Utilisation of Ion Chromatography and Tandem Mass Spectrometry (IC/MSMS) for the Quantitative Determination of Highly Polar Anionic Pesticide Residues in Fruit and Vegetables.

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Introduction

SASA is an Official UK laboratory that on behalf of the Scottish Government, participates in statutory UK and EU annual surveillance programmes that monitor UK and imported food & drink for residues of pesticides and their metabolites.

The determination of multiple pesticide residues that could remain in or on our food and drink requires the simultaneous detection, identification and quantitation of hundreds of different pesticides. The variation in chemical and physical properties of individual pesticides and sample matrices combined with ppb residue levels, presents a considerable and relentless challenge for mass spectrometrists and chromatographers.

Highly polar anionic compounds such as chlorate and perchlorate have come under close scrutiny in recent years. The European Food Safety Authority (EFSA) have found that current levels of chlorate in drinking water and in food could negatively impact iodine uptake especially among infants and children and chronic dietary exposure to perchlorate is also of potential concern. These compounds are particularly challenging to analyse since they have poor retention in reversed-phase LC.

This poster presents details of an effective and sensitive ion chromatography and tandem mass spectrometry (IC/MSMS) method developed earlier this year for the quantitative determination of multiple residues of highly polar anionic pesticides (chlorate, ethephon, perchlorate) in various fruit and vegetables (e.g. melon, peas without pods, pineapple). The method validation data and results from the statutory UK/EU Pesticide Residues in Food 2018 surveys and FAPAS proficiency testing scheme quality control samples demonstrate the routine and successful application of the method.

Experimental

Sample preparation

Isotopically labelled internal standards of chlorate $^{18}O_3$ and perchlorate $^{18}O_3$ were added to 10g of cryogenically milled fruit and vegetable samples, shaken with water and methanol and centrifuged at 4000 rpm for 10 minutes (matrix concentration ≡ 0.5 g ml^{-1}). No clean-up was employed. Melon and pea sample extracts were diluted 20 times in water and pineapple sample extracts were diluted 30 times in water and filtered into vials for IC/MSMS (0.2 µm polyethersulfone (PES)).
Calibration standards were prepared in appropriate (organic) fruit or vegetable matrix.

IC/MS set-up

Thermo Scientific Integrion HPIC system with eluent generation and electrolytic suppression coupled with a SCIEX 6500 QTRAP mass spectrometer

IC Mobile phase: 18.2 MΩ water with automatic generation of KOH, gradient elution
Analytical Column: Dionex IonPac AS19 4 µm (2 mm x 250 mm) with Dionex IonPac AG19 Guard Column 4 µm (2 mm x 50 mm)
Anion suppressor: ADRS 600 dynamically regenerated electrolytic suppressor (2 mm) operated in constant voltage mode
Flow rate of external water through suppressor: 0.4 mlmin^{-1}
Post column make up solvent (acetonitrile) flow rate: 0.2 mlmin^{-1}
Eluent flow rate: 0.30 mlmin^{-1}
Oven Temperature: 40°C
Injection Volume: 100 µl

Gradient:

Time (min)	Concentration KOH (mM)	Curve
Equilibration -8	35	5
8	35	5
10	80	8
20	80	5

Stop Time (min): 26
MS Acquisition: Electrospray, negative ionisation, multiple reaction monitoring (MRM).

Collision gas: Nitrogen

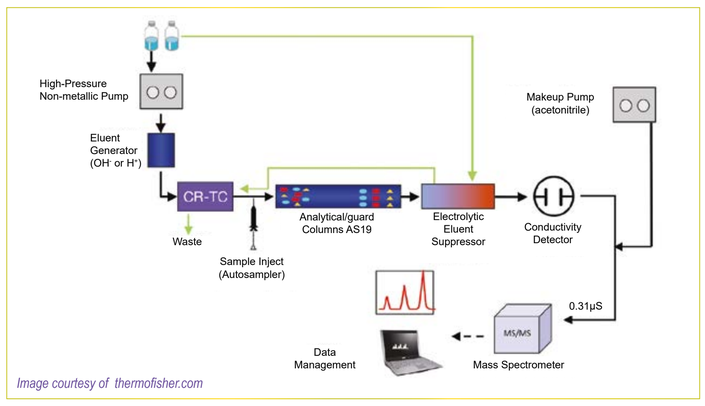
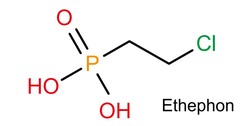
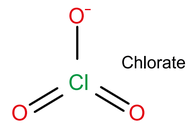


Figure 1. IC/MSMS set-up. * Continuously Regenerated Trap Column (CR-TC)





