Determination of Cadmium and Lead in honey by Electrothermal Atomization Atomic Absorption Spectrometry with Zeeman Effect Correction after microwave (MW) assisted sample digestion

NOTICE:

This method:

- has to be considered only as guideline to help the NRLs to approach the analytical problems of this matrix;
- is developed according to the validation scheme for method used for checking the sufficient homogeneity for PT test items (internal procedure PGVM2.00-11);
- has been developed by the EURL-CEFAO using the facilities available in its laboratories;
- digestion of samples has been performed by means of MILESTONE-ETHOS microwave oven equipped with a probe for control of the temperature. The effectiveness of digestion must be checked by each laboratory setting the parameters of the available equipment properly;
- procedural digested blanks are performed for each vessel to be used in the sample digestion (see 5.1). The mixture and the temperature programme as in the sample digestion are used. Therefore, these blanks are not performed in the same digestion run as that of the samples; The digested procedural blanks are diluted up to 20 ml with ultrapure water. The vessels used are carefully rinsed with ultrapure water before digesting the samples and then they are left to dry, paying attention to any contamination sources;
- Procedural digested blanks are mixed together (Pool of Digested Blanks-PDBs) to prepare the calibration standards and to dilute the samples, when necessary.

The laboratories have to study the instrumental conditions appropriate for their own instrumentation and verify that the differences between the slopes of the calibration curves and the standard addition curves are not significant. If the matrix effect occurs, the method of standard addition is to be preferred. If necessary, the blank sample sent together with the PT items can be used for this scope.
1. **SCOPE**
The method describes the procedure for the determination of cadmium and lead in honey by Z-ETA-AAS, after microwave (MW) assisted sample digestion.

2. **ACRONYMS AND CONVENTIONS**

<table>
<thead>
<tr>
<th>Z-ETA-AAS</th>
<th>Electrothermal Atomization Atomic Absorption Spectrometry with Zeeman Effect Correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>sp</td>
<td>Suprapure grade</td>
</tr>
<tr>
<td>up</td>
<td>Ultrapure grade</td>
</tr>
<tr>
<td>d</td>
<td>density</td>
</tr>
<tr>
<td>c</td>
<td>Concentration expressed in units of milligrams/micrograms per litre</td>
</tr>
<tr>
<td>RSDr</td>
<td>Relative Standard Deviation under repeatability conditions</td>
</tr>
<tr>
<td>EDL</td>
<td>Electrodeless Discharge Lamp</td>
</tr>
<tr>
<td>THGA</td>
<td>Transversely Heated Graphite Atomizer Tubes</td>
</tr>
<tr>
<td>Pool of Digestion Blanks</td>
<td>PDBs</td>
</tr>
</tbody>
</table>

3. **METHOD DESCRIPTION**
The determination of Cd and Pb are performed by Z-ETA-AAS after microwave assisted sample digestion.
Instrumental quantification is based on the external calibration approach.
The samples are digested according to the procedure described at point 5.1.
The calibration curve is prepared as specified in section 6.1.3. for Cd and 6.2.3 for Pb.

**WARNING:** the application of the method includes the use of hazardous materials, operations and equipment. The user is responsible for the appropriate use of safety tools.

3.1 **Apparatus and Equipment**
- Z-ETA-AAS (AAnalyst 800, Perkin Elmer)
- Graphite Tubes THGA
- EDL Lamps for Cd and Pb
- Microwave oven: (MILESTONE-ETHOS equipped with a probe for control of the temperature).
- Analytical scale (0.1 mg resolution)

4. **REAGENTS**
The reagents must have the required adequate purity. Sp or up grade guarantees that the concentration of the analyte in reagents and water is negligible compared with the level of concentration to be determined.

4.1 **HNO₃ sp, not less than 65% mass fraction, d(HNO₃) ~1.4 g/ml**

4.2 **Hydrogen peroxide sp, w(H₂O₂) =30%**

4.3 **Up-water**, specific resistance ≥17 MΩ cm for all samples preparation and dilutions

4.4 **Certified Element Standard Solutions Cd, c = 1000 µg/ml**

#### 4.4.1 **Cd 1 mg/l**
Pipette 0,1 ml of Cd stock solution (4.4) and make up to 100 ml with up-water (4.3) in a certified decontaminated volumetric flask.
Prepare these standard solutions in triplicate, transfer to a plastic decontaminated storage bottle (e.g. Kartell), thoroughly mix.

