Analysis of Chlorates and Perchlorate Residues in Milk and Powders

Martin Danaher, Clement Douillet,
Mohammad Hossain
Food Safety Department,
Teagasc, Dublin, Ireland

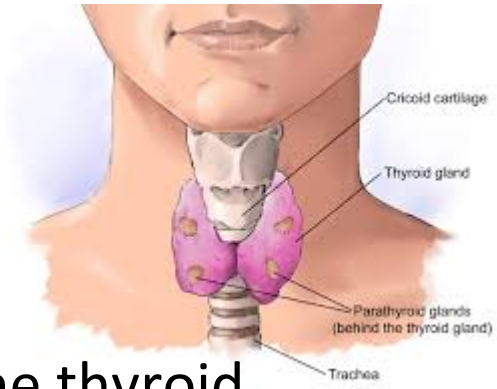
Chlorate background

- Chlorate (ClO_3^-) is a substance that is no longer approved as a pesticide (CD 2008/865/EC).

- ClO_3^- 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 ClO_3^- 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.

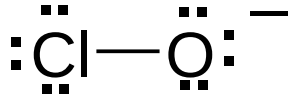
- Can cause damage to red blood cells.

Interpretation for Infant Formula (IF)

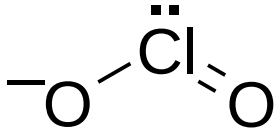
- MRL for Reconstituted IF = 0.01 mg/kg
- Reconstituted IF = 25.2 g powder + 180 mL H2O.
- Dilution factor (w/w) = (25.2g + 180 g)/25.2 g = 8.14
- 0.01 mg/kg Recon. IF ~ 0.0814 mg/kg IF (powder).
- IF contains approx. 50% SMP, ~0.1628 mg/kg (SMP)
- Milk and SMP need to be < 0.02 and <0.16 mg/kg, resp.

Analytical methodology

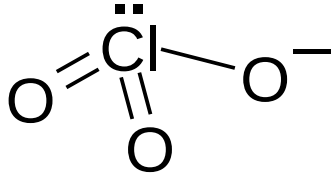
Analytical challenge



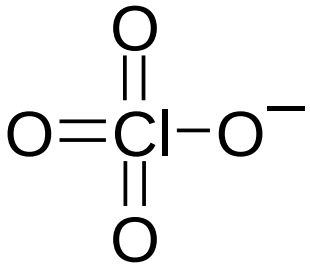
Hypochlorite



Chlorite



Chlorate



Perchlorate

- 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.
- EURL method available using an alternative Hypercarb LC column.
- The best methods are unpublished.

Sample Preparation Procedure for Milk

Weigh 5g of Milk

Add 100 µL Isotopically labelled internal standard

Centrifuge 10 min @ 3500 rpm

Take 2ml (~2g) skimmed milk carefully avoiding the fat layer at the top

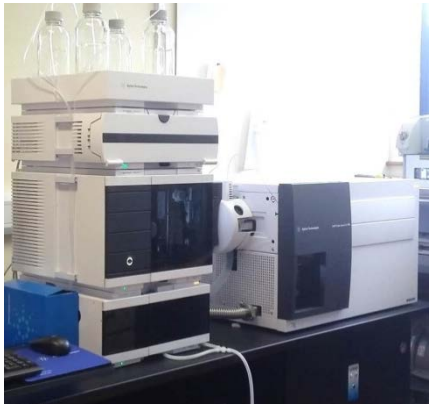
Add 8 ml ACN and 100 µl acetic acid

Shakie at 200 rpm for 20 min

Centrifuge 10 min @ 3500 rpm

Take 5 ml and Conc. under N_2, @ 30 °C

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, 11L/min.
Nebuliser:	45 psi
Capillary:	2000V
Nozzle:	0

