Sequential Determination of Cd, Cu, Pb, Co and Ni in Marine Invertebrates by Zeeman Graphite Furnace Atomic Absorption Spectroscopy

Application Note

Atomic Absorption

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Introduction

In marine biomonitoring, metal concentrations in different species of aquatic invertebrates are used to assess their role in the biogeochemical cycle within aquatic environments, to evaluate their suitability as monitors of the bio-available environmental supply or to analyze the internal exposure to potentially toxic substances as a basis for an effect monitoring. Many environmental monitoring programmes record results of past events without understanding the underlying physiological and ecological processes. There is, however, an increasing demand for prospective approaches to detect potential human impact on ecosystems, based on a sound understanding of these processes [1,2,3]. Natural and anthropogenic metal inputs influence the bio-available metal supply, which cannot be detected directly by routine analytical procedures for example, by measuring metal concentrations in the soluble phase. The bio-available fraction can only be assessed by determining incorporated metal levels in organisms, which is the main goal in biomonitoring [4,5]. This involves field investigations as well as bioaccumulation experiments. In recent studies, these aspects have been evaluated in detail by investigating various aquatic invertebrates and habitats such as marine zooplankton from the Arctic [6,7,8,9] freshwater zooplankton and benthos from lakes [10], benthic invertebrates from German coastal waters and estuaries [11,12,13,14] and crustaceans from the Antarctic marine environment [15]. Zooplankton organisms may contribute to the transfer of metals to higher trophic levels and have been chosen, amongst others, as recommended organisms in baseline studies for the marine environment for example, within the scope of the Arctic Monitoring and Assessment Programme [16].

When determining zooplankton samples from the field or from bioaccumulation experiments, there is normally only a limited amount of biomass available. This typically requires the use of micro digestion procedures in combination with multi-element determinations. In the past we employed such procedures, involving sequential multi-element graphite furnace atomic absorption spectroscopy with deuterium background correction, first using ammonium dihydrogen phosphate as matrix modifier for Cd and Pb [17] and since some years a palladium nitrate – magnesium nitrate modifier [18,19].

Agilent Technologies

This paper outlines the corresponding optimization of a graphite furnace (GFAAS) methodology for the Agilent SpectrAA-880 Zeeman atomic absorption spectrometer, suitable for the determination of the trace metals Cd, Co, Cu, Ni and Pb in various aquatic invertebrates including zooplankton and small zoobenthos. The certified reference materials Tort 2, Lobster hepatopancreas (National Research Council Canada) and CRM No 278R, Mussel Tissue: Mytilus edulis (Community Bureau of Reference) were used to optimize the methods and assess the precision and validity of the methods. These certified reference materials were selected as these samples are similar in matrix to the various aquatic invertebrates being determined.

Experimental

Sample Preparation

The samples of marine organisms are normally freeze-dried and then homogenized using a small boron carbide mortar and pestle or a ball mill made of agate. The certified reference materials used in this study did not require this treatment since they have already undergone these procedures.

Aliquots of about 10 mg dried material were digested for 3 hours at 80 °C with 100 µL HNO_3 (65%, suprapure Merck) in tightly closed 2 mL "safe lock" Eppendorf reaction tubes. The digests were allowed to cool down slowly and were subsequently made up to a volume of 2 mL using bidistilled water. After appropriate dilution, final sample and standard solutions were adjusted to concentrations of 3.25% HNO_3.

Quality Assurance

Quality assurance was performed in line with German GLP regulations [20] using the following documented criteria: stability of instrumental recalibration, precision of parallel injections (normally showing a coefficient of variation of 1–5%) and analytical blanks (also reflecting the digestion procedure). Furthermore, precision and validity was evaluated using certified reference materials randomly allocated within routine determinations. Limits of detection were calculated as mean blank (eventually set to zero) plus 2.6 times the standard deviation of a "low value" [21].

Instrument Parameters

Metal determinations were performed using an Agilent SpectrAA-880 Zeeman atomic absorption spectrometer equipped with the GTA-110 Zeeman graphite tube atomizer. All elements were measured in the absorbance and concentration calibration mode using wall atomization with Zeeman background correction. Calibration graphs were obtained using the New Rational calibration algorithm with 4–5 standards (depending on linearity) prepared from a single bulk standard using the auto-mix capabilities of the PSD (Programmable Sample Dispenser). Every ten samples a Reslope measurement was run automatically. The Reslope standard used was either the second or third standard from the calibration. The elements were determined in the order of increasing ashing and atomization temperatures. After completion of one element, a tube clean was applied to ensure an appropriate Cal Zero value. Under normal conditions, 300–600 firings could be completed before the tube had to be changed. Nitrogen gas of grade 5.0 was used.

The Rinse solution contained 0.002% Triton X -100 and 0.065% HNO_3 (suprapure Merck). The Rinse solution was prepared from 2–3 days old bidistilled water and the rinse vessel was kept at least half full. The sample dispensing system was bled before each automatic run to remove any gas bubbles in the syringe and capillary. The outside surfaces of the dispensing capillary were frequently wiped with a soft tissue soaked with isopropyl alcohol to prevent adhesive effects due to the matrix modifier used. Sample cups and the Reslope container were sealed with slitted lids to minimize evaporation and to prevent the formation of drops on the capillary during the determinations. With those lids on the sample cups, careful alignment of the dispensing capillary to the centre of the sample cups was necessary.

