POSTER NOTE

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The analysis of polar ionic pesticides by ion-exchange chromatography tandem mass spectrometry

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INTRODUCTION

Polar ionic pesticides, such as glyphosate, chlorate, and perchlorate, often occur as residues in food, but are not always included in pesticide monitoring programs, simply because they are not 'amenable' to generic multi-residue methods. The introduction of the Quick Polar Pesticides (QuPPe) Method by the European Reference Laboratory for single residue methods (EURL-SRM) has enabled more laboratories to conduct analysis for at least some of the polar pesticides. Still, the absence of a liquid partitioning step, or clean-up step, results in 'dirty extracts' containing high concentrations of matrix co-extractives. Thus, the separation and accurate quantification of analytes in QuPPe extracts is challenging. Analysts attempt to mitigate these issues by analyzing a single extract a number of times, using different chromatographic columns and conditions. These separation conditions are often less than ideal and the large amounts of co-extractives often contaminate the low capacity columns to cause variation in retention time and a decrease in the ruggedness of the method.

The application of high resolution ion-exchange chromatography, coupled to a triple quadrupole mass spectrometer can overcome the issues experienced with other chromatographic techniques.

MATERIALS AND METHODS

Sample Preparation:
QuPPe extraction method, acidified methanol extraction (1).

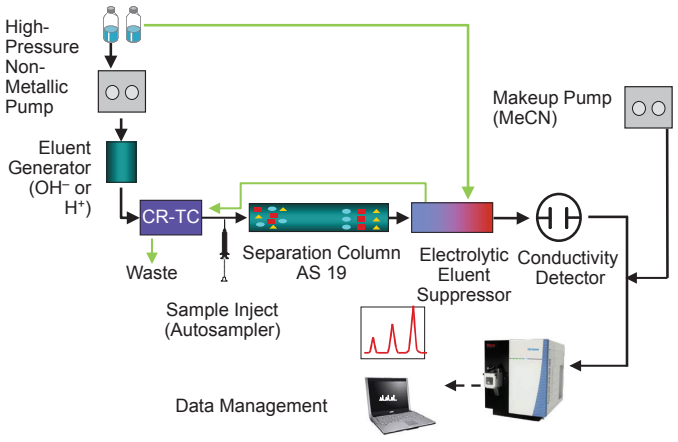
Ion Chromatography:
Thermo Scientific™ Dionex™ ICS-5000⁺ HPIC system with a Thermo Scientific™ Dionex™ IonPac™ AS19-4µm column

Mass Spectrometery:
Thermo Scientific™ TSQ Quantiva™ Triple Quadrupole Mass Spectrometer

Data Analysis Software:
Thermo Scientific™ TraceFinder™ Software

The IC-MS/MS system is shown in **Figure 1.**

Figure 1. IC-MS/MS system configuration.



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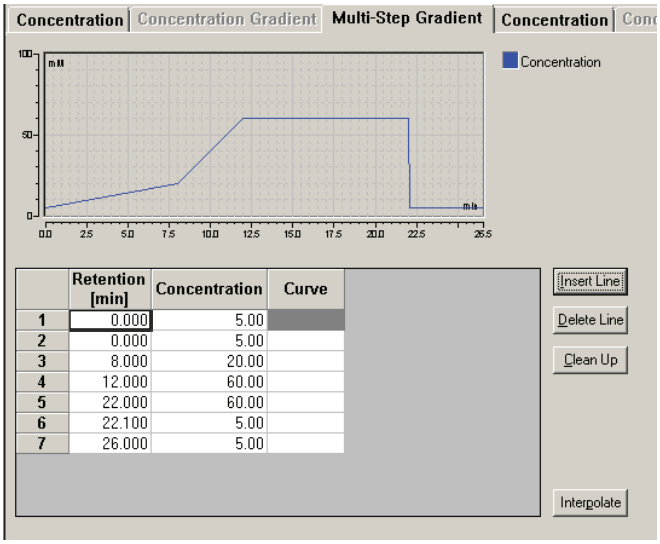
MATERIALS AND METHODS (cont.)

Figure 2. KOH gradient program.



