Analysis of Chlorates and Perchlorate Residues in Milk and Powders

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Chlorate background

- Chlorate (ClO₃⁻) is a substance that is no longer approved as a pesticide (CD 2008/865/EC).

- ClO₃⁻ is formed as a by-product when using chlorine, chlorine dioxide or hypochlorite for the disinfection of drinking water, water for food production and surfaces coming into contact with food.

- WHO guideline of 0.7 mg/L (700 ppb) for ClO₃⁻ in drinking water.
Toxicological concern

- Concern because chlorates are a competitive inhibitor of iodine uptake in the thyroid.

- Its presence in food a potential health concern for vulnerable groups, particularly infants, pregnant women and people with thyroid dysfunction.

- Can cause damage to red blood cells.
Interpretation for Infant Formula (IF)

- MRL for Reconstituted IF = 0.01 mg/kg
- Reconstituted IF = 25.2 g powder + 180 mL H2O.
- Dilution factor (w/w) = (25.2g + 180 g)/25.2 g = 8.14
- 0.01 mg/kg Recon. IF ~ 0.0814 mg/kg IF (powder).
- IF contains approx. 50% SMP, ~0.1628 mg/kg (SMP)
- Milk and SMP need to be < 0.02 and <0.16 mg/kg, resp.
Analytical methodology
Analytical challenge

- Very small polar molecules, which make it difficult to achieve selective analysis.
- Need selective detection i.e. MS or MS/MS to achieve low levels of detection.
- Due to high water solubility speciality chromatographic columns or ion chromatography is required.
Analytical methods

- Very few published methods available for milk or dairy powders.

- Most methods use Ion chromatography coupled to mass spectrometry.

- EUROL method available using an alternative Hypercarb LC column.

- The best methods are unpublished.
Sample Preparation Procedure for Milk

1. Weigh 5g of Milk
2. Add 100 μL isotopically labelled internal standard
3. Centrifuge 10 min @ 3500 rpm
4. Take 2ml (~2g) skimmed milk carefully avoiding the fat layer at the top
5. Take 5 ml and Conc. under N₂, @ 30 °C
6. Centrifuge 10 min @ 3500 rpm
7. Shake at 200 rpm for 20 min
8. Add 8 ml ACN and 100 μl acetic acid
9. Filter (0.2 μm PTFE) and inject in UHPLC-MS/MS
LC Separation Conditions

Column: Poroshell PFP 120, 50 x 2.1mm (1.9 µm)

Temp: 40°C

Mobile phase A: 1% Acetic Acid in Water
Mobile phase B: Methanol

Flow: 0.6 mL/min

Gradient: 0 min 100% A
0.99 min 100% A
1.0 min 0% A
1.79 min 0% A
1.80 min 100%A
2.8 min 100%A

Run Time: 2.8 min

Injection Volume: 2 µL

Needle Wash: Methanol:Water (50:50, v/v)
QqQ MS Conditions

Electrospray ionisation with Jet Stream Source

- Drying Gas: 150°C, 8 L/min
- Sheath Gas: 400°C, 11 L/min.
- Nebuliser: 45 psi
- Capillary: 2000 V
- Nozzle: 0

MS Conditions
- ESI Polarity: Negative
- Scan Type: Dynamic MRM
- Cycle time: 500 ms
- ΔEMV: 0 V

<table>
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<tr>
<th>Compound</th>
<th>Transition (m/z)</th>
<th>Dwell</th>
<th>FV</th>
<th>CE</th>
<th>CAV</th>
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<tbody>
<tr>
<td>Chlorate</td>
<td>84.9 &gt; 68.9 82.9 &gt; 66.9</td>
<td>124</td>
<td>50</td>
<td>19</td>
<td>4</td>
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<tr>
<td>18O3-Chlorate</td>
<td>89 &gt; 71</td>
<td>124</td>
<td>50</td>
<td>27</td>
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<td></td>
<td></td>
<td>4</td>
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<tr>
<td>Perchlorate</td>
<td>101 &gt; 84.9 99 &gt; 92.9</td>
<td>124</td>
<td>128</td>
<td>31</td>
<td>4</td>
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<tr>
<td>18O3-Chlorate</td>
<td>107 &gt; 88.9</td>
<td>124</td>
<td>128</td>
<td>35</td>
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</table>
Method Sensitivity: Chlorate

Calibration standard 1: 0.001 mg/kg in milk.

Lower Limit of reporting: 0.002 mg/kg in milk.
Method Sensitivity: Perchlorate

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Lower Limit of reporting: 0.002 mg/kg in milk.

Quantitation  Qualification  Internal Standard
# Accuracy and Precision

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Fortification Level (µg/kg)</th>
<th>Between days study (n =2 x 10d)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (µg/kg)</td>
<td>S.D. (µg/kg)</td>
<td>CV (%)</td>
<td>Trueness (%)</td>
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<tr>
<td>Chlorate</td>
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<td>2.04</td>
<td>0.18</td>
<td>8.6</td>
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<td>99.0</td>
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<td>95-105</td>
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<tr>
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<td>0.13</td>
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<td></td>
<td>100</td>
<td>98.8</td>
<td>1.46</td>
<td>1.48</td>
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