For the determinations of Cd, Pb, Ni and Cr, palladium and magnesium nitrate modifiers were applied [22,23,24]. Both modifiers must be kept in separate vessels on the PSD to prevent any chemical effects which may disturb the injection of samples in the graphite tube. Detailed information regarding the methods employed for the various elements are listed in Appendices 1–5 as original printouts from the automatic runs, including signal and calibration graphs. General instrument parameters are listed in Table 1. They were optimized based on the recommended or default instrument parameters provided in the software (Version 3.0) for the Agilent SpectrAA 880 Zeeman AAS.

Table 1. General Instrument Parameters and Matrix Modifiers Used

Measurement mode	Cd PROMT height	Cu PROMT area	Pb PROMT height	Co PROMT area	Ni PROMT area	Cr PROMT height
Wavelength (nm)	228.8	327.4	283.3	242.5	232.0	357.9
Lamp current (mA)	4.0	4.0	10.0	7.0	4.0	7.0
Slit width (mm)	0.5	0.5	0.5	0.2	0.2	0.2
No. of calibration standards	5	5	5	5	4	4
Sample volume (µL)	5	10	20	40	20	10
Modifier 1* (µL)	5	–	5	–	8	5
Modifier 2** (µL)	5	–	5	–	4	5
Calibration range (µg/L)	1–5	5–25	20–150	3–12	2–10	6–25

* Modifier 1: 0.4 mg/mL $Pd(NO_3)_2$

** Modifier 2: 2 g/L $MgNO_3$

Statistical Analysis

To assess the accuracy and the reproducibility of the methods, quality control charts were produced, taking into account daily measures of independent standard reference samples (6–7 replicates per day). Furthermore, it was tested whether the means between days differ significantly. First, the hypothesis of normal distribution was tested using the Lilliefors test provided in SYSTAT 8.0 for Windows [25]. Since this hypothesis had not to be rejected in most cases ($\alpha = 0.01$) further statistical evaluation was performed using BMDP Dynamic program 7d [26]. First, global null hypotheses (equality of means) were tested either by classical ANOVA (assuming equality of variances) or by non-classical Welch Test (not assuming equality of variances). The adequate procedure was selected after testing equality of variances by Levene Test. Null hypotheses were rejected at 95% significance level ($P < 0.05$). Second, heterogeneity was analysed in more detail using the Student-Newman-Keuls Multiple Range Test ($\alpha = 0.05$). The robust NK procedure involves an adjusted significance level for each group of ordered means.

Results

The measured results for the certified reference materials are listed in Table 2. It is noted there is good agreement between the measured and certified values. There were no certified values listed for Co and Ni in the CRM No 278R, Mussel Tissue: Mytilus edulis (Community Bureau of Reference). Also included in the Table are the measured limits of detection [21].

The long term stability of the analytical methodology is demonstrated in the quality control charts (Figures 1–2), which display the daily measured results for both certified reference materials. The statistical evaluation of the QA procedure is summarized in Tables 3–5.

Table 2. Measured Results for the Certified Reference Materials

Element	Measured limits of detection (mg/kg)	Tort-2; lobster hepatopancreas National Research Council Canada				CRM No 278R Mussel tissue: Mytilus edulis; Community Bureau of Reference			
	(dry weight)	Measured	n	Certified value	% Recovery	Measured	n	Certified value	% Recovery
Cd	0.10	25.7 ± 0.92	45	26.7 ± 0.6	96	0.31 ± 0.01	54	0.348 ± 0.007	90
Cu	3.5	109 ± 4	50	106 ± 10	103	9.1 ± 0.4	53	9.45 ± 0.13	96
Pb	0.32	0.36 ± 0.04	47	0.35 ± 0.13	103	1.8 ± 0.1	51	2.00 ± 0.04	91
Co	0.13	0.55 ± 0.02	49	0.51 ± 0.09	107	0.34 ± 0.01	56	n/a	n/a
Ni	0.39	2.30 ± 0.05	49	2.5 ± 0.19	92	0.94 ± 0.04	52	n/a	n/a

Values listed are the mean value ± 95% confidence intervals (mg/kg d.w.) regarding the completed data set.

n: number of independent determinations.

