

Direct determination of Cd in NaCl containing metallothionein fractions  
of different red mullet tissues by GF-AAS

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**Table 1.** Precision and recovery of Cd measurement in standard solutions of Cd in 0.9% NaCl (n = 10)

<b>Concentration of Cd, µg/L</b>	<b>Mean measured Cd concentration, µg/L</b>	<b>SD µg/L</b>	<b>RSD %</b>	<b>Recovery %</b>
<b>0.050</b>	0.041	0.006	13.5	82.0
<b>0.050</b>	0.055	0.004	7.6	110.0
<b>0.150</b>	0.150	0.004	2.8	100.0
<b>0.300</b>	0.309	0.007	2.4	103.0
<b>0.500</b>	0.508	0.009	1.7	101.6
<b>0.800</b>	0.793	0.018	2.3	99.1
<b>1.000</b>	1.044	0.013	1.2	104.4
<b>1.150</b>	1.160	0.073	6.3	100.9

**Table 2.** Precision of Cd measurement in heat-treated cytosol (S50) of different red mullet tissues (n = 10)

<b>Tissue sample</b>	<b>Mean measured Cd concentration, µg/L</b>	<b>SD µg/L</b>	<b>RSD %</b>
<b>Brain sample 1</b>	0.068	0.006	8.7
<b>Brain sample 2</b>	0.115	0.005	4.4
<b>Brain sample 3</b>	0.129	0.005	3.9
<b>Liver sample 1</b>	0.387	0.006	1.6
<b>Liver sample 2</b>	0.644	0.006	1.0
<b>Liver sample 3</b>	0.517	0.008	1.5
<b>Kidney sample 1</b>	0.420	0.010	2.3
<b>Kidney sample 2</b>	0.469	0.007	1.5
<b>Kidney sample 3</b>	0.222	0.011	4.8
<b>Intestine sample 1</b>	0.464	0.009	1.9
<b>Intestine sample 2</b>	0.333	0.007	2.2
<b>Intestine sample 3</b>	1.064	0.008	0.7
<b>Intestine sample 4</b>	0.228	0.005	2.1

**Table 3.** Recovery of Cd in spiked heat-treated cytosol of different red mullet tissues. The samples were spiked in such a way that one volume of sample was mixed with one volume of appropriate standard solution.

<b>Tissue sample</b>	<b>Cd sample concentration µg/L (*n=10; **n=5)</b>	<b>Cd standard solution concentration µg/L</b>	<b>Expected Cd concentration in spiked sample µg/L</b>	<b>Measured Cd concentration µg/L n = 5</b>	<b>RSD %</b>	<b>Recovery %</b>
<b>Brain sample 1</b>	*0.068 ± 0.006	0.150	0.109	0.160 ± 0.007	4.7	146.8
<b>Brain sample 2</b>	*0.115 ± 0.005	0.300	0.208	0.220 ± 0.010	4.7	105.8
<b>Brain sample 3</b>	*0.129 ± 0.005	0.300	0.215	0.227 ± 0.007	3.0	105.6
<b>Brain sample 4</b>	**0.138 ± 0.006	0.300	0.219	0.215 ± 0.005	2.4	98.2
<b>Brain sample 5</b>	**0.080 ± 0.003	0.300	0.190	0.200 ± 0.005	2.3	105.3
<b>Brain sample 6</b>	**0.075 ± 0.007	0.150	0.113	0.126 ± 0.004	3.1	111.5
<b>Liver sample 1</b>	*0.387 ± 0.006	1.150	0.769	0.844 ± 0.016	1.9	109.8
<b>Liver sample 2</b>	*0.644 ± 0.006	1.400	1.022	0.984 ± 0.027	2.8	96.3
<b>Liver sample 3</b>	*0.517 ± 0.008	1.400	0.959	0.833 ± 0.020	2.5	86.9
<b>Liver sample 4</b>	**0.449 ± 0.004	1.400	0.925	0.863 ± 0.027	3.1	93.3
<b>Liver sample 5</b>	**0.835 ± 0.007	1.400	1.118	0.994 ± 0.040	4.0	88.9
<b>Liver sample 6</b>	**0.409 ± 0.004	1.150	0.780	0.734 ± 0.025	3.5	94.1
<b>Kidney sample 1</b>	*0.420 ± 0.010	1.150	0.785	0.793 ± 0.031	3.9	101.0
<b>Kidney sample 2</b>	*0.469 ± 0.007	1.150	0.810	0.800 ± 0.009	1.1	98.8
<b>Kidney sample 3</b>	*0.222 ± 0.011	0.500	0.361	0.384 ± 0.005	1.4	106.4
<b>Intestine sample 1</b>	*0.464 ± 0.009	1.400	0.932	0.937 ± 0.013	1.4	100.5
<b>Intestine sample 2</b>	*0.333 ± 0.007	1.000	0.667	0.687 ± 0.010	1.4	103.0
<b>Intestine sample 4</b>	*0.228 ± 0.005	0.800	0.514	0.511 ± 0.011	2.1	99.4

