The routine analysis polar ionic pesticides by ion chromatography tandem mass spectrometry

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INTRODUCTION

Polar ionic pesticides, such as glyphosate, glufosinate, ethephon, and the like, 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 extracts containing high concentrations of matrix co-extractives, which means 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 overload the low capacity columns causing 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. Using the ion chromatography-tandem mass spectrometry (IC-MS/MS) approach for direct analysis of QuPPe extracts, low limits of quantification (typically < 5 ng/g), and associated repeatability (typically < 20%) have been achieved for glyphosate, and its metabolites AMPA (aminomethylphos-phonic acid) and N-acetyl AMPA), glufosinate (and its metabolites, glufosinate, N-acetyl glufosinate, 3-MPPA (3 methylphosphinico-propionic acid), fosetyl-Al, phosphonic acid, ethephon, chlorate, perchlorate, and more, in a single analysis.

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 Spectrometry:
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.**

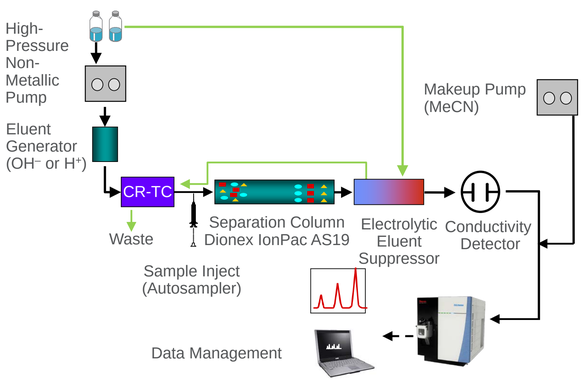


Figure 1. IC-MS/MS System Configuration.

IC Conditions

- Column: Dionex IonPac AS19 250 x 2mm, 4 µm particle size
  - Column flow: 0.35 mL/min
  - Column temp: 40° C
  - Suppressor: Thermo Scientific™ Dionex™ AERS™ 500 Anion Electrolytically Regenerated Supressor, 2mm (in external water mode)
    - External water flow: 0.5 mL/min
    - Suppressor current: 52 mA
  - Eluent: KOH gradient with EGC 500 (see Figure 2).
  - Injection volume: 100 µL
  - Post column MeCN flow (make-up): 0.2 mL/min

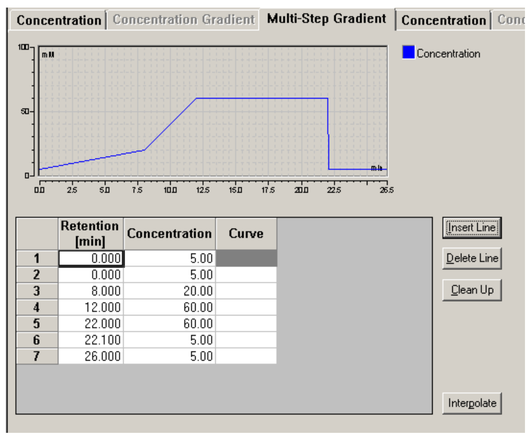


Figure 2. KOH Gradient Program.

RESULTS AND DISCUSSION

Analysis of Polar Pesticides in Wheat (Flour)

A matrix of wheat (flour) was validated for polar pesticides including glyphosate (and metabolites AMPA and N-acetyl-AMPA) glufosinate (and metabolites 3-MPPA and N-acetyl-glufosinate), ethephon, fosetyl, clopyralid, perchlorate, and chlorate. The mean recovery and %RSD for each concentration (five replicate spikes) are shown in **Table 1.** In each case, these pass the criteria set out in the SANTE guidelines (2). Retention time stability of glyphosate during the analytical run is shown in **Figure 3.**

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-Acetyl-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

Table 1. Recovery and %RSD for Pesticides Spiked into Wheat Flour before Extraction.

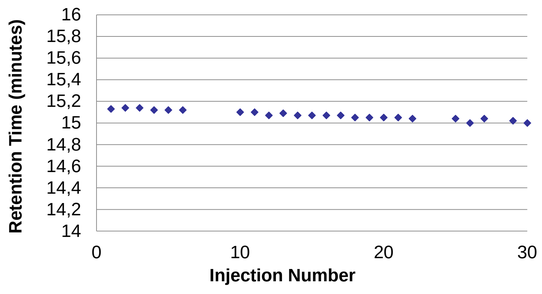


Figure 3. Retention Time Stability of Glyphosate in Wheat Flour During 30 Consecutive Injections.

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.