Table 3. Statistical Analysis of Daily Measurements of Reference Materials

Tort-2					CRM No 278 R				
Measurement	Mean ± 95%CI	N	LIP	Grp	Measurement	Mean ± 95%CI	N	LIP	Grp
Cd 1	22.5 ± 4.1	6	0.182	|	Cd 1	0.30 ± 0.03	7	0.249	|
Cd 2	27.2 ± 2.4	6	0.966	|	Cd 2	0.30 ± 0.03	7	0.937	|
Cd 3	27.1 ± 2.1	6	1.000	|	Cd 3	0.34 ± 0.05	7	0.816	|
Cd 4	25.5 ± 1.4	6	0.632	|	Cd 4	0.32 ± 0.02	7	0.068	|
Cd 5	25.2 ± 1.1	6	0.420	|	Cd 5	0.34 ± 0.04	7	0.018	|
Cd 6	25.4 ± 1.9	6	0.569	|	Cd 6	0.32 ± 0.03	7	0.759	|
Cd 7	26.7 ± 4.3	7	0.801	|	Cd 7	0.28 ± 0.04	8	0.378	|
Cu 1	98.0 ± 10.3	5	0.626	|	Cu 1	8.7 ± 1.4	6	0.051	|
Cu 2	113 ± 12	5	0.948	|	Cu 2	9.3 ± 0.3	6	1.000	|
Cu 3	112 ± 18	5	0.802	|	Cu 3	9.1 ± 0.8	6	0.164	|
Cu 4	116 ± 8	6	1.000	|	Cu 4	8.7 ± 0.9	6	0.553	|
Cu 5	109 ± 8	6	0.144	|	Cu 5	8.9 ± 0.6	6	0.996	|
Cu 6	103 ± 14	6	1.000	|	Cu 6	9.3 ± 2.3	6	0.002	|
Cu 7	111 ± 13	5	1.000	|	Cu 7	9.6 ± 2.0	5	0.376	|
Pb 1	0.39 ± 0.21	6	0.088	|	Pb 1	1.9 ± 0.4	7	0.009	|
Pb 2	0.27 ± 0.03	6	0.096	|	Pb 2	2.0 ± 0.3	7	1.000	|
Pb 3	0.40 ± 0.13	6	0.659	|	Pb 3	1.9 ± 0.1	7	0.915	|
Pb 4	0.37 ± 0.06	6	0.851	|	Pb 4	1.8 ± 0.2	7	0.521	|
Pb 5	0.31 ± 0.11	6	0.282	|	Pb 5	1.8 ± 0.1	7	0.494	|
Pb 6	0.33 ± 0.11	7	0.148	|	Pb 6	1.5 ± 0.1	6	0.028	|
Pb 7	0.43 ± 0.10	7	0.426	|	Pb 7	1.7 ± 0.2	6	1.000	|
Co 1	0.53 ± 0.08	6	1.000	|	Co 1	0.36 ± 0.07	7	0.211	|
Co 2	0.56 ± 0.09	6	0.048	|	Co 2	0.32 ± 0.04	7	0.827	|
Co 3	0.50 ± 0.02	6	0.801	|	Co 3	0.34 ± 0.03	7	1.000	|
Co 4	0.54 ± 0.03	7	0.365	|	Co 4	0.33 ± 0.02	7	1.000	|
Co 5	0.52 ± 0.06	7	0.135	|	Co 5	0.32 ± 0.05	8	0.876	|
Co 6	0.57 ± 0.08	7	0.102	|	Co 6	0.37 ± 0.03	8	1.000	|
Co 7	0.61 ± 0.08	7	0.690	|	Co 7	0.35 ± 0.02	8	0.321	|
Ni 1	2.3 ± 0.3	6	0.948	|	Ni 1	0.9 ± 0.1	7	0.720	|
Ni 2	2.3 ± 0.1	6	1.000	|	Ni 2	1.0 ± 0.1	7	1.000	|
Ni 3	2.3 ± 0.1	6	1.000	|	Ni 3	0.9 ± 0.1	7	0.515	|
Ni 4	2.3 ± 0.2	6	0.067	|	Ni 4	0.9 ± 0.1	7	1.000	|
Ni 5	2.2 ± 0.1	6	1.000	|	Ni 5	1.0 ± 0.2	6	0.021	|
Ni 6	2.4 ± 0.2	7	0.277	|	Ni 6	1.0 ± 0.2	7	0.777	|
Ni 7	2.3 ± 0.2	7	0.637	|	Ni 7	0.9 ± 0.1	6	0.065	|

Values listed are all shown as mg/kg dry weight.

Notes: 95% CI: 95% confidence intervals; N: number of independent samples; LIP: Lilliefors probabilities (2-tailed), test of normality ($\alpha = 0.01$); Grp: homogeneous groups according to the Student-Newman-Keuls multiple range test, means which do not differ significantly ($\alpha = 0.05$) are marked by vertical bars.

Table 4. Test of Global Null Hypothesis for Daily Measurements of the Reference Material Tort–2 (Lobster Hepatopancreas)

Element	LS	P	Global null hypothesis		P	df
Cd	3.83	0.005	WS	1.63	0.204	6; 16
Cu	1.18	0.343	F	1.99	0.098	6; 31
Pb	1.84	0.119	F	1.45	0.221	6; 37
Co	1.33	0.269	F	1.70	0.147	6; 39
Ni	1.62	0.170	F	0.89	0.511	6; 37

Notes: LS: Levene statistic; WS: Welch statistic; F: classical ANOVA; P: tail probability (corresponding null hypotheses are rejected when $P < 0.05$); df: degrees of freedom (for LS = strata-1; total of determinations-strata).

Table 5. Test of Global Null Hypothesis for Daily Measurements of Reference Material CRM No 278 R (Mussel Tissue)

Element	LS	P	Global null hypothesis		P	df
Cd	1.08	0.388	F	2.11	0.072	6; 43
Cu	2.32	0.055	F	0.40	0.873	6; 34
Pb	1.89	0.107	F	2.24	0.059	6; 40
Co	4.51	0.001	WS	1.55	0.212	6; 20
Ni	2.66	0.029	WS	2.25	0.088	6; 17

Notes: LS: Levene statistic; WS: Welch statistic; F: classical ANOVA; P: tail probability (corresponding null hypotheses are rejected when $P < 0.05$); df: degrees of freedom (for LS = strata-1; total of determinations-strata).

