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

Zrinka Dragun* and Biserka Raspor

Ruder Bošković Institute, Center for Marine and Environmental Research, POBox 180, 10002 Zagreb, Croatia

* phone: xx385-1-4680216; fax: xx385-1-4680242; e-mail: zdragun@rudjer.irb.hr
### Table 1. Precision and recovery of Cd measurement in standard solutions of Cd in 0.9% NaCl (n = 10)

<table>
<thead>
<tr>
<th>Concentration of Cd, µg/L</th>
<th>Mean measured Cd concentration, µg/L</th>
<th>SD µg/L</th>
<th>RSD %</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.050</td>
<td>0.055</td>
<td>0.006</td>
<td>13.5</td>
<td>82.0</td>
</tr>
<tr>
<td>0.050</td>
<td>0.055</td>
<td>0.004</td>
<td>7.6</td>
<td>110.0</td>
</tr>
<tr>
<td>0.150</td>
<td>0.150</td>
<td>0.004</td>
<td>2.8</td>
<td>100.0</td>
</tr>
<tr>
<td>0.500</td>
<td>0.509</td>
<td>0.007</td>
<td>2.4</td>
<td>103.0</td>
</tr>
<tr>
<td>0.800</td>
<td>0.508</td>
<td>0.009</td>
<td>1.7</td>
<td>101.6</td>
</tr>
<tr>
<td>1.000</td>
<td>0.793</td>
<td>0.018</td>
<td>2.3</td>
<td>99.1</td>
</tr>
<tr>
<td>1.150</td>
<td>1.144</td>
<td>0.013</td>
<td>1.2</td>
<td>104.4</td>
</tr>
</tbody>
</table>

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

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

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

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

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

a) ▲ Slope of a calibration straight line created using bulk standard solution of Cd in 0.9% NaCl (c_{Cd} = 0.8 µg L⁻¹) and EDTA (pH ∼7.0) as a modifier; ∆ Background absorbance recorded at calibration zero.

b) ○ Cd concentration in heat-treated cytosol of red mullet kidney; ● Cd concentration in standard solution of Cd in 0.9% NaCl (c_{Cd} = 0.5 µg L⁻¹); □ 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_{Cd} = 0.8 µg 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.

![Graph showing different modifier concentrations and volumes for EDTA pH 4.5 and 7.](Image)

- **a)** $C_{Cd} = 1.5 \mu g L^{-1}$
  - Abs = 0.8468 ± 0.0052
  - BG = 0.7658 ± 0.0163
- **b)** $C_{Cd} = 1.5 \mu g L^{-1}$
  - Abs = 0.8029 ± 0.0004
  - BG = 1.0540 ± 0.0046
- **c)** $C_{Cd} = 1.5 \mu g L^{-1}$
  - Abs = 0.8468 ± 0.0028
  - BG = 1.0050 ± 0.0005
- **d)** $C_{Cd} = 1.5 \mu g L^{-1}$
  - Abs = 0.8306 ± 0.0053
  - BG = 0.8265 ± 0.0349

Figure 2.

![Graph showing relationship between Cd concentration and temperature](Image)

- **a)** Slop of the calibration straight line vs. background absorbance
- **b)** Cd concentration vs. temperature
Figure 3.
Figure 4.

![Graph showing absorbance vs. Cd concentration](image)

- Calibration zero:
  - Abs = 0.0038 ± 0.0038
  - RSD = 7.2%

- Standard 1:
  - Abs = 0.1984 ± 0.0076
  - RSD = 3.9%

- Standard 2:
  - Abs = 0.2221 ± 0.0081
  - RSD = 3.7%

- Standard 3:
  - Abs = 0.4803 ± 0.0047
  - RSD = 0.9%

The equation is given by:

\[
\text{Abs} = a + b \times c_{\text{Cd}}
\]

- For Standard 3:
  - \(a = 0.01043 \pm 0.00040\) (RSD = 38.8%)
  - \(b = 0.3384 \pm 0.01081\) (RSD = 3.2%)
  - \(R^2 = 0.8985 \pm 0.0003\)
  - \(n = 11\)

Figure 5.

![Bar graph showing background absorbance and metallothionein (MT) concentration](image)

- Background absorbance (BG) and MT concentration in heat treated (55°C, 15 min)

- Sample groups: K-BG, K-MT, IN-BG, IN-MT, L-BG, L-MT, B-BG, B-MT

- Data points with error bars for each group

- Inset graph:
  - MT concentration vs. absorbance
  - Linear regression:
    - \(r = 0.9610, p < 0.0001\)
    - \(n = 20\)