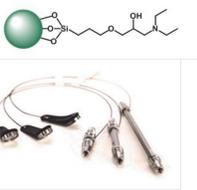


DETERMINATION OF CHLORATE AND PERCHLORATE USING A NOVEL HILIC COLUMN CHEMISTRY BY LC-MS/MS

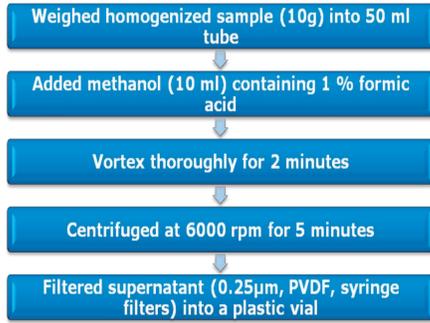
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INTRODUCTION

Food can be contaminated by chlorate and perchlorate during different stages of production. Perchlorate can be present in food via the use of fertilisers, while chlorate can be present due to the use of chlorinated water during irrigation, crop washing or disinfection of surfaces during food production. In 2008, chlorate was banned for use as a pesticide and the maximum residue limit (MRL)¹ for chlorate was set at 0.01mg/kg (under revision)². There are currently no regulatory maximum limits for perchlorate in food in Europe. The European Commission introduced reference levels for perchlorate³ (0.1 to 1.0 mg/kg depending upon commodity), to support trade, but is looking at setting maximum levels to replace the current levels for intra-community trade. Chlorate and perchlorate have traditionally been analysed by ion chromatography, requiring the use of specialised equipment. More recent methods involve LC-MS/MS, utilising columns highlighted in the QuPPE methodology⁴. In this poster we highlight an alternative LC-MS/MS method with chromatographic separation achieved on a novel hydrophilic interaction liquid chromatography (HILIC) column, applying an ammonium formate mobile phase gradient.



METHODS



Samples of various food commodities were extracted using the QuPPE method as shown in Figure 1. Filtered extracts were then analysed using the liquid chromatography, mass spectrometry method highlighted below.

Liquid chromatography

LC system: ACQUITY UPLC I-Class
Column: Torus DEA 2.1 x 50 mm
Mobile phase A: 50 mM ammonium formate pH 2.9
Mobile phase B: 0.9% formic acid in acetonitrile
Strong Wash: 10:90 acetonitrile : water
Weak Wash: 90:10 acetonitrile : water
Column temperature: 50 °C
Sample temperature: 10 °C
Injection volume: 5 µL
Flow rate: 0.5 mL/min
Runtime: 9.0 minutes



Mass Spectrometry

MS system: Xevo TQ-XS
Ionisation mode: ESI negative
Capillary: 0.5 kV
Desolvation temp.: 600°C
Desolvation gas flow: 1000 L/hr
Source temp.: 150 °C
Acquisition: MRM with at least 2 transitions per compound. Primary transition reported in Figure 2.

Figure 1: Summary of QuPPE extraction procedure utilised.

RESULTS AND DISCUSSION

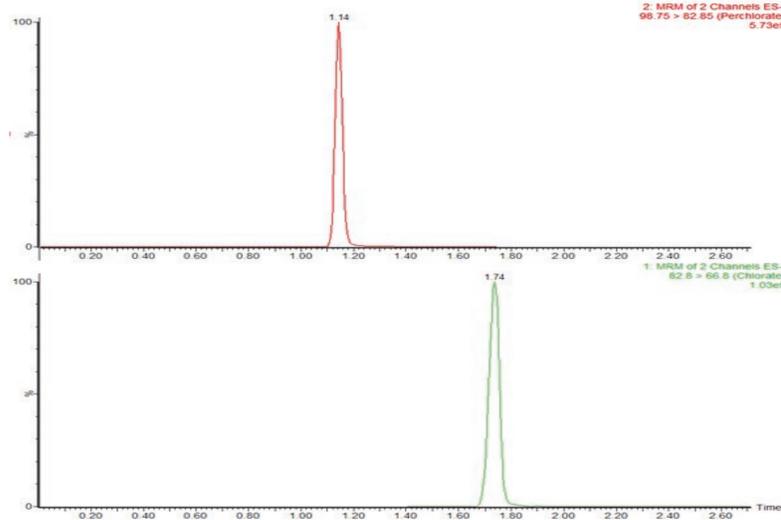


Figure 2: Example of retention and separation of chlorate and perchlorate in infant food (0.010mg/kg) on the TORUS DEA column using the ammonium formate gradient.

Compound name: Perchlorate
Correlation coefficient: $r = 0.999965$, $r^2 = 0.999931$
Calibration curve: $1379.09 \cdot x + 4303.03$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None
Standard Addition Concentration: 3.12019

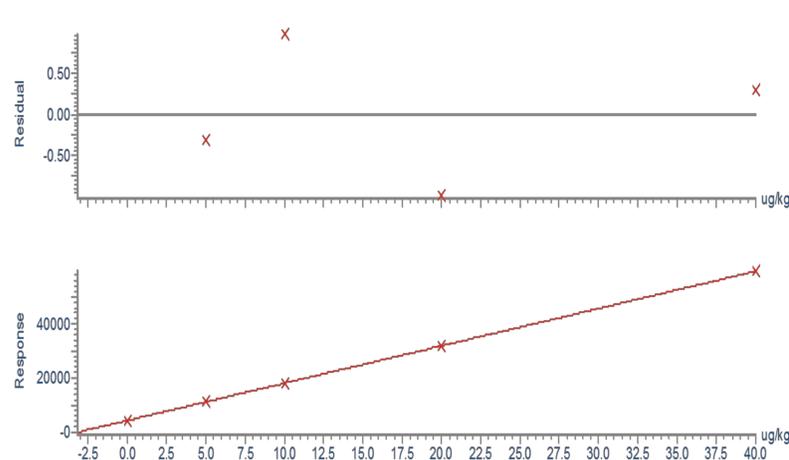


Figure 6: Standard addition plot for the quantification of incurred perchlorate residue in infant food, calculated at a concentration of 0.003 mg/kg (1.5ng/ml in vial).