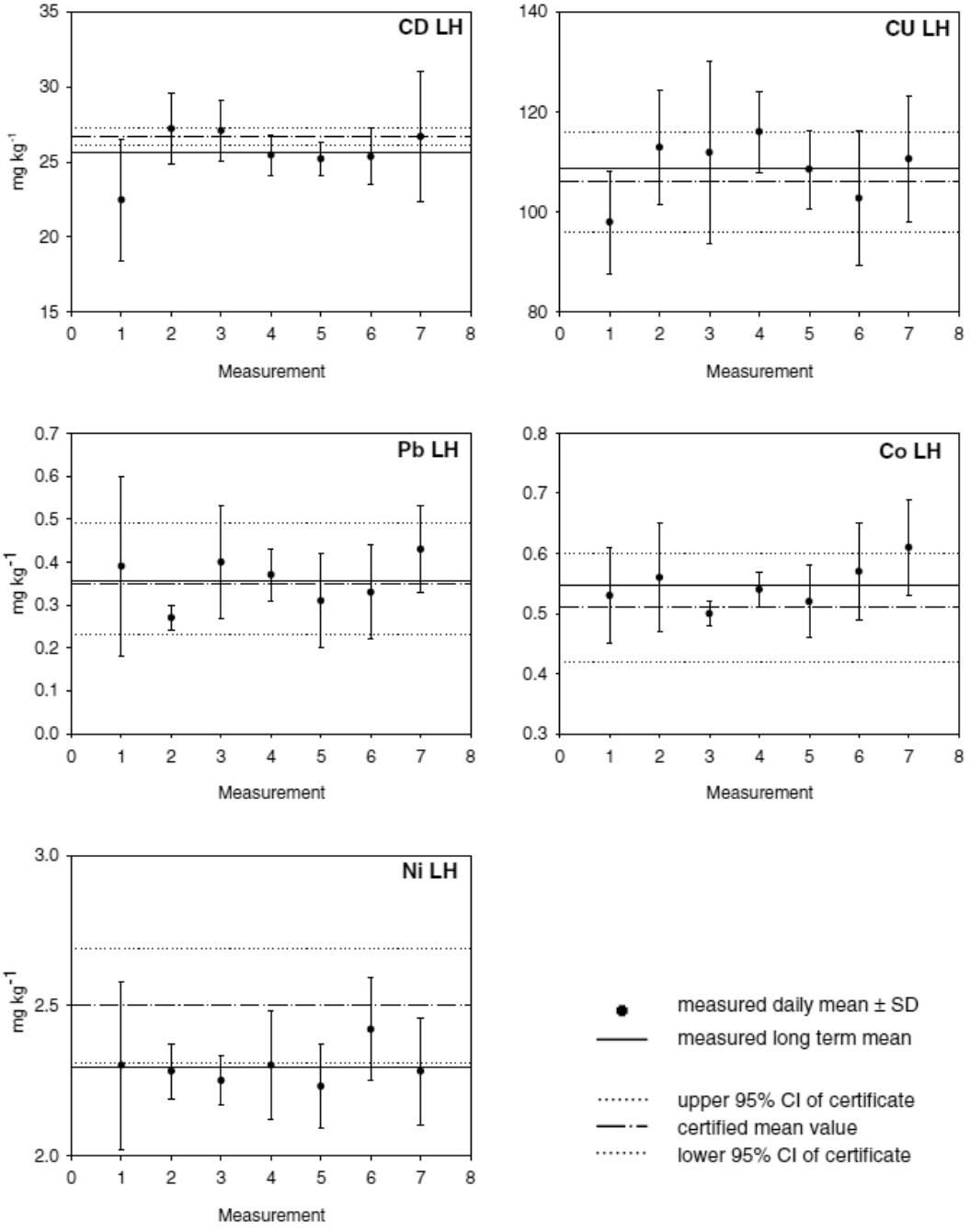

Figure 1. Quality control charts for daily measurements of the reference material Tort-2 (Lobster hepatopancreas, LH; mg/kg dry weight).

