

The Multi-Residue Analysis of Polar Anionic Pesticides using IC-MS/MS

Richard J. Fussell¹, Qilei Guo², Yingchen Li², Fausto Pigozzo³ Thermo Fisher Scientific, ¹Hemel Hempstead – UK, ²Beijing – China, ³Milan – Italy,

ABSTRACT

Purpose: This presentation provides information on the development and validation of a new integrated 'sample-to-result' workflow for the reliable and sensitive quantitation of polar anionic pesticides and contaminants in wheat.

Methods: A homogenous sample is extracted by water and methanol. After freezing and centrifugation, the extract is diluted ten fold, cleaned-up and filtered. The IC-MS/MS analysis was performed using a Thermo Scientific™ Dionex™ Integri™ HPIC system coupled to a Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer. The ion chromatography separation column was a Thermo Scientific™ Dionex™ IonPac™ AS19 column maintained at 40 °C. The eluent flow rate was 0.35 mL/min with acetonitrile as a makeup solvent. The KOH eluent was neutralized using a Thermo Scientific™ Dionex™ ADRS 600 suppressor. The injection volume was 25 µL of the extract.

Results: At the optimized sample preparation and chromatographic conditions, the separation of target polar pesticides was rapidly achieved within 21 minutes. Based on the optimization of RF lens, collision energy and ion source parameters, SRM transitions are used for the quantifier and qualifier for each compound. The established quantitative method demonstrates excellent accuracy (recoveries are between 70–120% at 2 spiked levels in wheat flour), excellent repeatability (RSDs are below 20%, n=5), good linearity ($r^2 > 0.995$), and excellent sensitivity that meets the MRL regulations of EU, America, Japan and China. The established IC-MS/MS method provides a robust tool for simultaneous determination of polar anionic pesticides in food with excellent repeatability, reliability, and high sensitivity, which can be used for quality control in the food industry.

INTRODUCTION

The group of polar anionic pesticides include some of the most frequently used pesticides worldwide such as glyphosate, glufosinate and fosetyl-aluminum. Previously, these pesticides were determined individually using special methods involving derivatization or ion pairing agents to overcome unwanted interactions during extraction and chromatographic separation. Due to the complexity of food samples and high polarity of the anionic pesticides, their simultaneous and sensitive analysis is rather challenging. In this study, a new sensitive, fast, and robust analytical method for polar anionic pesticides and metabolites in wheat flour based on a modified QuPPe (Quick Polar Pesticides) extraction procedure coupled to ion chromatography-tandem mass spectrometry (IC-MS/MS) was developed. This development is important because polar anionic pesticides and contaminants such as glyphosate, perchlorate, chlorate and the like, often occur as residues in food; but are not always included in food safety monitoring programs, simply because they are not 'amenable' to more conventional generic multi-analyte methods. The IC-MS/MS workflow approach enables aggregation of separate methods into a single analysis improving productivity, while the high capacity ion exchange columns can withstand higher sample loading enabling the analysis of lower concentrations of polar analytes in the more difficult, but relevant matrices, such as cereals and cereal products.

MATERIALS AND METHODS

Sample Preparation

Extraction of the samples was based on a modification of the EURL-SRM QuPPe Method. Samples of wheat flour obtained from retail sources in Beijing, China, were analyzed as received.

Instruments

Thermo Scientific™ Dionex™ Integri™ HPIC system, Reagent-Free™ IC (RFIC™) model, with CD conductivity detector, and Thermo Scientific™ Dionex™ AS-AP Autosampler

Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer

Thermo Scientific™ Dionex™ IonPac™ AS19-4µm Analytical, 2 × 250 mm column, AG19-4µm Guard, 2 × 50 mm

Data Analysis

Thermo Scientific™ TraceFinder™ software 4.1, Thermo Scientific™ FreeStyle™ 1.5.

The IC-MS/MS configuration is shown in Figure 1.

Figure 1. IC-MS/MS configuration

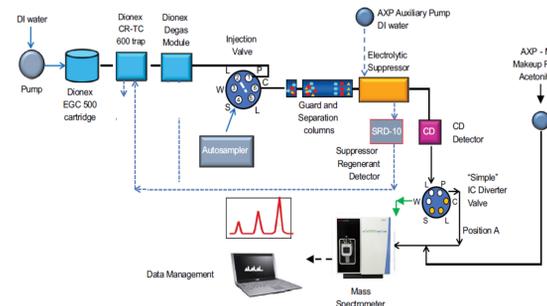
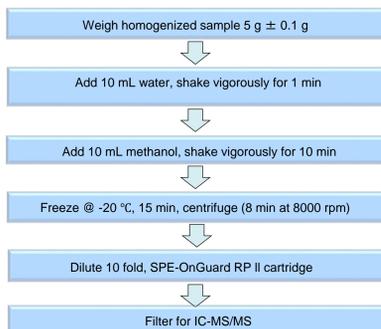


