration in water intended for human consumption, which is 1 µg/l. For mineral water, Romanian Norm HG 1020/2005 imposes the same limit (1 µg/l). Since mercury concentrations in waters are expected to be very low, high sensitivity methods with low detection and quantification limits, high accuracy and precision are required. Different analytical techniques have been used for mercury determination at low concentrations including: cold vapor atomic absorption spectrometry, cold vapor atomic fluorescence spectrometry (CV-AFS), flow injection-inductively coupled plasma optical emission spectrometry and inductively coupled plasma mass spectrometry [4-8]. CV-AFS is used for the determination of mercury due to its high sensitivity and high selectivity. However, the cost of such instrumentation may still be prohibitive to many laboratories.

In the paper are presented the working conditions for determination of Hg from water samples using cold vapor atomic fluorescence spectrometry with two different enrichment methods using one, respectively two gold traps.

Experimental part

Apparatus

Mercury Analyzer Tedelyne type Cetac QuickTrace M-800 equipped with continuous flow vapour generator, gas liquid separator and a humidity removal system (dryer cartridge); ASX-520 CETAC Autosampler, ENC 500+ Enclosure; Milli Q water Purification System with Q-POD Element for trace element analysis.

Quality control and assurance

The purity of all reagents used is critical when determining low levels of hydride elements. Only ultra-pure analytical grade quality reagents were used: Certified Reference Material solution for calibration (Standard TraceCERT for ICP, 1000 mg/L Hg, Fluka, Lot BCBM 1790V); Tin (II) chloride ehydrate (max 0.000001% Hg); Potassium bromide purris, Sigma-Aldrich; Potassium bromate purris, Sigma-Aldrich; L-ascorbic acid purris, Sigma-Aldrich; ultra-pure nitric acid (69% Fluka quality), ultra-pure hydrochloric acid (30% Fluka quality) argon 99.996% purity, ultra-pure water was used for all aqueous solutions and cleaning procedures. The quality control of the data was carried out according to Certified Reference Material CertiPur, 1000 mg/L Hg, Lot HC 42627826, produced by Merck.

Sample collection

Mercury vapour can diffuse through various plastics, for this reason glass or special plastics tubing like FEP (fluorinated ethylene-propylene) should be used. Tap,

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surface and waste waters were collected in borosilicate glasses. All materials used for sampling activities were previously washed overnight with a 10% HNO_3 solution and rinsed with ultrapure water. Samples were preserved with potassium bromide/bromated and hydrochloric acid and analyzed after reception in the lab. If preserved samples must to be stored, the analysis need to be performed within seven days after collection.

Sample digestion and standard calibration preparation

Sample preparation is a key step for the accurate determination of mercury in different water samples. For total mercury determination, sample digestion prior to analysis is usually neded for the decomposition of organic matrix.

Mono, divalent mercury and organo-mercury compounds are converted to divalent mercury by oxidation with $KbrO_3$-KBr. Prior the analysis, Br excess is eliminated with L-ascorbic acid. The Hg^{2+} is reduced to the elemental form using tin chloride, mercury vapor being carried by a carrier gas (argon), whide water vapors are retained on a Nafion Dryer Cratridge. Atomic mercury vapors is captured on one or two gold traps for enrichment and released in a thermal process. Using a photomultiplier detector and an ozone-free Hg lamp, the content of mercury is quantified.

Calibration standards were daily prepared, the concentrations were situated in the range 10 ng/L to 100 ng/L (10; 30; 50; 70; 100 ng/L). For preservation it was used reagents blank, prepared with 150 mL ultrapure nitric acid 120g/kg and 20 mL $KbrO_3$-KBr mixture, (0.0333 mol/L $KbrO_3$; 0.2 mol/L KBr; mixing report =1:1(v/v)). For each set of standards was prepared a blank sample using the same procedure and control standard samples in order to verify the linearity of the calibration curve. For the reaction were used 20 g/L tin chloride solution and 100 g/L L-ascorbic acid. The reagents were added to standards and to all the analyzed samples using an ASX-520 CETAC Autosampler according to the recommendations of SR EN ISO 17852:2008 standard [9].