4.5 **Certified Element Standard Solutions Pb, c = 1000 µg/ml**

#### 4.5.1 **Pb 1mg/l**
Pipette 0.1 ml of Pb stock solution (4.5) and make up to 100 ml with up-water (4.3) in a certified decontaminated volumetric flask. Prepare these standard solutions in triplicate, transfer to a plastic decontaminated storage bottle (e.g. Kartell), thoroughly mix.

4.6 Ammonium dihydrogen phosphate: \( \text{NH}_4\text{H}_2\text{PO}_4 \) (99.99 sp)

4.6.1 Ammonium dihydrogen phosphate (\( \text{NH}_4\text{H}_2\text{PO}_4 \)) 100 g/l

Example: weigh 10 g of \( \text{NH}_4\text{H}_2\text{PO}_4 \) (4.6) and make up to 100 ml with water in a volumetric flask.

4.7 Magnesium nitrate: \( \text{Mg(NO}_3\text{)}_2 \cdot 6\text{H}_2\text{O} \) (99.99 sp)

4.7.1 Magnesium nitrate (\( \text{Mg(NO}_3\text{)}_2 \)) 5 g/l

Example: weigh 0.5 g of \( \text{Mg(NO}_3\text{)}_2 \) (4.7) and make up to 100 ml with water in a volumetric flask.

4.8 Triton X-100: TX: reagent for analysis.

4.8.1 Triton X-100 1% (TX1)

Example: weigh 1 g of TX (4.8) and make up to 100 ml with water in a volumetric flask.

5. SAMPLE TREATMENT

5.1 Microwave assisted digestion

Weigh about 1.5 g of sample in Teflon digestion vessels. Afterwards add 4 ml H\(_2\)O (4.3), 5 ml HNO\(_3\) (4.1) and let the vessels closed under a hood at room temperature for an overnight pre-digestion treatment. Then, add 1 ml H\(_2\)O\(_2\) (4.2), seal the vessels and start the digestion programme (table 1).

At the end of the digestion process, dilute the resulting solutions up to 20 ml with up-water in 50 ml Falcon tubes.

NOTICE: weigh Falcon tubes before transferring the digested solution (\( P_i \)) and weigh them again after taking to the final volume (\( P_f \)).

<table>
<thead>
<tr>
<th>Step</th>
<th>Time (min)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>120</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>120</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>170</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>170</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>Vent</td>
</tr>
</tbody>
</table>

Table 1. Digestion program

During sample digestion an increase of temperature could be visible as an anomalous peak on the temperature profile (at roughly 80 °C). This effect is a consequence of the heat generated from the exothermic reaction of sugary matrix: the overnight pre-digestion treatment is necessary to keep this growth as low as possible.

Alternatively to the overnight pre-digestion it is also possible to perform a microwave assisted treatment so as to make the procedure less time-consuming: after adding the digestion mixture, seal the vessels and start the pre-digestion program (table 2). Let vessels
cool and open them in order to remove fumes. Seal the vessels again and start the digestion program (table 1).

Table 2. Pre-digestion program

<table>
<thead>
<tr>
<th>Step</th>
<th>Time (min)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>Vent</td>
</tr>
</tbody>
</table>

6. INSTRUMENTAL ANALYSIS

a) please note:
New tips and cuvettes were used and they were rinsed at least once with up-water, before their use.
Cuvettes are also decontaminated before their use by letting them stand overnight filled with a 10% HNO₃ aqueous solution.
The micropipette used for mixing shall not be the same used for dilution, nor one used for sample/standard preparation purposes (e.g.: declassified micropipette and used only for mixing).