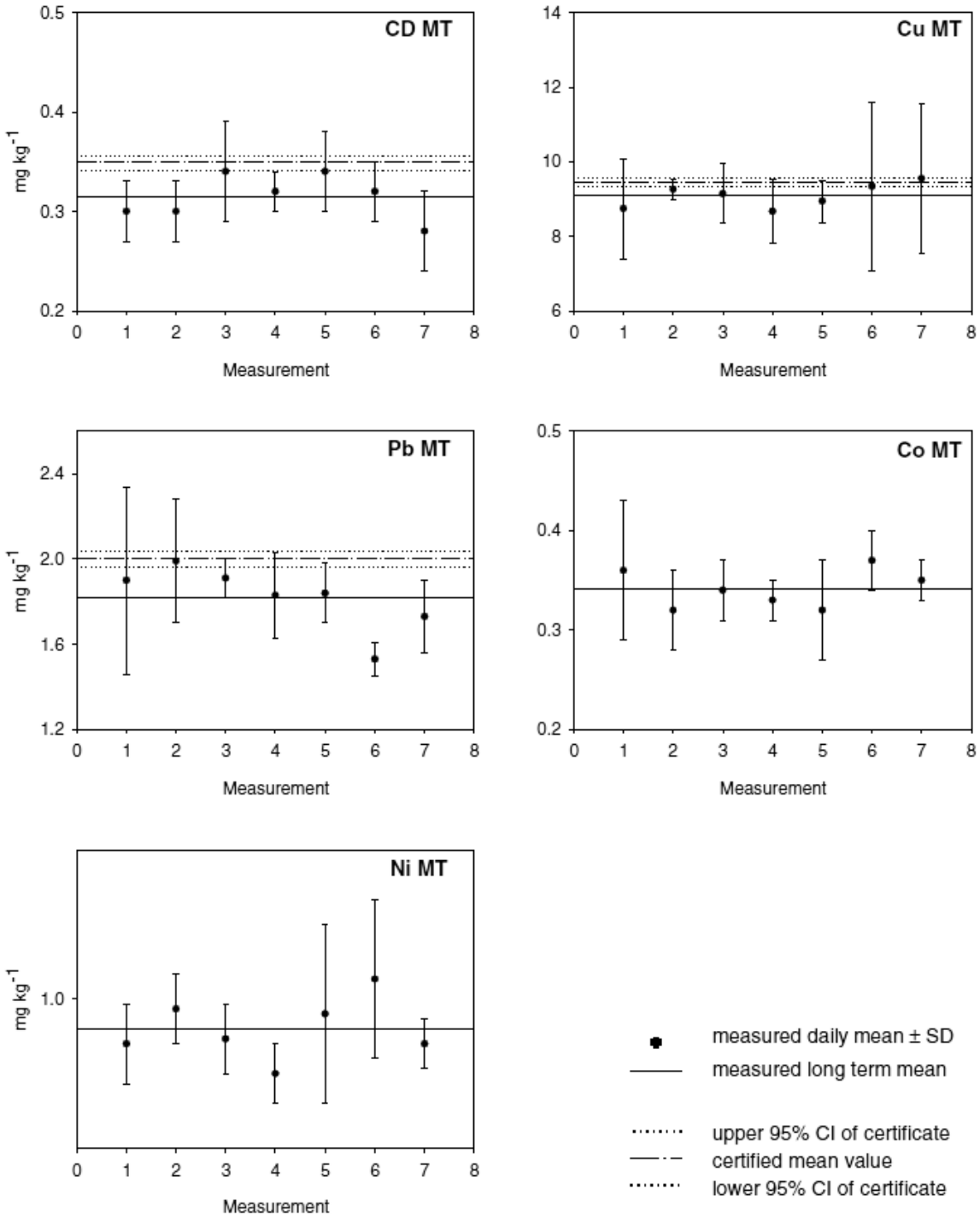

Figure 2. Quality control charts for daily measurements of the reference material CRM No 278R (Mussel tissue; MT; mg/kg dry weight).

Discussion

The graphite furnace methodology presented in this paper yields accurate and long term stable results with a high precision. This is evident from the quality charts (Figures 1–2) and the statistical analyses of daily measurements. The global null hypotheses (equality of means) cannot be rejected in any case (Table 4-5) and consequently, all daily obtained means are belonging to one group according to the Student-Newman-Keuls multiple range test (Table 3). Furthermore, the Lilliefors probabilities (> 0.01) indicate that the hypothesis of normal distribution cannot be rejected for each daily dataset. The overall recovery rate is high for all elements determined in both reference materials (Table 2) and the limits of detection are sufficiently low to make the methodology suitable for the analysis of aquatic invertebrates. This was likewise the case in previous studies employing graphite furnace AAS with deuterium background correction [8]. Somewhat surprising is that the current limits of detection are not lower than those previously reported despite the fact the instrument used in this study (SpectrAA-880 Zeeman AAS) seems to be more sensitive than the instrument used before (Agilent AA-975 equipped with the GTA-95 graphite tube atomizer). However since the method [21] relies on the variability of a "low value", the micro-digestion procedure will be the limiting factor due to unavoidable inhomogeneities in the samples.

It is noteworthy to mention that the peak appearance times largely show good agreement between the calibration standards and both reference materials for all elements (see Appendix 1–5) indicating that there were no severe matrix effects. It was also noted that there was no significant drift observed in the calibration or background signals, As a result, determinations using the concentration calibration mode are preferred to the method of standard additions due to the simplified analyses i.e. reduced analysis time and less material effort. This result is most likely due to the application of chemical modifiers and Zeeman background correction [23,27,28].

In order to obtain as most information as possible from small sample volumes, the capability to measure multi-element sequences within one automatic run is necessary. This reduces analytical effort and prevents digests from ageing. However, the concentration ranges of calibration curves, sample volumes and volume reduction factors have to be selected carefully. Thus, drying and ashing stages must be adjusted to the different sample volumes. To prevent any possible over range signals occurring, it is strongly recommended that the concentration range of samples being determined should be approximately known.

The utilization of slitted lids for the sample cups was also important in this application, as this helped to reduce the evaporation of the digest to a minimum.

Conclusion

The method presented in this paper is applicable to the analysis of various marine invertebrates (for example, zooplankton or benthos) where often only small amounts of biomass are available, especially when bioaccumulation experiments are considered.

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Appendix. Instrumental Parameters, Calibration Graphs and Selected Signal Graphics

Appendix 1. Method: Ni (Zeeman)

Instrument mode	Absorbance
Sampling mode	Automix
Calibration mode	Concentration
Measurement mode	PROMT height
Replicates standard	3
Replicates sample	3
Total volume	37 µL
Sample volume	20 µL
Volume reduction factor	5
Bulk conc	20.00 µg/L
Modifier 1 mode	Co inject
Modifier 1 volume	8 µL
Modifier 2 mode	Co inject
Modifier 2 volume	4 µL

Furnace parameters

Step	Temp (C)	Time (s)	Flow (L/min)	Gas type	Read	Signal storage
1	100	5.0	3.0	Normal	No	No
2	115	40.0	3.0	Normal	No	No
3	130	10.0	3.0	Normal	No	No
4	800	5.0	3.0	Normal	No	No
5	800	1.0	3.0	Normal	No	No
6	800	1.3	0.0	Normal	No	Yes
7	2700	1.4	0.0	Normal	Yes	Yes
8	2700	1.2	0.0	Normal	Yes	Yes
9	2800	1.0	3.0	Normal	No	Yes

Calibration

Sample id	Conc (µg/L)		%Prec	Mean abs
Cal zero	0.00	m	40.6	0.0017
Standard 1	6.00	m	5.1	0.0532
Standard 2	12.00	m	0.4	0.1048
Standard 3	18.00	m	0.7	0.1520
Standard 4	25.00	m	0.1	0.2094

Curve fit = New rational
r = 1.0000

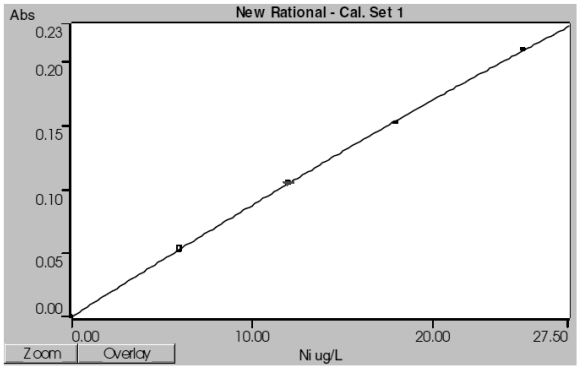

Figure 3. Calibration graph.