RESULTS AND DISCUSSION

Analysis of Polar Ionic Analytes in Wheat (Flour)

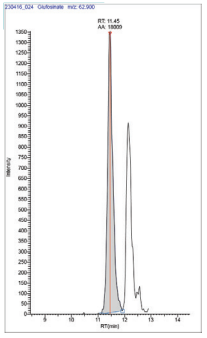
A matrix of wheat (flour) was validated for several polar pesticides including Glyphosate (and metabolites AMPA and N-Acetyl-AMAP) Glufosinate (and metabolites 3-MPPa and N-Acetyl-Glufosinate), Perchlorate, Chlorate and Ethephon, The mean recovery and %RSD for each concentration (five replicate spikes) is shown in **Table 1,** which pass the criteria set out in SANTE (2). Retention time stability of glyphosate is demonstrated during the analytical run as shown in **Figure 3.**

Table 1. Recovery & % RSD from analysis of flour samples (n=5) spiked with polar ionic analytes at 3 concentrations.

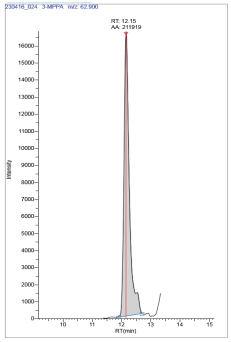
Compound	Concn (µg/kg)	Mean Recovery (n=5)	Mean % RSD
Glyphosate (IS)	10	112	15
	50	108	12
	100	111	7
AMPA	10	92	22
	50	98	13
	100	97	3
N-Aectyl-AMPA (IS)	10	85	7
	50	82	10
	100	86	2
Glufosinate (IS)	10	100	16
	50	109	11
	100	109	8
3-MPPA (IS)	10	106	17
	50	108	13
	100	111	7
N-Acetyl-Glufosinate (IS)	10	88	6
	50	88	9
	100	91	3
Perchlorate (IS)	10	95	6
	50	90	7
	100	92	9
Chlorate (IS)	10	93	5
	50	88	2
	100	87	4
Ethephon (IS)	10	95	11
	50	86	4
	100	85	4
Clopyralid	50	70	5
	100	89	6
Fosetyl Al	200	60	4
	1,000	71	4
	2,000	72	2
Phosphonic acid	200	106	5
	1,000	94	4
	2,000	97	2
Cyanuric acid (IS)	50	75	31
	100	88	13

Selected chromatograms for several of the spiked polar pesticides in flour matrix are shown in **Figure 4.** The MS/MS transition used for quantitation is shown in the upper left hand corner of each extracted ion chromatogram.

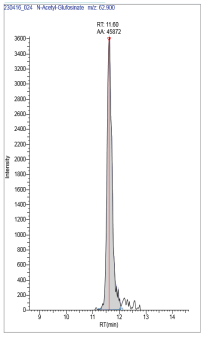
Figure 4. Selected chromatograms for six of the polar pesticides and metabolites spiked into flour matrix at 50 µg/kg.



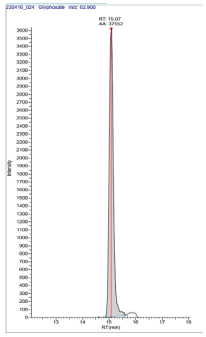
Glufosinate @ 50 µg/kg



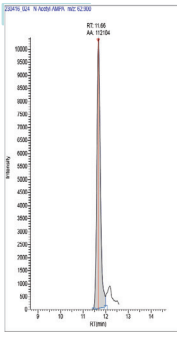
3-MPPA @ 50 µg/kg



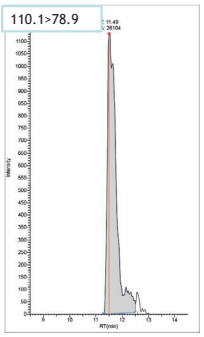
N-Acetyl-Glufosinate @ 50 µg/kg



Glyphosate @ 50 µg/kg



N-Acetyl-AMPA @ 50 µg/kg

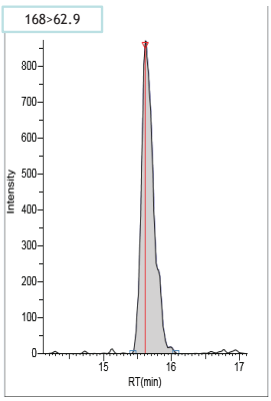


AMPA @ 50 µg/kg

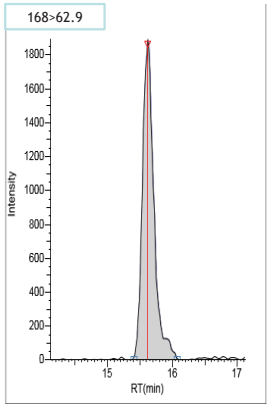
Analysis of Glyphosate in Infant Food

A sample of organic infant food was spiked at 3 different concentrations. The mean recovery and %RSD for each concentration (five replicates is shown in **Table 2**, which are compliant with the criteria set out in SANTE (2) The chromatograms for glyphosate spiked in infant food are shown in **Figure 5**.