6.1 Determination of Cd

6.1.1 Working standard solutions

These solutions shall be daily prepared

6.1.1.1 \( c(Cd) = 0.025 \text{ mg/l} \), into a 1ml cuvette for instrumental analysis (example):
- pipette 1.0 ml of up water (4.3)
- take off 25µl
- add 25µl of Cu (4.4.1)
- mix the solution by pipetting several times (see 6 a)

6.1.1.2 \( c(Cd) = 0.050 \text{ mg/l} \), into a 1ml cuvette for instrumental analysis (example):
- pipette 1.0 ml of up water (4.3)
- take off 50µl
- add 50 µl of Cu (4.4.1)
- mix the solution by pipetting several times (see 6 a)

6.1.2 Matrix modifier Cd: 10g/l \( NH_4H_2PO_4 \) + 0.6g/l \( Mg(NO_3)_2 \) in 0.01% TX
- Example: pipette 5ml of \( NH_4H_2PO_4 \) 100 g/l (4.6.1), 6 ml of \( Mg(NO_3)_2 \) 5 g/l (4.7.1) and 0.5 ml of TX1 1% (4.8.1) and make up with up-water in a 50ml volumetric flask.
6.1.3 Calibration Standards

Table 3. Scheme of preparation calibration curve for Cd in honey:

<table>
<thead>
<tr>
<th>Standard</th>
<th>PDBs (µl)</th>
<th>Cd (6.1.1.1) (µl)</th>
<th>Cd (6.1.1.2) (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>1000</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Std 1 (0.25 µg/l)</td>
<td>1980</td>
<td>20</td>
<td>--</td>
</tr>
<tr>
<td>Std 2 (0.5 µg/l)</td>
<td>980</td>
<td>20</td>
<td>--</td>
</tr>
<tr>
<td>Std 3 (1.0 µg/l)</td>
<td>980</td>
<td>--</td>
<td>20</td>
</tr>
<tr>
<td>Std 4 (1.5 µg/l)</td>
<td>960</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Std 5 (2.0 µg/l)</td>
<td>960</td>
<td>--</td>
<td>40</td>
</tr>
</tbody>
</table>

6.1.3.1 Preparation of calibration standards

6.1.3.1.1 Blank: in a cuvette for instrumental analysis (example):
- pipette 1.0 ml of Pool of Digested Blanks (PDBs)

6.1.3.1.2 Standard 1: in a 5ml falcon tube (example):
- pipette 2000 µl of Pool of Digested Blanks (PDBs)
- take off 20µl
- add 20µl of Cd 0.025 mg/l (6.1.1.1) and mix thoroughly
- pipette 1ml of the obtained solution in a cuvette for instrumental analysis.

6.1.3.1.3 Standards 2, 3, 4, 5: in a cuvette for instrumental analysis (example):
- pipette 1000 µl of Pool of Digested Blanks (PDBs)
- take off the amount of standard to be added (e.g. for standard 2, take off 20µl)
- add the calibration solution (e.g. for standard 2, add 20µl of Cd 0.025 mg/l (6.1.1.1))
- mix thoroughly by pipetting (see 6 a).

6.1.4 Sample Dilution

Samples can be run undiluted; when necessary dilute as follows:
Dilution 1:4: into a 1 ml cuvette for instrumental analysis (example):
- pipette 750µl of Pool of Digested Blanks (PDBs)
- add 250µl of sample and mix thoroughly by pipetting (see 6 a).

6.2 Determination of Pb

6.2.1 Working standard solutions

These solutions shall be daily prepared

6.2.1.1 c (Pb) = 0.25 mg/l, into a 1ml cuvette for instrumental analysis (example):
- pipette 750 ml of up water (4.3)
- add 250 ml of Pb (4.5.1)
- mix the solution by pipetting several times (see 6 a).

6.2.1.2 c (Pb) = 0.50 mg/l, into a 1ml cuvette for instrumental analysis (example):
- pipette 0.50 ml of up water (4.3)
- add 0.50 ml of Pb (4.5.1)
- mix the solution by pipetting several times (see 6 a).