Validation of the method

In terms of maximum permissible limits of mercury content in water samples, limits imposed by the laws, the analytical methods applied must comply from the point of view of performance characteristics. Table 1 presents the maximum values of LOD, LOQ, precision, accuracy and measurement uncertainty, values evaluated at the maximum imposed limit by each law.

In order to evaluate the performance of the method (linearity of the calibration curve, detection limit LOD, quantitation limit LOQ, repeatability, intermediate precision, recovery, accuracy) validation experiments were performed [10 - 12].

To establish the performance parameters of the methods (linearity, working range, detection limit, quantification limit, repeatability, and recovery), different tests were performed (table 2).

The Cetac QuickTrace M-8000 model allows Hg determination using three different methods: without enrichment and with enrichment on one or two gold traps. Figure 1 shows the optimized parameters set for Hg determinination with a gold trap enrichment, respectively with two-gold traps (fig. 2). According to QuickTrace M-8000 Mercury Analyzer Operator's Manual, the application range for method without enrichment is 1 to 50 μg/L, to high for the criteria listed in table 1.

In figure 3 is presented the peak shape for one-gold trap method at the highest value of the calibration curve range.

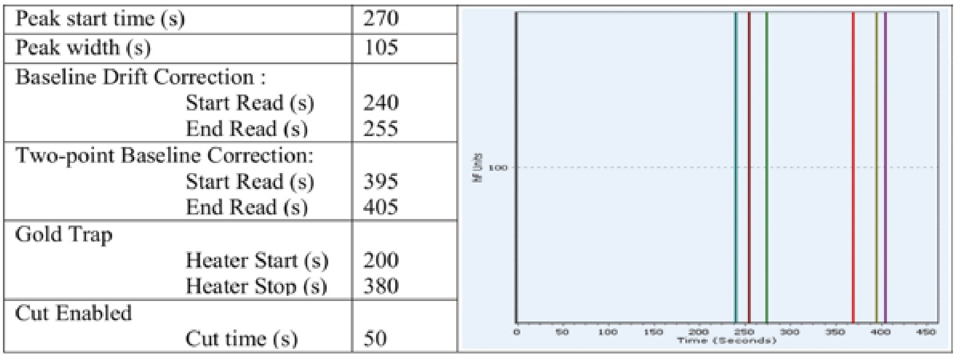
Results and discussions

Parameter, ng/L	Criteria according to **Directive 2013/39 / EU**	Criteria according to **Low 458(r1)/2002**	Criteria according to **HG 1020/2005**
MAV	50	1000	1000
LOD	≤ 5	<100	<100
LOQ	≤ 15	-	-
Precision	≤ 5	<100	<100
Accuracy	≤ 5	<100	<100
Uncertainty	≤ 25	-	-