## Figure captions

**Figure 1.** Cd and background signals obtained after addition of various volumes of EDTA ( $6.0 \text{ g L}^{-1}$ ), of two different pH values: a) pH of EDTA solution was  $\sim 4.5$ , and volume  $5 \text{ }\mu\text{L}$ ; b) pH of EDTA solution was  $\sim 4.5$ , and volume  $10 \text{ }\mu\text{L}$ ; c) pH of EDTA solution was  $\sim 4.5$ , and volume  $15 \text{ }\mu\text{L}$ ; d) pH of EDTA solution was  $\sim 7.0$ , and volume  $5 \text{ }\mu\text{L}$ . All signals were obtained using solution of Cd in  $0.9\%$  NaCl ( $c_{\text{Cd}} = 1.5 \text{ }\mu\text{g L}^{-1}$ ).

**Figure 2.** Effect of atomisation temperature on Cd and background signals.

a)  $\blacktriangle$  Slope of a calibration straight line created using bulk standard solution of Cd in  $0.9\%$  NaCl ( $c_{\text{Cd}} = 0.8 \text{ }\mu\text{g L}^{-1}$ ) and EDTA (pH  $\sim 7.0$ ) as a modifier;  $\Delta$  Background absorbance recorded at calibration zero.

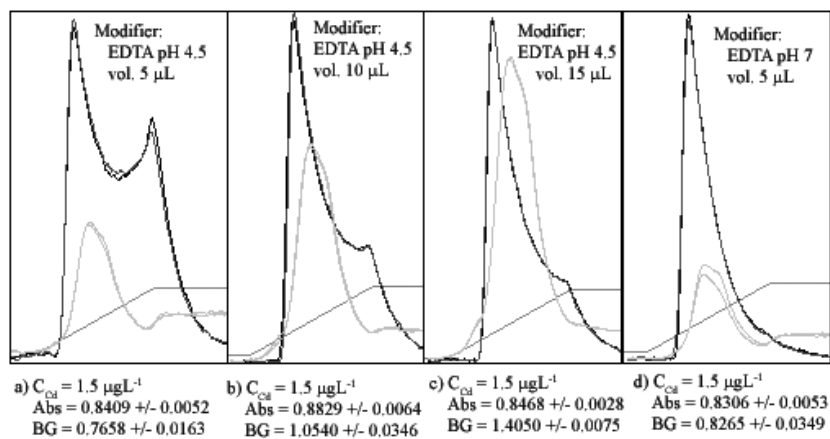
b)  $\circ$  Cd concentration in heat-treated cytosol of red mullet kidney;  $\bullet$  Cd concentration in standard solution of Cd in  $0.9\%$  NaCl ( $c_{\text{Cd}} = 0.5 \text{ }\mu\text{g L}^{-1}$ );  $\square$  Cd concentration in heat-treated cytosol of red mullet intestine.

**Figure 3.** Background signals recorded at calibration zero: a) signal obtained using blank solution of  $0.9\%$  NaCl with addition of EDTA; b) signal obtained using only blank solution of  $0.9\%$  NaCl, without addition of EDTA; c) signal obtained using Milli-Q water with addition of EDTA.

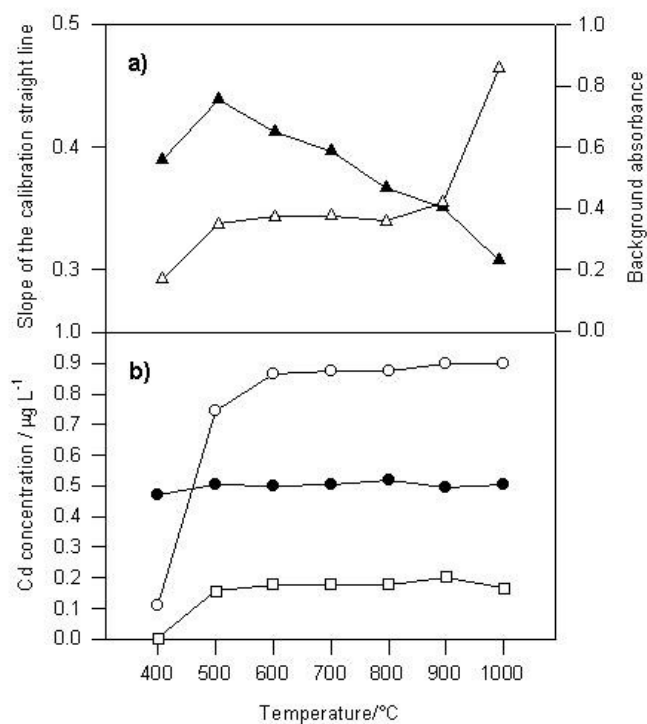
**Figure 4.** Calibration straight line created at 11 different occasions with addition of EDTA, using bulk standard solution of Cd in  $0.9\%$  NaCl ( $c_{\text{Cd}} = 0.8 \text{ }\mu\text{g L}^{-1}$ ) and heating program from Table 1.