6.2.2 Matrix Modifier Pb: 6g/l NH₄H₂PO₄ + 0.5g/l Mg(NO₃)₂+ 0.01% TX.
- Example: pipette 3ml of NH₄H₂PO₄ 100 g/l (4.6.1), 5 ml of Mg(NO₃)₂, 5g/l (4.7.1) and 0.5 ml of TX1 1% (4.8.1) and make up with up-water in a 50ml volumetric flask.
6.2.3 Calibration Standards

Table 4. Scheme of preparation calibration curve for Pb in honey:

<table>
<thead>
<tr>
<th>Standard</th>
<th>PDBs (µl)</th>
<th>Pb (6.2.1.1) (µl)</th>
<th>Pb (6.2.1.2) (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>1000</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Std 1 (0.25 µg/l)</td>
<td>1980</td>
<td>20</td>
<td>--</td>
</tr>
<tr>
<td>Std 2 (0.5 µg/l)</td>
<td>980</td>
<td>20</td>
<td>--</td>
</tr>
<tr>
<td>Std 3 (1.0 µg/l)</td>
<td>980</td>
<td>--</td>
<td>20</td>
</tr>
<tr>
<td>Std 4 (1.5 µg/l)</td>
<td>960</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Std 5 (2.0 µg/l)</td>
<td>960</td>
<td>--</td>
<td>40</td>
</tr>
</tbody>
</table>

6.2.3.1 Preparation of calibration standards

6.2.3.1.1 Blank: in a cuvette for instrumental analysis (example):
- pipette 1.0 ml of Pool of Digested Blanks (PDBs)

6.2.3.1.2 Standard 1: in a 5ml falcon tube (example):
- pipette 2000 µl of Pool of Digested Blanks (PDBs)
- take off 20µl
- add 20µl of Pb 0.25 mg/l (6.2.1.1) and mix thoroughly
- pipette 1ml of the obtained solution in a cuvette for instrumental analysis.

6.3.1.3 Standards 2, 3, 4, 5: in a cuvette for instrumental analysis (example):
- pipette 1000 µl of Pool of Digested Blanks (PDBs)
- take off the amount of standard to be added (e.g. for standard 2, take off 20µl)
- add the calibration solution (e.g. for standard 2, add 20µl of Pb 0.25 mg/l (6.2.1.1))
- mix thoroughly by pipetting (see 6 a).

6.1.4 Sample Dilution

Samples can be run undiluted; when necessary dilute as follows:
Dilution 1:4: into a 1 ml cuvette for instrumental analysis (example):
- pipette 750µl of Pool of Digested Blanks (PDBs)
- add 250µl of sample and mix thoroughly by pipetting (see 6 a).

6.3 Drift Check

Drift is checked by analyzing the second and the fourth standard:
- after each series of 8 samples;
- at the end of an analysis, if more than 3 samples have been run after the last check.
If the instrumental drift is higher than the maximum value set in the method parameters, repeat the calibration curve and read the previous samples up to the last acceptable check.

6.4 Spectrometer Settings

Optimize the instrument in accordance with the recommendation described in the internal procedures.
The instrumental method is reported in Annex 1 for Cd and in Annex 2 for Pb.

7. CALCULATION

Calculate the content, w, of the chemical element as mass fraction mg/kg as follows:
\[ w = \frac{D \times a \times V}{m \times 1000} \]

where:
D = dilution of the test solution before the instrumental determination
a = is the concentration of the element in the test solution in \( \mu g/l \)
V = final volume of the solution in ml
m = weight of the sample in g

Calculate V as follows:
\[ V = \frac{(P_f - P_i)}{d} \]

where:
P_i = weight of the falcon empty tubes
P_f = weight of the falcon tubes containing the digested solution
d = weight of 1 ml of digested solution (please note, if you perform this procedure after digestion allow sample to reach room temperature)

8. EXPRESSION OF RESULTS

Express the final result as mean of three independent sample preparations

9. PARAMETERS OF THE EURL-CEFAO METHOD

LoD and LoQ were determined using 21 aliquots of 3 different samples of honey purchased on the market. The pooled standard deviation was used as basis of calculation both for Cd and Pb. Repeatability was evaluated using honey spiked with Cd and Pb: 10 aliquots were quantified twice on the same day, in two different analytical runs.