Table 1
MINIMUM IMPOSED PERFORMANCE CHARACTERISTICS OF THE APPLIED METHOD

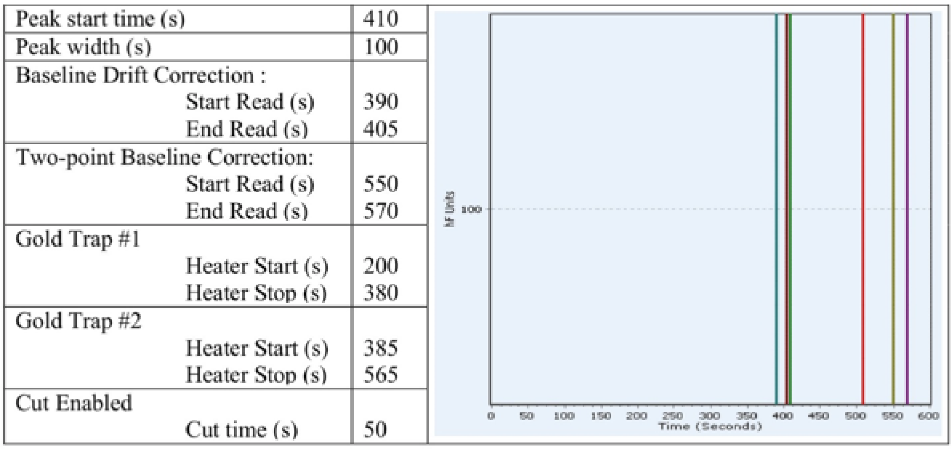
Parameters	**Experiments**
Linearity test for homogeneity of the variance, working range	- Standard solutions 10 ng/L, 30 ng/L, 50 ng/L, 70 ng/L, 100 ng/L Hg; - 10 independent standard solution of 10 ng/L, respectively 100 ng/L
LOD and LOQ	- 5 independent fortified blank solutions (2 ng/L Hg)
Repeatability (precision tests) on standard solutions	- 10 independent standard solution of 10 ng/L concentration, respectively100 ng/L, performed by one chemist in the same day
Repeatability (precision tests) on real samples	- 10 samples of surface water fortified with 50 ng/L Hg, performed by one chemist in the same day; - 10 samples of wastewater fortified with 100 ng/L Hg, performed by one chemist in the same day.
Intermediate precision	- 4 experiments (3 samples per experiment analyzed by two chemist in four different days), 12 independent samples of surface water fortified with 50 ng/L Hg, respectively wastewater fortified with 100 ng/L Hg
Recovery	- 5 independent drinking water (dw) samples fortified with 10 ng/L, 50 ng/L, respectively 100 ng/L Hg.

Table 2
IN HOUSE VALIDATION EXPERIMENTS, TESTS USED FOR DETERMINATION OF PERFORMANCE PARAMETERS OF THE METHOD



Peak start time (s)	270
Peak width (s)	105
Baseline Drift Correction : Start Read (s) End Read (s)	 240 255
Two-point Baseline Correction: Start Read (s) End Read (s)	 395 405
Gold Trap Heater Start (s) Heater Stop (s)	 200 380
Cut Enabled Cut time (s)	 50

Fig. 1. Method Editor Conditions Settings for One-Gold Trap QuickTrace M-8000



Peak start time (s)	410
Peak width (s)	100
Baseline Drift Correction : Start Read (s) End Read (s)	 390 405
Two-point Baseline Correction: Start Read (s) End Read (s)	 550 570
Gold Trap #1 Heater Start (s) Heater Stop (s)	 200 380
Gold Trap #2 Heater Start (s) Heater Stop (s)	 385 565
Cut Enabled Cut time (s)	 50

Fig. 2. Method Editor Conditions Settings for Two-Gold Traps QuickTrace M-8000

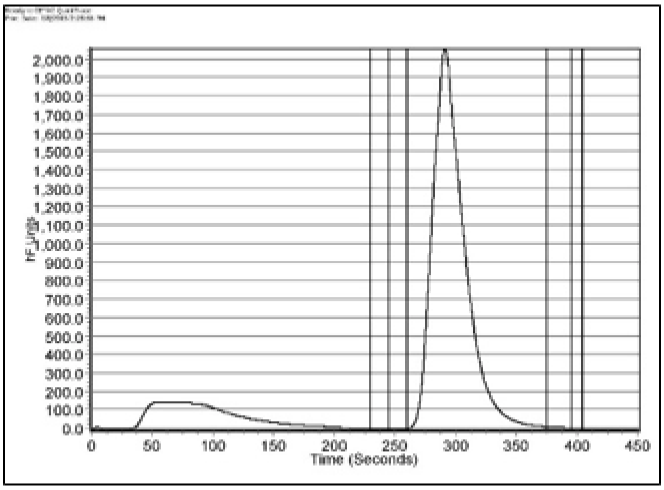


Fig. 3. Typical Peak from One Gold Trap Settings (100 ng/L)

In order to evaluate the performance of both methods (one, respectively two-gold trap enrichment), LOD, LOQ, linearity, precision and accuracy were perfomed. The obtained results indicated that one-gold trap enrichment method allows Hg determination at the lowest level imposed by the legislation.