MS Conditions

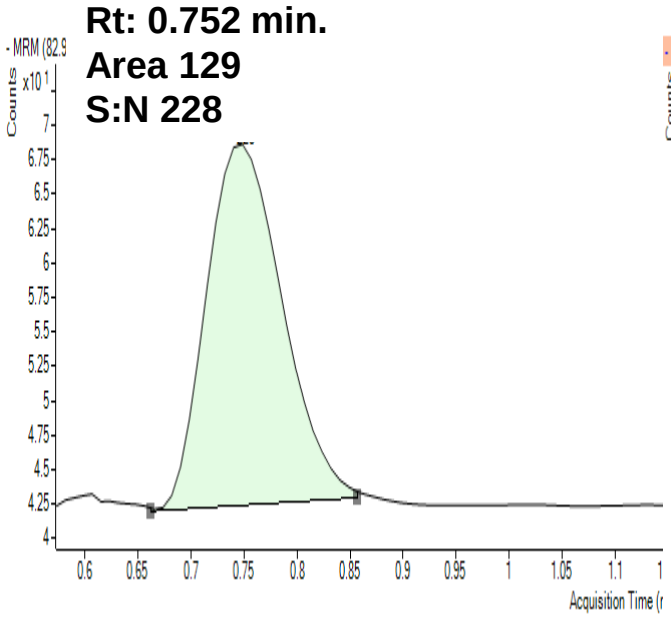
ESI Polarity:	Negative
Scan Type:	Dynamic MRM
Cycle time:	500 ms
ΔEMV:	0V

Compound	Transition (m/z)	Dwell	FV	CE	CAV
Chlorate	84.9>68.9 82.9 > 66.9	124	50	19 27	4 4
$^{18}O_3$-Chlorate	89 > 71	124	50	27	4
Perchlorate	101 >84.9 99>92.9	124	128	31 31	4 4
$^{18}O_3$-Chlorate	107 > 88.9	124	128	35	4

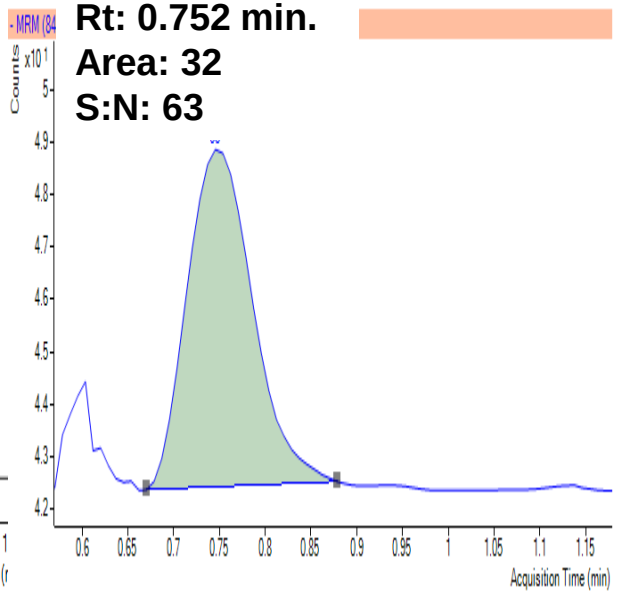
Method Sensitivity: Chlorate

Calibration standard 1: 0.001 mg/kg in milk.

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



Quantitation



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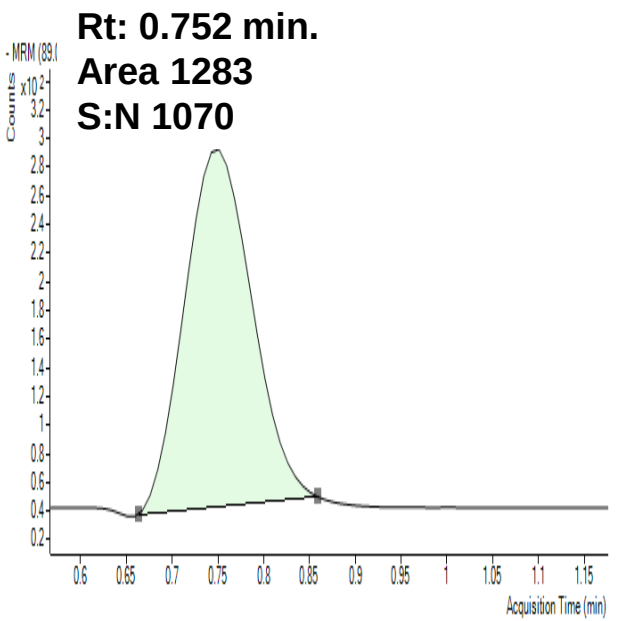