The parameters obtained during the method development are summarized as follows:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cd</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>LoD (=3*SD) ( \mu g/kg )</td>
<td>0.3</td>
<td>6.7</td>
</tr>
<tr>
<td>LoQ (=10*SD) ( \mu g/kg )</td>
<td>1.1</td>
<td>22.4</td>
</tr>
<tr>
<td>RSDr %</td>
<td>2.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Maximum Acceptable Drift %</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>
Annex 1: Instrumental Settings for Cd
The annex is the instrumental program for Cd in paper form.
Method Name: Cd Honey

Define Element

Method Description : NH₄H₂PO₄ 10 g/l + Mg(NO₃)₂ 0.6 g/l in Triton 0.05%

Spectrometer
  Element : Cd
  Wavelength (nm) : 228.8
  Slit Width (nm) : 0.7L

Signal
  Type : AA - BG  Measurement : Peak Area

Settings

Read Parameters
  Time (sec) : 3.0  Delay Time (sec) : 0.0  BOC Time (sec) : 2

Replicates ... Same for All Samples : 2

Lamp Current
  Use value entered in Lamp Setup window

Furnace Program

<table>
<thead>
<tr>
<th>Step</th>
<th>Temp(°C)</th>
<th>Ramp Time</th>
<th>Hold Time</th>
<th>Internal Flow</th>
<th>Gas Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>120</td>
<td>1</td>
<td>30</td>
<td>250</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>140</td>
<td>15</td>
<td>30</td>
<td>250</td>
<td>Normal</td>
</tr>
<tr>
<td>3</td>
<td>700</td>
<td>10</td>
<td>20</td>
<td>250</td>
<td>Normal</td>
</tr>
<tr>
<td>4</td>
<td>1550</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>2450</td>
<td>1</td>
<td>3</td>
<td>250</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Read Step : 4  Injection Temperature(°C) : 20

No extra furnace cleanout.

Furnace Autosampler

Sample
  Volume : 20 uL  Diluent Volume : 0 uL  Diluent Location : 1

Matrix Modifiers
  #1 Volume : 5 uL  Location : 20
    Add to calibration blank and standards.
    Add to reagent blank and samples.
  #2 Volume : 0 uL  Location : 1
    Add to calibration blank and standards.
    Add to reagent blank and samples.

Autosampler and Furnace Sequence

Step  Actions and Parameters
A    Pipet diluent + modifier1 + spike + sample/std
B    Run furnace steps 1 to end
Method Name: Cd Honey

Pipet Speed ... Take up : 100%   Dispensing : 100%

-------------------------------------------------------------------

Calibration Equation and Units

Equation : Linear, Calculated Intercept
Maximum Decimal Places : 3   Maximum Significant Figures : 4
Calibration Units : ug/L
Sample Units : ug/L

-------------------------------------------------------------------

Calibration Standard Concentrations

<table>
<thead>
<tr>
<th>ID</th>
<th>Concentration</th>
<th>A/S</th>
<th>Loc</th>
<th>Stock(uL)</th>
<th>Diluent(uL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calib Blank</td>
<td>Blk</td>
<td></td>
<td>1</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Standard 1</td>
<td>Calib Std 1</td>
<td>0.25</td>
<td>2</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Standard 2</td>
<td>Calib Std 2</td>
<td>0.5</td>
<td>3</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Standard 3</td>
<td>Calib Std 3</td>
<td>1</td>
<td>4</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Standard 4</td>
<td>Calib Std 4</td>
<td>1.5</td>
<td>5</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Standard 5</td>
<td>Calib Std 5</td>
<td>2</td>
<td>6</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

-------------------------------------------------------------------

Initial Calibration Options

When opening this method manually:
Clear calibration curve(s) and construct a new calib curve.

When using this method in a multimethod sequence:
Start by constructing new calibration curves.

-------------------------------------------------------------------

Calibration Check

Correlation coefficient checking is not enabled in this method.

-------------------------------------------------------------------

Recalibration

Periodic recalibration is not enabled in this method.

Analyze standards at end of analysis is not enabled in this method.