Figure 5. Selected chromatograms for glyphosate spiked into infant food matrix.



Glyphosate @ 5 µg/kg



Glyphosate @ 10 µg/kg

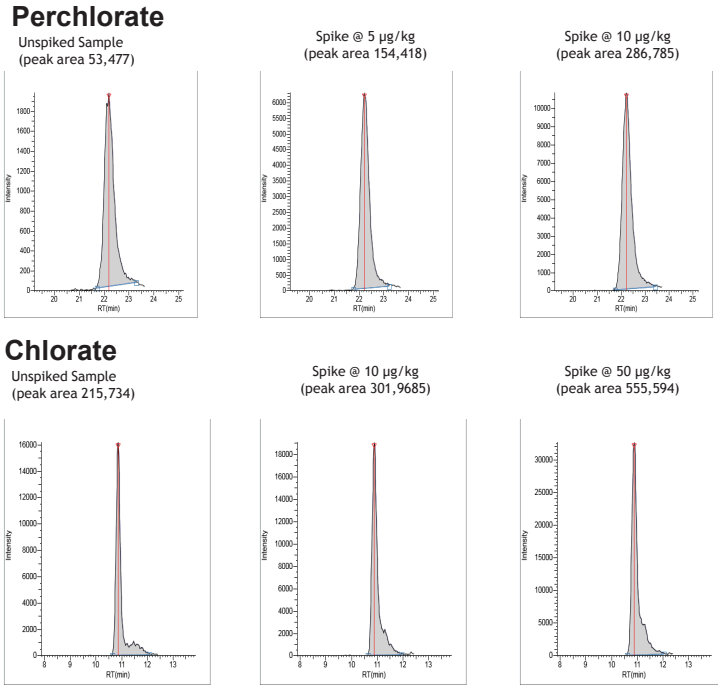
Table 2. Results of analysis of Infant food spiked with glyphosate at 3 different concentrations

Compound	Concn (µg/kg)	Mean Recovery (n=5)	Mean % RSD
Glyphosate (IS)	5	110	5
	10	120	12
	50	102	4

Analysis of Perchlorate and Chlorate in Infant Food

A sample of organic infant food was spiked with perchlorate and chlorate. The unspiked samples were found to contain both perchlorate and chlorate. To determine the amount of perchlorate and chlorate in the infant food, a standard addition curve was constructed. The amount of perchlorate and chlorate calculated in the organic infant food was calculated to be 2 µg/kg and 38 µg/kg, respectively. The chromatograms for perchlorate and chlorate spiked in infant food as well as the unspiked samples are shown in **Figure 6**.

Figure 6. Chromatograms of infant formula for perchlorate and chlorate.



CONCLUSIONS

IC-MS/MS shows good selectivity and sensitivity for analyzing for polar pesticides when combined with the QuPPe extraction method. There is 'good' retention of analytes on the column coupled with 'good' sensitivity and selectivity by using a tandem mass spectrometry for detection. The validation data presented supports that IC-MS/MS is the solution for problematic polar pesticide analysis. The results presented demonstrates that single residue methods can be incorporated into a polar multi-residue method with a suitable extraction method such as the QuPPe. This approach is routinely used for chlorate and perchlorate analysis with plans to incorporate other analytes as required. There are plans to further validate this technique with other polar pesticides in other matrices and to expand the capability and investigate cationic polar pesticides analysis using this approach.

REFERENCES

1. Quick Method for the Analysis of numerous Highly Polar Pesticides in Foods of Plant Origin via LC-MS/MS invoving Simultaneous Extraction with Methanol (QuPPe-Method). Version 8.1. M. Anastassiades, et al. http://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?LabID=200&CntID=1005&Theme_ID=1&Pdf=False&Lang=EN

2. SANTE/11945/2015, Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. http://ec.europa.eu/food/plant/docs/plant_pesticides_mrl_guidelines_wrkdoc_11945_en.pdf

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