In table 3 are listed the values for lineary tests and calibration curve obtained with One-Gold Trap Method (A) and Two-Gold Traps Method (B).

The working range is linear between 10 and 100 ng/L Hg for both applied methods. In the tests for the homogeneity of the variance, PG values are lower than the Fischer-Snedecor F value. The values of coefficient of variation (0.93% method A and 1.04% method B), less than 2% according to the Horwitz function [10], indicate that both methods are as good as spectrometric method. In figure 4 is inserted a sequence from The QuickTrace™ Mercury Software (calibration curve, peak, results) for Two-Gold Traps Method.

In terms of LOD and LOQ, the results indicated that both methods are suitable for the purpose (table4).

The precision experiments performed on mercury standard solution at two different concentrations (10ng/L, respectively 100 ng/L) presented in table 5, demonstrate that precision is lower than 5 ng/L.

In the study have been performed intermediate precision experiments on surface water samples using both methods, the results being comparable (table 6).

Considering the fact that the gold trap is an expensive consumable (life time around 2-3 months of weekly use), the following experimental data were focused on one-gold trap method (A). Also, the analysis time per sample is lower, approximately 7 min, while for two-gold trap method (B) is 10 min.

Experimental data regarding intermediate precision and repeatability obtained with

Method A on real samples (surface water, wastewater) are presented in tables 7 and 8.

The accepted values of relative standard deviation (RSD) depend on the analyte concentrations. For 1 μg/L (the lowest level), acceptable RSD value according to Horwitz is less than 45% and according to AOAC Peer Verified Methods is less than 30% [10]. As shown in tables 11 and 12, the recorded RSD values are below 4%.

Recovery tests were carried out on all type of matrices (sw, ww, drinking water – dw) and have led to the conclusion that method A is fitt for the purpose. Recovery percentages were situated in the range 104-113% for all types of matrices 4. The accepted range for 1 μg/L (the lowest level) is 40-120% according to Taverniers and colaborators [10]. Accuracy values ranged below 5 ng/L (as specified in table 9).

Following the validation studies it resulted that the performance parameters obtained by both methods are situated below the minimum required limits, imposed by the most restrictive law for the Hg determination from water samples (table 10).

Method A *Calibration Curve Parameters*	x_i (ng/L)	10	30	50	70	100
	y_i (peak area)	68688	174194	291216	396228	553485
	y = 15448 + 5E+06 x					
	a = 15448	b = 5409838 $ng^{-1}L$	R = 0,9998			
	S_{Y1} = 2605 ng/L	S_{x01} = .,0005 ng/L	V_{x01} = 0.93 %			
Test for the homogeneity of the variance for linear calibration curve						
y 1i	y 10i					
64239	676063	(Variance y1)^2 = 33184423 (Variance y10)^2 = 176644564 PG 10/1 = 5.323 PG 1/10 = 0.188 $F_{9,9;0,99}$ = 5.35 PG < $F_{9,9;0,99}$ 5.323 < 5.35				
66704	697971					
68832	694063					
68413	655884					
75651	692996					
83836	681027					
75276	696596					
74129	698060					
75740	693235					
75400	690256					
Method B *Calibration Curve Parameters*	x_i (ng/L)	10	30	50	70	100
	y_i (peak area)	82168	212821	267996	513083	734454
	y = 2545+ 7E+06 x					
	a = 2545	b = 7299216 $ng^{-1}L$	R = 0,9998			
	S_{Y1} = 3937 ng/L	S_{x01} = 0,0005 ng/L	V_{x01} = 1,04 %			
Test for the homogeneity of the variance for linear calibration curve						
y 1i	y 10i					
77408	728570	(Variance y1)^2 = 5516039 (Variance y10)^2 = 21730890 PG 10/1 = 3.94 PG 1/10 = 0.254 $F_{9,9;0,99}$ = 5.35 PG < $F_{9,9;0,99}$ 3.94 < 5.35				
75227	733139					
76421	744206					
82206	736602					
80784	728977					
81627	734852					
79240	729648					
78925	735248					
80711	736912					
81039	734208					