-------------------------------------------------------------------

Precision Checks

Precision checking is not enabled in this method.

-------------------------------------------------------------------

Beyond Calibration Range

Beyond calibration range checking is not enabled in this method.

-------------------------------------------------------------------

Matrix Recovery
Matrix recovery checking is not enabled in this method.

---

Automatic Recovery

Automatic recovery checking is not enabled in this method.

---

QC Sample Definition

<table>
<thead>
<tr>
<th>QC Sample ID</th>
<th>A/S</th>
<th>Count as</th>
<th>Subtract</th>
<th>Loc</th>
<th>Sample</th>
<th>Reagent Blank</th>
</tr>
</thead>
</table>

---

QC Sample Concentrations and Limits

---

Schedule for QC Analyses

<table>
<thead>
<tr>
<th>QC Sample ID</th>
<th>After Init Cal</th>
<th>After Recal</th>
<th>Periodic Timing of Analyses</th>
<th>Frequency</th>
<th>Count</th>
<th>Samples</th>
</tr>
</thead>
</table>

- Frequency ... Same for all QC's : 1
- Count : Samples

---

Failure Actions for After-Calibration QC's

| QC Sample ID | Times to Retry QC | When All Tries Fail | Additional Message |

Failure Actions for Periodic QC's

| QC Sample ID | Times to Retry QC | When All Tries Fail | Additional Message |

Failure Actions for At-End QC's

| QC Sample ID | Times to Retry QC | When All Tries Fail | Additional Message |

Maximum Retries After QC Failure

After a group of standards or unknowns has been reanalyzed 1 times, then Continue

---

Options

Include in Results Display and Printed Log:

Headers:
- Analytical Header
- Method Header (Short)
Method Name: Cd Honey

* Sample Header (Short)
  Start each sample on a new page

Sample Data Items:
* Replicate Data
* Means and Statistics
  Transient Peak Plots (Last)

Summary Items:
Analysis List
* Matrix Test Reports
* Calibration Summary
* Calibration Curves

Save with Results:
* Transient Peak Profiles

Remarks:
MM: Cd Offal; pos 20
Campioni TQ o se diluiti in BM
Annex 2: Instrumental Settings for Pb
The annex is the instrumental program for Pb in paper form.
Method Name: Pb Honey

Define Element

Method Description : Pb Honey

Spectrometer
  Element : Pb
  Wavelength (nm) : 283.3
  Slit Width (nm) : 0.7L

Signal
  Type : AA - BG  Measurement : Peak Area

Settings

Read Parameters
  Time (sec) : 5.0  Delay Time (sec) : 0.0  BOC Time (sec) : 2

Replicates ... Same for All Samples : 2

Lamp Current
  Use value entered in Lamp Setup window

Furnace Program

<table>
<thead>
<tr>
<th>Step</th>
<th>Temp(°C)</th>
<th>Ramp Time</th>
<th>Hold Time</th>
<th>Internal Flow</th>
<th>Gas Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>110</td>
<td>1</td>
<td>30</td>
<td>250</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>130</td>
<td>15</td>
<td>30</td>
<td>250</td>
<td>Normal</td>
</tr>
<tr>
<td>3</td>
<td>600</td>
<td>15</td>
<td>20</td>
<td>250</td>
<td>Normal</td>
</tr>
<tr>
<td>4</td>
<td>1700</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>2450</td>
<td>1</td>
<td>3</td>
<td>250</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Read Step : 4  Injection Temperature(°C) : 20

No extra furnace cleanout.

Furnace Autosampler

Sample
  Volume : 20 uL  Diluent Volume : 0 uL  Diluent Location : 1

Matrix Modifiers
  #1 Volume : 5 uL  Location : 20
    Add to calibration blank and standards.
    Add to reagent blank and samples.
  #2 Volume : 0 uL  Location : 1

Autosampler and Furnace Sequence

Step  Actions and Parameters
  A  Pipet diluent + modifier1 + spike + sample/std
  B  Run furnace steps 1 to end

Pipet Speed ... Take up : 100%  Dispensing : 100%
Method Name: Pb Honey

Calibration Equation and Units

Equation : Linear, Calculated Intercept
Maximum Decimal Places : 3   Maximum Significant Figures : 4
Calibration Units : ug/L
Sample Units : ug/L