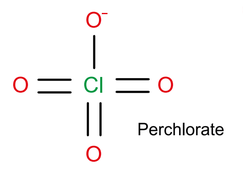


Table 1. MS/MS parameters

Compound	Transition Type	Precursor (m/z)	Product (m/z)	Declustering Potential (DP) V	Collision Energy (CE) V	Collision Exit Potential (CXP) V
Chlorate	Quantifier	82.9	66.9	-105	--28	-9
	Qualifier 1	82.9	50.8	-105	-42	-5
	Qualifier 2	84.9	52.6	-90	-38	-23
	Qualifier 3	84.9	68.7	-90	-28	-9
Chlorate IS $^{18}O_3$	Quantifier	89	71	-70	-32	-1
Ethephon	Quantifier	143	107.1	-40	-12	-7
	Qualifier 1	143	78.7	-40	-24	-9
	Qualifier 2	145	107.1	-40	-12	-7
	Qualifier 3	145	78.7	-40	-24	-9
Perchlorate	Quantifier	98.8	50.9	-110	-62	-7
	Qualifier 1	98.8	66.8	-110	-50	-9
	Qualifier 2	98.8	82.9	-110	-34	-9
Perchlorate IS $^{18}O_3$	Quantifier	145	78.7	-200	-38	-13

Results

Validation data and results must comply with the requirements of European Commission guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed (SANTE/11813/2017).

Validation data presented in Table 2 satisfy these requirements.

Table 2. 2018 Melon, pea and pineapple validation data

Pesticide	Reporting level (RL) mgkg^{-1}	Fortification level RL (n=6)						Fortification level 2RL (n=6)					
		Melon		Pea		Pineapple		Melon		Pea		Pineapple	
		Mean recovery %	% RSD	Mean recovery %	% RSD	Mean recovery %	% RSD	Mean recovery %	% RSD	Mean recovery %	% RSD	Mean recovery %	% RSD
Chlorate	0.01	93	3	98	4	102	6	100	3	101	6	104	7
Ethephon	0.05	98	10	102	5	99	9	96	6	100	3	98	10
Perchlorate	0.01	107	8	101	6	103	4	109	14	98	8	100	5

The chromatogram in Figure 2 shows very good signal to noise for a matrix-matched standard in pea at the lowest calibration level equivalent to 2.5 ppb chlorate and perchlorate and 12.5 ppb ethephon.

The method was used to analyse a FAPAS proficiency testing scheme quality control material on ethephon in pineapple. The results from extraction and analysis in two separate batches were within the acceptable range specified on the material data sheet (Table 3).

Table 3. Results of FAPAS Pineapple QC material (T19239QC)

Ethephon result (µgkg^{-1}) in FAPAS Pineapple QC material		Assigned value (µgkg^{-1})	Range for acceptable z-score (|z| ≤ 2)
Batch 1	Batch 2		
621	654	788	526 - 1050

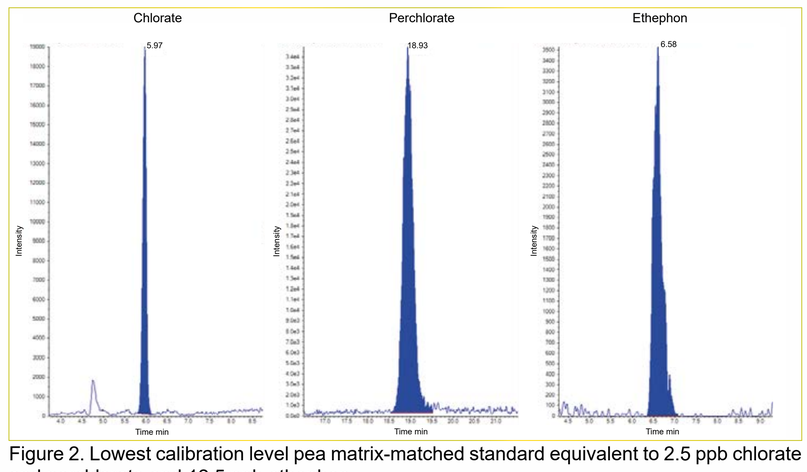


Figure 2. Lowest calibration level pea matrix-matched standard equivalent to 2.5 ppb chlorate and perchlorate and 12.5 ppb ethephon

To date the method has been successfully applied to statutory UK/EU samples of melon (60), peas (36) and pineapple (48) in 2018. In quarter one, 24% of samples had positive findings of chlorate, ethephon or perchlorate in the range 0.010 to 0.13 mgkg^{-1}.

Results obtained have been submitted to the UK regulatory authority and are awaiting official publication in September 2018 (www.hse.gov.uk/pesticides) and therefore cannot be presented here.

Conclusions

This effective and sensitive IC/MSMS method and experimental set-up has enabled quantification of three highly polar pesticides of interest in 2018 statutory surveys of fruit and vegetables. Validation data and analysis of a FAPAS QC material were within the required range. Following significant optimisation of the IC to MS hyphenation it has been very easy to switch between routine LC/MSMS and IC/MSMS assays on the same mass spectrometer. Further work on this method will be undertaken to validate other food commodities and to include more anionic pesticides of interest and their metabolites such as fosetyl aluminium, phosphonic acid, glyphosate, aminomethylphosphonic acid (AMPA), N-acetylglyphosate and maleic hydrazide.

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