Table 3
LINEARITY RESULTS FOR BOTH METHODS

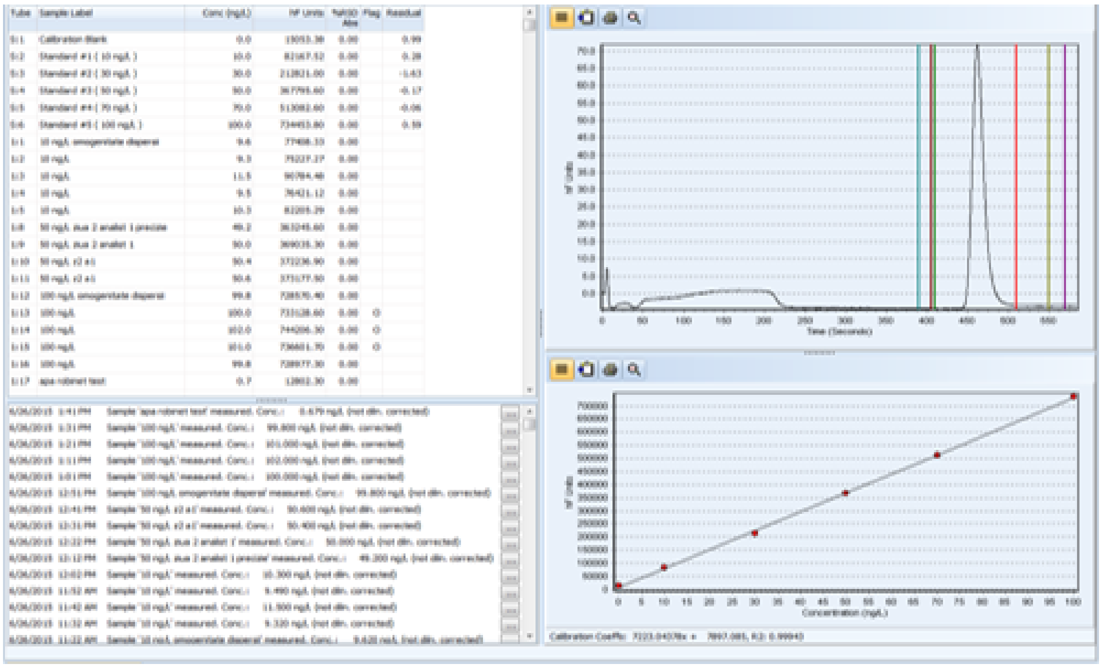


Fig. 4. The calibration curve and the peak shape for two-gold trap method (B)

Method A						
$x_{measured}$	ng/L	1.8	0.5	0.9	1.0	1.7
LOD	ng/L	**2.0**	*LOQ*		ng/L	**6.0**
Method B						
$x_{measured}$	ng/L	1.7	1.3	1.9	1.8	1.4
LOD	ng/L	**1.0**	*LOQ*		ng/L	**3.0**