Calibration Standard Concentrations

<table>
<thead>
<tr>
<th>ID</th>
<th>Concentration</th>
<th>A/S</th>
<th>Loc</th>
<th>Stock (uL)</th>
<th>Diluent (uL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calib Blank</td>
<td>Calib Blank 1</td>
<td>1</td>
<td>20</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Standard 1</td>
<td>Calib Std 1</td>
<td>2.5</td>
<td>2</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Standard 2</td>
<td>Calib Std 2</td>
<td>5</td>
<td>3</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Standard 3</td>
<td>Calib Std 3</td>
<td>10</td>
<td>4</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Standard 4</td>
<td>Calib Std 4</td>
<td>15</td>
<td>5</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Standard 5</td>
<td>Calib Std 5</td>
<td>20</td>
<td>6</td>
<td>20</td>
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</tr>
</tbody>
</table>

Initial Calibration Options

When opening this method manually:
  Clear calibration curve(s) and construct a new calib curve.

When using this method in a multimethod sequence:
  Start by constructing new calibration curves.

Calibration Check

Correlation coefficient checking is not enabled in this method.

Recalibration

Periodic recalibration is not enabled in this method.

Analyze standards at end of analysis is not enabled in this method.

Precision Checks

Precision checking is not enabled in this method.

Beyond Calibration Range

Beyond calibration range checking is not enabled in this method.

Matrix Recovery

Matrix recovery checking is not enabled in this method.
Method Name: Pb Honey

Automatic Recovery

Automatic recovery checking is not enabled in this method.

QC Sample Definition

<table>
<thead>
<tr>
<th>QC</th>
<th>Sample ID</th>
<th>A/S</th>
<th>Count as</th>
<th>Subtract</th>
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</thead>
<tbody>
<tr>
<td>Loc</td>
<td>Sample</td>
<td>Reagent Blank</td>
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</table>

QC Sample Concentrations and Limits

Schedule for QC Analyses

<table>
<thead>
<tr>
<th>QC</th>
<th>Sample ID</th>
<th>After</th>
<th>After</th>
<th>After</th>
<th>Periodic</th>
<th>Freq</th>
<th>At End</th>
</tr>
</thead>
<tbody>
<tr>
<td>Init Cal</td>
<td>Recal</td>
<td>Periodic</td>
<td>Freq</td>
<td>At End</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Periodic Timing of Analyses

Frequency ... Same for all QC's : 1

Count : Samples

Failure Actions for After-Calibration QC's

<table>
<thead>
<tr>
<th>QC</th>
<th>Sample ID</th>
<th>Times to</th>
<th>When All</th>
<th>Additional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retry QC</td>
<td>Tries Fail</td>
<td>Message</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Failure Actions for Periodic QC's

<table>
<thead>
<tr>
<th>QC</th>
<th>Sample ID</th>
<th>Times to</th>
<th>When All</th>
<th>Additional</th>
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<tr>
<td>Retry QC</td>
<td>Tries Fail</td>
<td>Message</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Failure Actions for At-End QC's

<table>
<thead>
<tr>
<th>QC</th>
<th>Sample ID</th>
<th>Times to</th>
<th>When All</th>
<th>Additional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retry QC</td>
<td>Tries Fail</td>
<td>Message</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Maximum Retries After QC Failure

After a group of standards or unknowns has been reanalyzed 1 times, then Continue

Options

Include in Results Display and Printed Log:

Headers:
Analytical Header
Method Header (Short)
* Sample Header (Short)
Start each sample on a new page
Method Name: Pb Honey

Sample Data Items:
* Replicate Data
* Means and Statistics
  Transient Peak Plots (First)

Summary Items:
  Analysis List
  * Matrix Test Reports
  * Calibration Summary
  Calibration Curves

Save with Results:
* Transient Peak Profiles

Remarks:
NH4H2PO4 6 g/l + Mg(NO3)2 0,5 g/l + Triton X 0.05%; posizione 20
Campioni TQ