**Figure 5.** The comparison of metallothionein level (MT) and background absorbance (BG) in heat-treated cytosol of four tissues of red mullet (K-kidney; IN-intestine; L-liver; B-brain). A small inserted figure represents linear regression graph illustrating high correlation between MTs and BG.

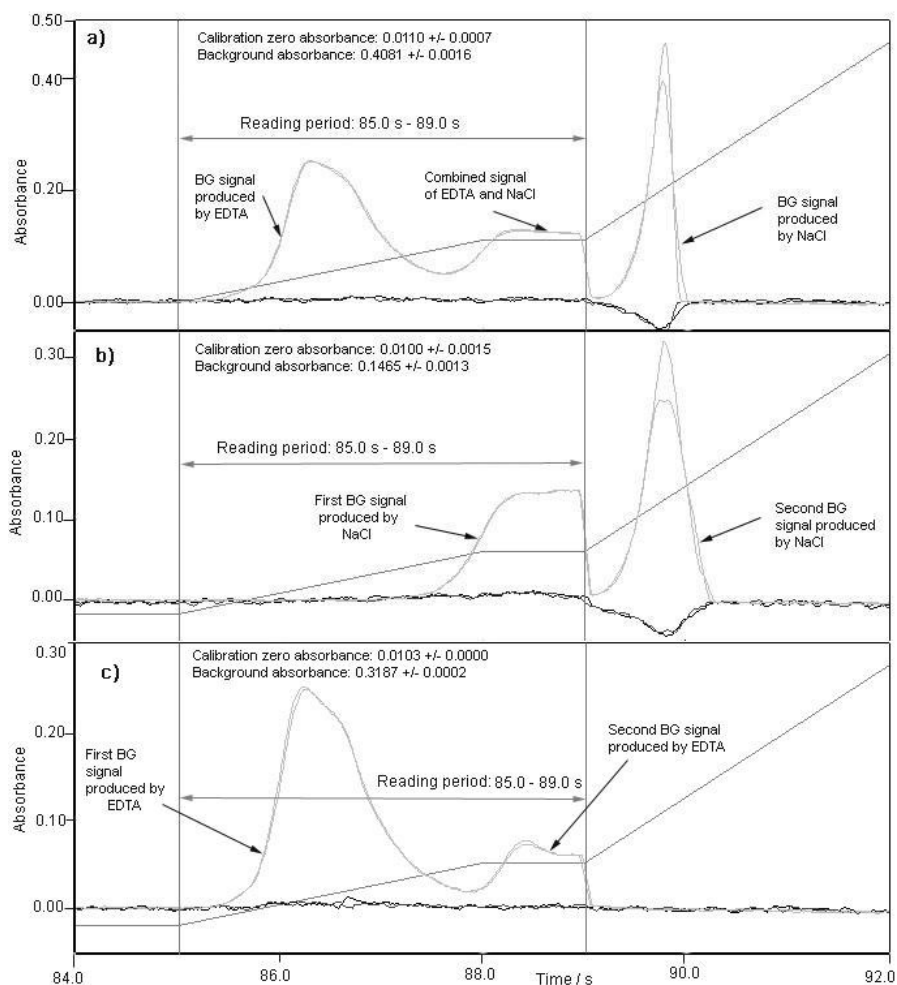
**Figure 1.**



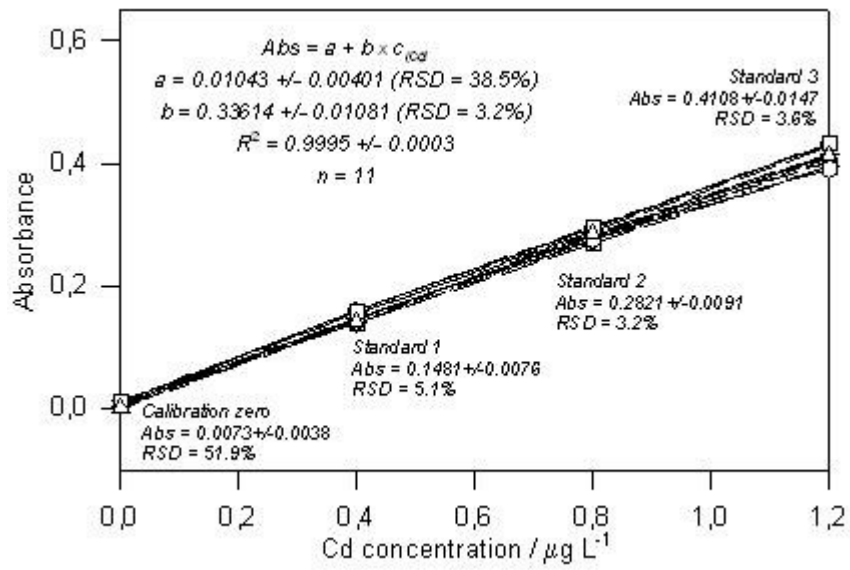
**Figure 2.**



**Figure 3.**



**Figure 4.**



**Figure 5.**