Calibration was assessed in various matrices and found to be acceptable, to SANTE guidelines 11813/2017. An example of a matrix matched calibration curve for organic carrot can be seen in Figure 3. Response was linear for both compounds over a range of 0.002 to 0.2 mg/kg ($R^2 > 0.995$, residuals < 20%).

Repeatability of the LC-MS/MS method was determined by injecting $n=6$ of the 0.010mg/kg level from the matrix matched curves. The % RSD data from carrot, grape and infant food can be seen in Figure 4.

An incurred residue of perchlorate was quantified in infant food, in the absence of isotopically labelled internal standard, using standard addition calibration within TargetLynx XS.

A chromatogram of an incurred residue of perchlorate is shown in Figure 5, retention time and ion ratio were acceptable to SANTE guidelines 11813/2017. An example of this standard addition plot is shown in Figure 6, where the incurred residue was quantified at 0.003 mg/kg in infant food. All residuals were back calculated against the known added concentration automatically within TargetLynx and all were < 6 %.

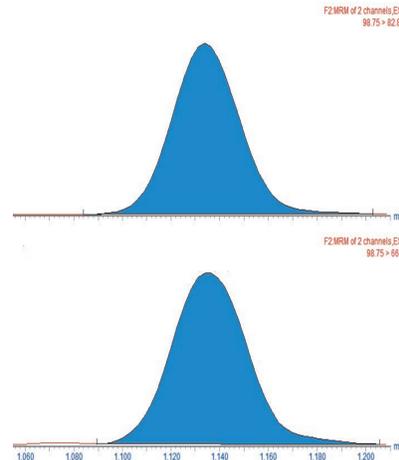


Figure 5: MRM Chromatogram of incurred perchlorate in infant food at 0.003mg/kg.

Compound name: Chlorate
Correlation coefficient: $r = 0.999458$, $r^2 = 0.998917$
Calibration curve: $42.9609 \cdot x + 28.3481$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

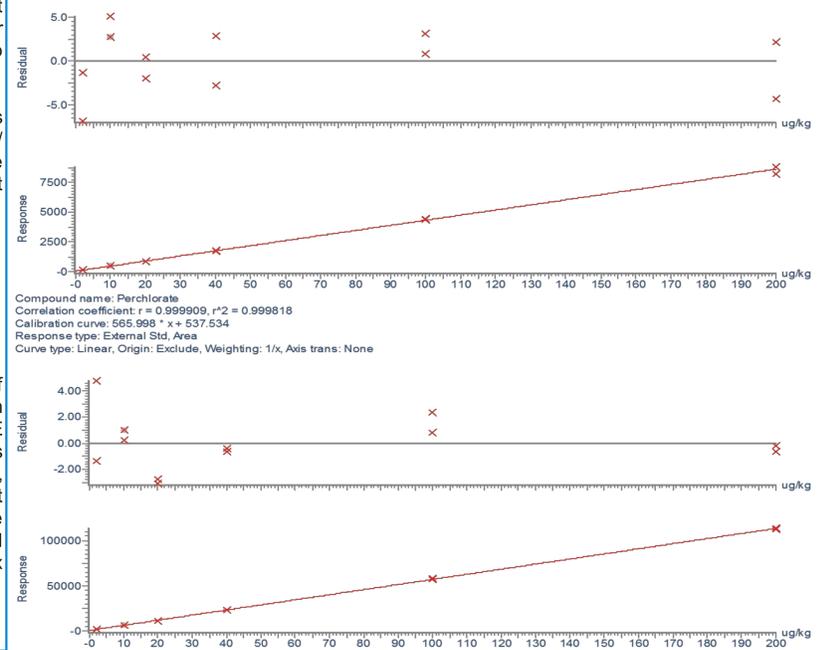


Figure 3: Bracketed matrix matched calibration curve in organic carrot, 0.002mg/kg - 0.2mg/kg (1.0 - 100 ng/ml in vial)

Extracted Matrix, Post Spiked Concentration 0.010mg/kg % RSD N=6		
Commodity	Chlorate	Perchlorate
White Grape	7.6	2.5
Carrot	4.8	2.3
Infant Food	2.1	2.1

Figure 4: Repeatability data for 0.010mg/ml calibration point (5ng/ml in vial).

CONCLUSIONS

- The **TORUS DEA** stationary phase, provided excellent retention, retention time stability and separation for the analysis of chlorate and perchlorate, in the commodities tested by LC-MS/MS.
- Excellent linearity obtained from 0.002mg/kg to 0.200mg/kg in the tested commodities.
- Acceptable repeatability at 0.010mg/kg for all three commodities, with %RSD below 8% without the use of labelled standards.
- An incurred residue of perchlorate in infant food was quantified using standard addition in the absence of labelled standards.
- Limits of quantification and detection below 0.001 mg/kg for each compound are likely to be achieved based on peak to peak signal to noise of the 0.002 mg/kg matrix matched standards.



MORE INFORMATION

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