Table 4
LOD AND LOQ VALUES FOR BOTH METHODS

Method A						
$x_{measured}$	ng/L	9.5	10.7	12.1	13.1	10.7
$x_{measured}$	ng/L	10.5	10.8	11.0	10.7	9.2
$x_{mean\ value}$	ng/L	**10.83**	s		ng/L	**1.21**
$x_{measured}$	ng/L	105	101	100	94,7	100
$x_{measured}$	ng/L	98,4	105	107	103	101
$x_{mean\ value}$	ng/L	**101.5**	s		ng/L	**3.78**
Method B						
$x_{measured}$	ng/L	10.1	10.5	9.6	9.8	10.3
$x_{measured}$	ng/L	10.3	10.7	9.9	10.2	10.1
$x_{mean\ value}$	ng/L	**10.15**	s		ng/L	**0.306**
$x_{measured}$	ng/L	101	103	98.5	102.6	100
$x_{measured}$	ng/L	102	101.7	99.3	103.5	105
$x_{mean\ value}$	ng/L	**101.7**	s		ng/L	**1.96**

Table 5
PRECISION TESTS PERFORMED ON MERCURY STANDARD SOLUTION

Method A							
$x_{measured}$ SW	ng/L	49.9	49.9	52.1	49.4	52.3	51.1
$x_{measured}$ SW	ng/L	51.6	51.8	54.6	49.2	52.8	51.9
$x_{mean\ value}$ SW	ng/L	**51.4**	*Intermediate precision*		ng/L	**4.42**	
s	ng/L	**1.58**	*RSDr*		%	**3.07**	
Method B							
$x_{measured}$ SW	ng/L	48.2	50.6	50.4	50.8	50.1	50.2
$x_{measured}$ SW	ng/L	49.2	50.6	50.7	50.4	49.2	53.0
$x_{mean\ value}$ SW	ng/L	**50.3**	*Intermediate precision*		ng/L	**3.25**	
s	ng/L	**1.16**	*RSDr*		%	**2.30**	

Table 6
INTERMEDIATE PRECISION PREFORMED ON SURFACE WATER SAMPLES (SW)

$x_{measured}$ WW	ng/L	104	105	102	111	99	112
$x_{measured}$ WW	ng/L	101	103	106	108	104	107
$x_{mean\ value}$ WW	ng/L	**105.2**	*Intermediate precision*		ng/L	**10.9**	
s	ng/L	**3.88**	*RSDr*		%	**3.69**	

Table 7
INTERMEDIATE PRECISION TESTS PREFORMED ON SWASTEWATER SAMPLES (WW)

$x_{measured}$ SW	ng/L	52.5	52.3	51.4	52.2	52.7
$x_{measured}$ SW	ng/L	52.4	52.0	52.9	53.3	52.1
$x_{mean\ value}$ SW	ng/L	**52.4**	*Repeatability*		ng/L	**1.47**
s	ng/L	**1.21**	*RSDr*		%	**1.00**
$x_{measured}$ WW	ng/L	106	106	103	102	101
$x_{measured}$ WW	ng/L	100	97.7	98.2	95.2	98.6
$x_{mean\ value}$ WW	ng/L	**100.8**	*Repeatability*		ng/L	**9.95**
s	ng/L	**3.55**	*RSDr*		%	**3.53**

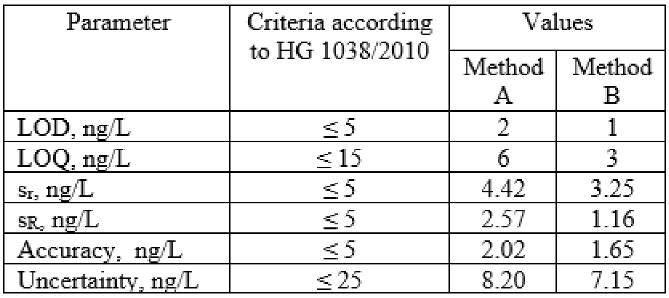
Table 8
REPEATABILITY TESTS PERFORMED ON REAL SAMPLES

$x_{measured}$ dw	ng/L	9.5	10.7	12.1	13.1	11.0
Recovery	%	**112.8**	**Accuracy**		ng/L	**1.28**
$x_{measured}$ SW	ng/L	49.9	53.6	51.1	49.2	56.3
Recovery	%	**104.04**	**Accuracy**		ng/L	**2.02**
$x_{measured}$ WW	ng/L	104	111	99	101	107
Recovery	%	**104.04**	**Accuracy**		ng/L	**4.40**