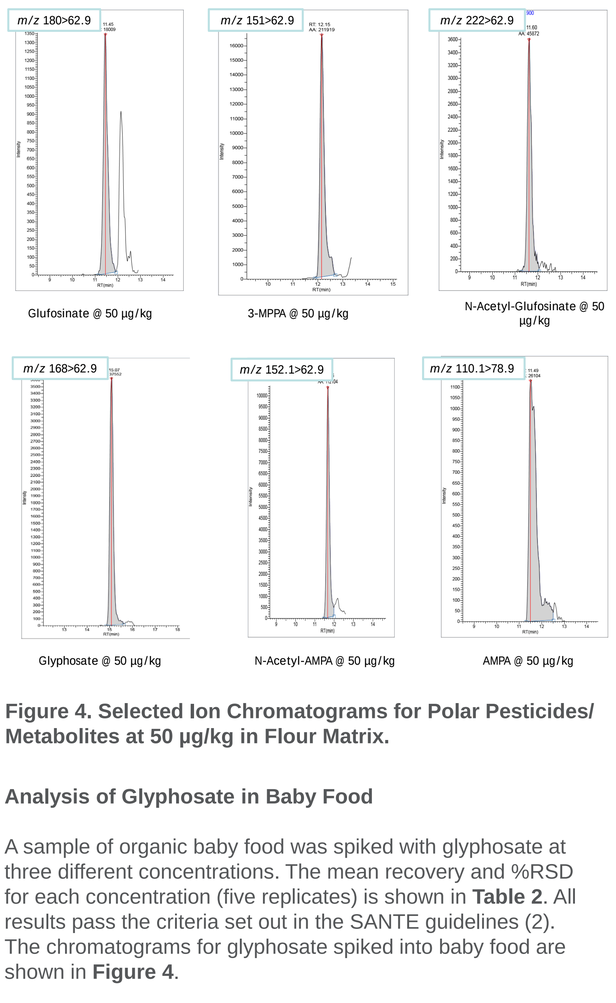


Figure 4. Selected Ion Chromatograms for Polar Pesticides/ Metabolites at 50 µg/kg in Flour Matrix.

Analysis of Glyphosate in Baby Food

A sample of organic baby food was spiked with glyphosate at three different concentrations. The mean recovery and %RSD for each concentration (five replicates) is shown in **Table 2**. All results pass the criteria set out in the SANTE guidelines (2). The chromatograms for glyphosate spiked into baby food are shown in **Figure 4**.

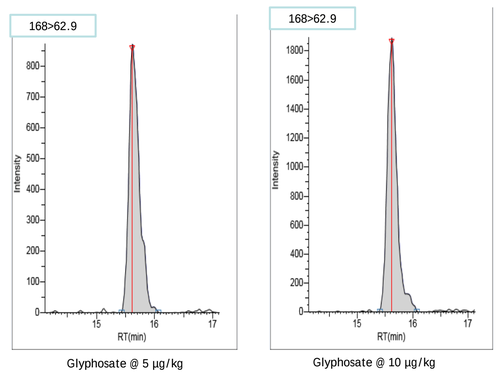


Figure 5. Ion Chromatograms for Glyphosate Spiked into Baby Food.

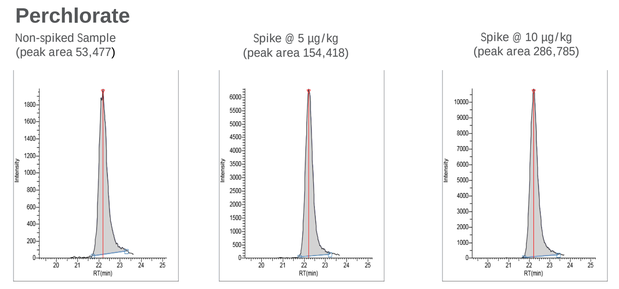
Compound	Concⁿ (µg/kg)	Mean Recovery (n=5)	Mean % RSD
Glyphosate (IS)	5	110	5
	10	120	12
	50	102	4

Table 2. Recovery and %RSD for Glyphosate Spiked into Baby Food.

Analysis of Perchlorate and Chlorate in Infant Formula

A sample of organic infant formula was spiked with perchlorate and chlorate. The non-spiked samples showed the presence of 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 non-spiked samples are shown in Figure 4.

Perchlorate



Chlorate

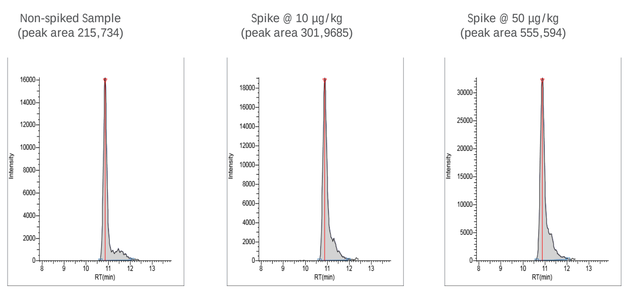


Figure 5. Chromatograms of Non-spiked and Spiked (Perchlorate and Chlorate) Infant Formula.

CONCLUSIONS

IC-MS/MS shows good selectivity and sensitivity for the analysis polar pesticides in QuPPe extracts. There is 'good' retention of analytes on the column coupled with 'good' sensitivity and selectivity when using tandem mass spectrometry for detection. The validation data presented support the idea that IC-MS/MS is the solution for problematic polar pesticide analysis. The results presented demonstrate that single residue methods can be incorporated into a polar multi-residue method if using a generic extraction method such as the QuPPe. 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/11813/2017, Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf

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