Internal Standard

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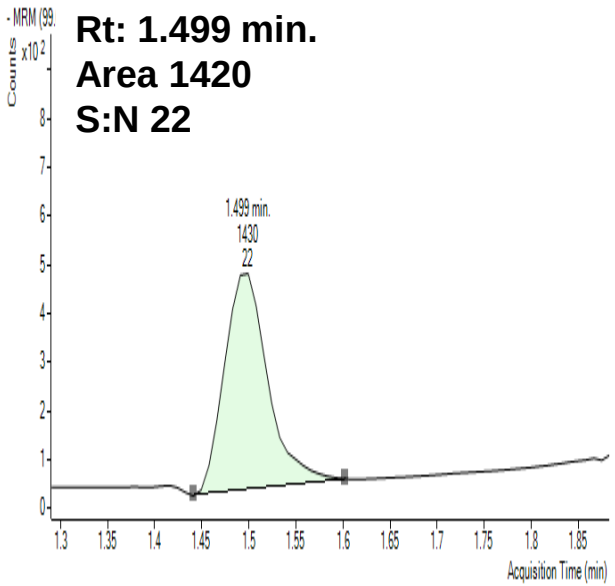

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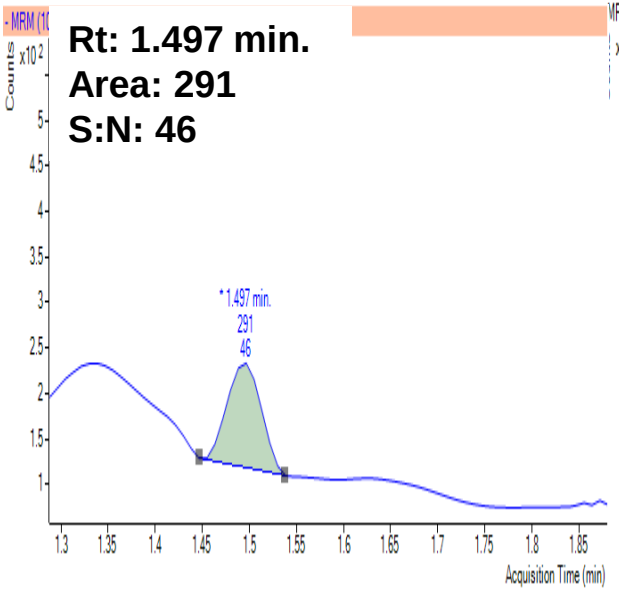

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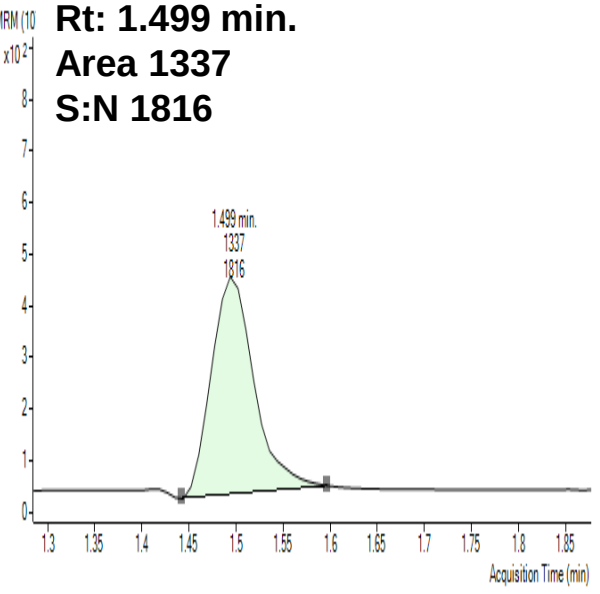

Internal Standard

Accuracy and Precision

Analyte	Fortification Level (µg/kg)	Between days study (n =2 x 10d) Mean (µg/kg)	S.D. (µg/kg)	CV (%)	Trueness (%)
Chlorate	2	2.04	0.18	8.6	92-112
	100	99.0	2.5	2.5	95-105
Perchlorate	2	2.04	0.13	6.2	95-108
	100	98.8	1.46	1.48	94-101

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