Table 9
RECOVERY AND ACCURACY TESTS PERFORMED ON REAL SAMPLES

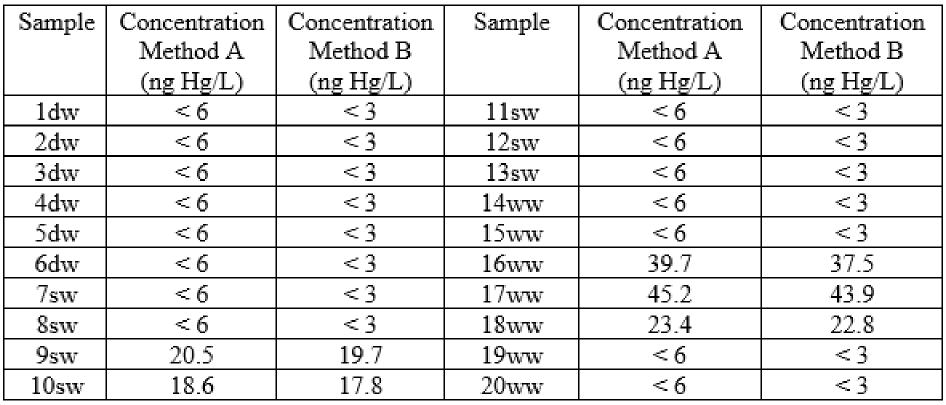
Analysis of Hg in water samples

Using the developed analytical methods was performed a comparative study concerning the determination of mercury from different types of water. In the study were included six drinking water samples collected from the tap (Bucharest, one from each district), seven surface water sampled from Danube River (Giurgiu, Oltenita, Calarasi, Tulcea, Murighiol, Uzlina, Sfantu Gheorghe), seven samples of wastewater discharged into Bucharest sewerage municipal system. All the results of drinking water were situated under the quantification limits of the methods. The surface water samples collected from Danube River (Calarasi - 9sw; Tulcea - 10sw) contain around 20 ng/L Hg, concentration detected with both methods. Also, in three wastewater samples discharged into Bucharest sewerage municipal system were detected mercury in the range 23 to 45 ng/L. The obtained results are presented in table 11.



Parameter	Criteria according to HG 1038/2010	Values	
		Method A	Method B
LOD, ng/L	≤ 5	2	1
LOQ, ng/L	≤ 15	6	3
s_r, ng/L	≤ 5	4.42	3.25
s_R, ng/L	≤ 5	2.57	1.16
Accuracy, ng/L	≤ 5	2.02	1.65
Uncertainty, ng/L	≤ 25	8.20	7.15

Table 10
RESULTS OF PERFORMANCE PARAMETERS COMPARED WITH ADMITTED VALUES



Sample	Concentration Method A (ng Hg/L)	Concentration Method B (ng Hg/L)	Sample	Concentration Method A (ng Hg/L)	Concentration Method B (ng Hg/L)
1dw	< 6	< 3	11sw	< 6	< 3
2dw	< 6	< 3	12sw	< 6	< 3
3dw	< 6	< 3	13sw	< 6	< 3
4dw	< 6	< 3	14ww	< 6	< 3
5dw	< 6	< 3	15ww	< 6	< 3
6dw	< 6	< 3	16ww	39.7	37.5
7sw	< 6	< 3	17ww	45.2	43.9
8sw	< 6	< 3	18ww	23.4	22.8
9sw	20.5	19.7	19ww	< 6	< 3
10sw	18.6	17.8	20ww	< 6	< 3

Table 11
CONCENTRATION OF MERCURY IN WATER SAMPLES
(dw -drinking water; sw - surface water; ww -wastewater)

Conclusions

The statistical interpretation of the experimental results proved that the proposed methods can be successfully used for the analytical control of mercury in slightly contaminated water samples, either are drinking water, surface water or wastewater.

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