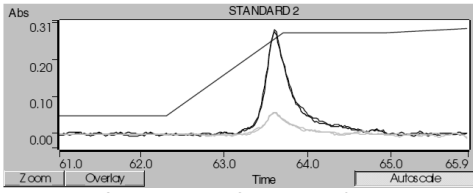

Figure 4. Signal graphics for standard 2.

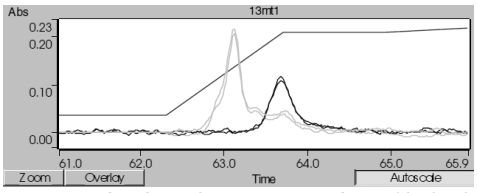

Figure 5. Certified reference material CRM 786R (MT).

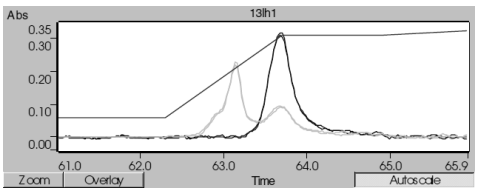

Figure 6. Certified reference material Tort-2 (LH).

Appendix 2. Method: Pb (Zeeman)

Instrument mode	Absorbance
Sampling mode	Automix
Calibration mode	Concentration
Measurement mode	PROMT height
Replicates standard	3
Replicates sample	3
Total volume	30 µL
Sample volume	20 µL
Volume reduction factor	5
Bulk conc	25.00 µg/L
Modifier 1 mode	Co inject
Modifier 1 volume	5 µL
Modifier 2 mode	Co inject
Modifier 2 volume	5 µL

Furnace parameters

Step	Temp (C)	Time (s)	Flow (L/min)	Gas type	Read	Signal storage
1	90	10.0	3.0	Normal	No	No
2	110	45.0	3.0	Normal	No	No
3	150	5.0	3.0	Normal	No	No
4	1000	5.0	3.0	Normal	No	No
5	1000	5.0	3.0	Normal	No	No
6	1000	1.5	0.0	Normal	No	Yes
7	2200	0.6	0.0	Normal	Yes	Yes
8	2200	1.0	0.0	Normal	Yes	Yes
9	2650	0.3	3.0	Normal	No	Yes
10	2650	0.8	3.0	Normal	No	Yes

Calibration

Sample id	Conc (µg/L)		%Prec	Mean abs
Cal zero	0.00	m	14.6	0.0029
Standard 1	5.00	m	2.5	0.0606
Standard 2	10.00	m	2.7	0.1305
Standard 3	15.00	m	0.1	0.1880
Standard 4	20.00	m	1.6	0.2448
Standard 5	25.00	m	1.5	0.3045

Curve fit = New rational
r = 1.0000

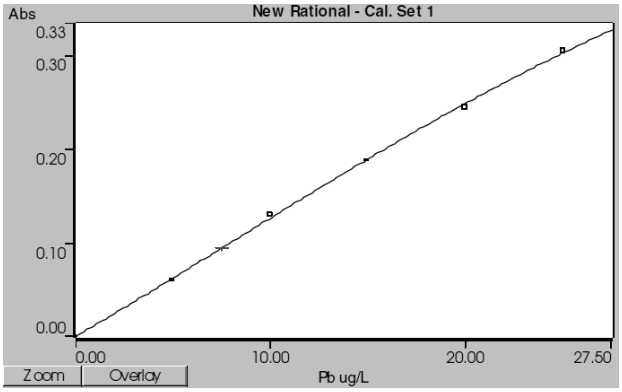

Figure 7. Calibration graph.

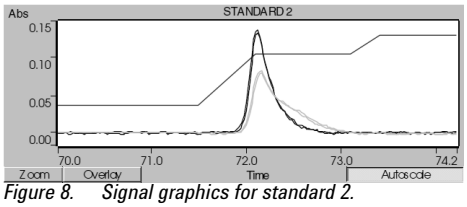

Figure 8. Signal graphics for standard 2.

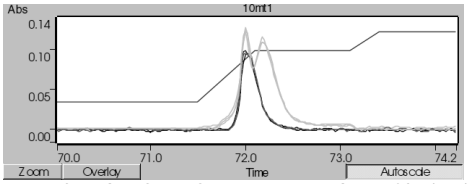

Figure 9. Certified reference material CRM 786R (MT).

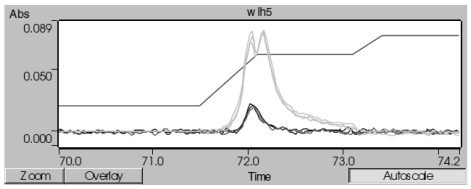

Figure 10. Certified reference material Tort-2 (LH).

Appendix 3. Method: Co (Zeeman)

Instrument mode	Absorbance
Sampling mode	Autonormal
Calibration mode	Concentration
Measurement mode	PROMT height
Replicates standard	3
Replicates sample	3
Total volume	40 µL
Sample volume	40 µL
Volume reduction factor	5
Bulk conc	10.00 µg/L

Furnace parameters

Step	Temp (C)	Time (s)	Flow (L/min)	Gas type	Read	Signal storage
1	100	5.0	3.0	Normal	No	No
2	110	20.0	3.0	Normal	No	No
3	130	8.0	3.0	Normal	No	No
4	800	5.0	3.0	Normal	No	No
5	800	1.5	3.0	Normal	No	No
6	800	1.2	0.0	Normal	No	Yes
7	2300	0.9	0.0	Normal	Yes	Yes
8	2300	1.4	0.0	Normal	Yes	Yes
9	2500	2.0	3.0	Normal	No	Yes

Calibration

Sample id	Conc (µg/L)	%Prec	Mean abs
Cal zero	0.00	16.4	0.0066
Standard 1	2.00	1.7	0.0440
Standard 2	4.00	2.6	0.0990
Standard 3	6.00	0.4	0.1497
Standard 4	8.00	2.3	0.2028
Standard 5	10.00	1.0	0.2480

Curve fit = New rational
r = 1.0000

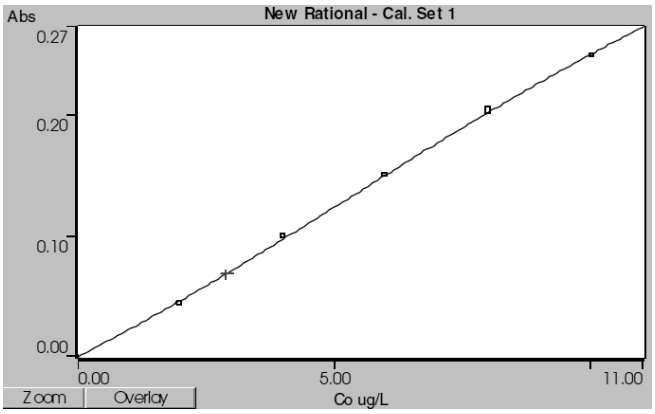

Figure 11. Calibration graph.

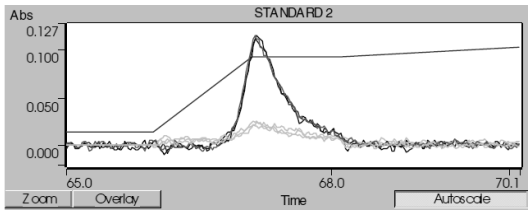

Figure 12. Signal graphics for standard 2.

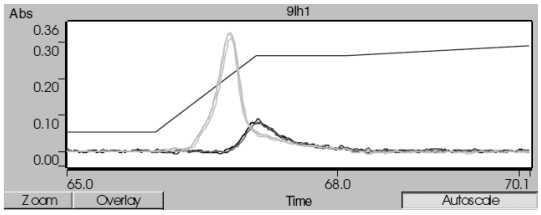

Figure 13. Certified reference material CRM 786R (MT).

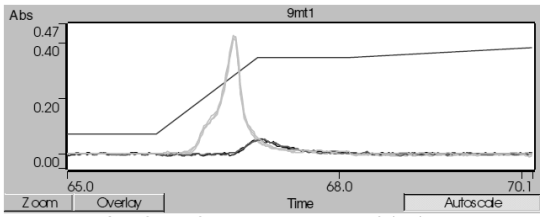

Figure 14. Certified reference material Tort-2 (LH).

Appendix 4. Method: Cu (Zeeman)

Instrument mode	Absorbance
Sampling mode	Autonormal
Calibration mode	Concentration
Measurement mode	PROMT area
Replicates standard	3
Replicates sample	3
Total volume	20 µL
Sample volume	10 µL
Volume reduction factor	5
Bulk conc	100.00 µg/L

Furnace parameters

Step	Temp (C)	Time (s)	Flow (L/min)	Gas type	Read	Signal storage
1	100	5.0	3.0	Normal	No	No
2	110	35.0	3.0	Normal	No	No
3	120	10.0	3.0	Normal	No	No
4	800	5.0	3.0	Normal	No	No
5	800	5.0	3.0	Normal	No	No
6	800	1.0	0.0	Normal	No	Yes
7	2300	1.1	0.0	Normal	Yes	Yes
8	2300	2.0	0.0	Normal	Yes	Yes
9	2650	2.5	3.0	Normal	No	Yes

Calibration

Sample id	Conc (µg/L)	%Prec	Mean abs
Cal zero	0.00	20.5	0.0045
Standard 1	20.00	0.4	0.0655
Standard 2	50.00	0.5	0.1469
Standard 3	80.00	0.3	0.2254
Standard 4	110.00	0.5	0.3027
Standard 5	150.00	0.0	0.3940

Curve fit = New rational
r = 1.0000

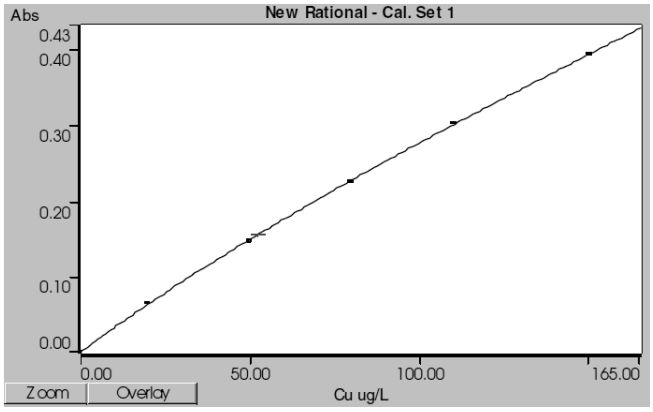

Figure 15. Calibration graph.

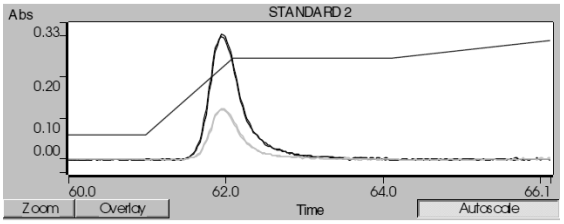

Figure 16. Signal graphics for standard 2.

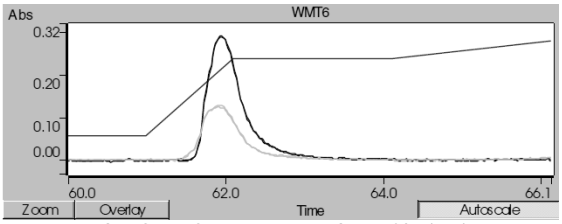

Figure 17. Certified reference material CRM 786R (MT).

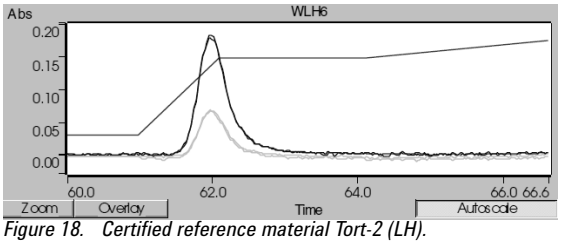

Figure 18. Certified reference material Tort-2 (LH).

Appendix 5. Method: Cd (Zeeman)

Instrument mode	Absorbance
Sampling mode	Autonormal
Calibration mode	Concentration
Measurement mode	PROMT height
Replicates standard	3
Replicates sample	3
Total volume	15 µL
Sample volume	5 µL
Volume reduction factor	5
Bulk conc	5.000 µg/L
Modifier 1 mode	Co inject
Modifier 1 volume	5 µL
Modifier 2 mode	Co inject
Modifier 2 volume	5 µL

Furnace parameters

Step	Temp (C)	Time (s)	Flow (L/min)	Gas type	Read	Signal storage
1	80	5.0	3.0	Normal	No	No
2	110	25.0	3.0	Normal	No	No
3	600	5.0	3.0	Normal	No	No
4	600	7.0	3.0	Normal	No	No
5	600	1.0	3.0	Normal	No	Yes
6	1800	0.8	0.0	Normal	Yes	Yes
7	1800	1.0	0.0	Normal	Yes	Yes
8	2400	0.3	0.0	Normal	No	Yes
9	2400	0.8	3.0	Normal	No	Yes

Calibration

Sample id	Conc (µg/L)		%Prec	Mean abs
Cal zero	0.000		36.6	0.0050
Standard 1	1.000		3.3	0.0609
Standard 2	2.000		1.5	0.1198
Standard 3	3.000	e	1.3	0.1617
Standard 4	4.000		1.3	0.2123
Standard 5	5.000	e	2.5	0.2625

Curve fit = New rational
r = 1.0000

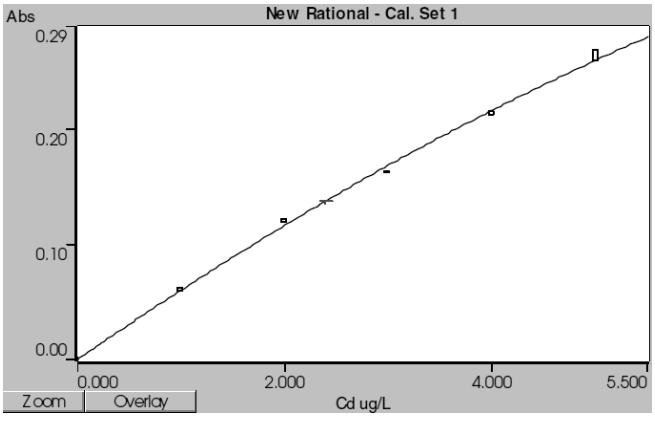

Figure 19. Calibration graph.

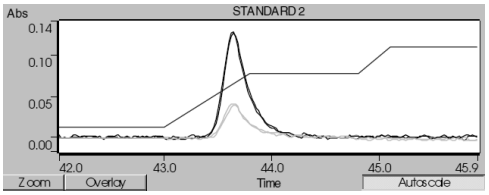

Figure 20. Signal graphics for standard 2.

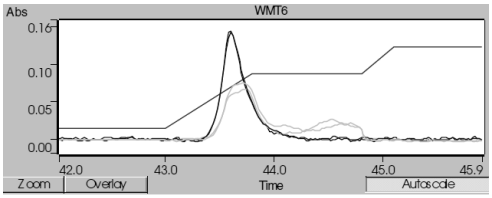

Figure 21. Certified reference material CRM 786R (MT).

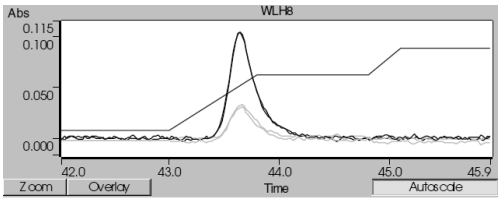

Figure 22. Certified reference material Tort-2 (LH).

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