Figure 2. Sample preparation procedure



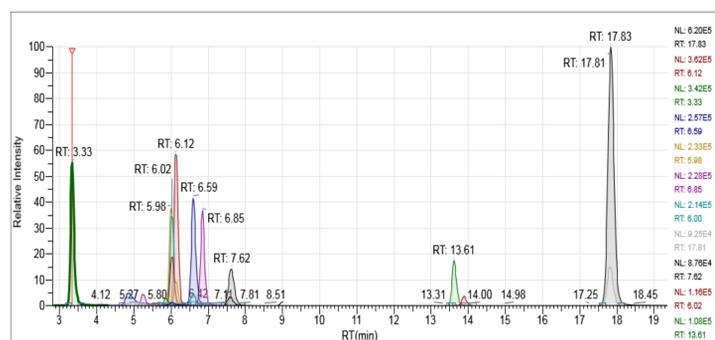
A Thermo Scientific™ Dionex™ ADRS 600 Anion Dynamically Regenerated Suppressor (2 mm) installed after the column converted the KOH to water before the eluent flow entered a conductivity detector and mass spectrometer which were connected in series. The Dionex ADRS 600 suppressor runs in constant current mode (74 mA) and external mode using DI water delivered by an AXP pump for the regeneration. The Thermo Scientific™ SRD-10 device was added between the *Regen Out* on the suppressor and the *Regen In* on the CR-TC. It was programmed to turn off the IC pump when the IC suppressor regenerant flow stops for >5 min. This is to prevent unsuppressed eluent from flowing into the MS. Acetonitrile solvent modifier at 0.23 mL/min is delivered by an auxiliary AXP-MS pump, via a T junction between the conductivity cell and mass spectrometer, to assist with more efficient desolvation and typically increased response for most analytes by 3-4 fold. The injection volume of cleaned-up extract was 25 µL. A Thermo Scientific™ Dionex™ 6-port 2 position valve kit is used to divert unwanted matrix away from the separation columns and MS.

RESULTS

Chromatographic separation and MS/MS response for polar anionic pesticides

A new multi-target HPIC-MS/MS method for the screening and quantitation of polar pesticides was developed using Dionex Integri™ HPIC system coupled to a TSQ Altis™ Triple Quadrupole Mass Spectrometer. Due to the large number of analytes and the expected matrix load caused by the crude solvent extraction, a total runtime of 21 min with gradient elution program were chosen and a better separation of the analytes was achieved. Figure 3 shows the chromatogram of a calibration sample with 15 analytes (pesticides and metabolites). To minimize the amount of matrix going into the spray chamber, the HPIC flow was diverted to waste for the first 2.5 min of the analysis, and then again from 19 min until the end of the analysis.

Figure 3. Separation of anionic pesticides and metabolites under optimized HPIC-MS/MS conditions: glyphosate (N-acetyl glyphosate, AMPA, N-acetyl AMPA), glufosinate, (N-acetyl glufosinate, MPPA), ethephon (HEPA), fosetyl-aluminium (phosphonic acid), bialophos, cyanuric acid, chlorate, and perchlorate.



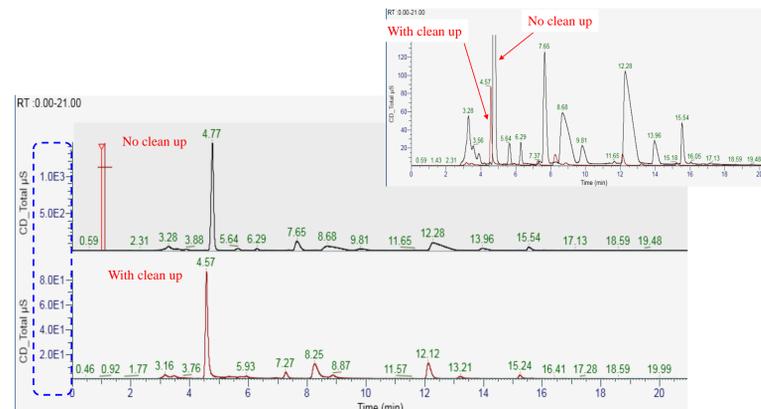
Analytes	RT / min
Fosetyl-AI	3.33
Bialophos	5.25
Glufosinate	5.85
AMPA	5.97
HEPA	5.98
N-acetyl- AMPA	6.00
N-Acetyl-Glufosinate	6.02
Chlorate	6.12
MPPA	6.59
Phosphonic acid	6.85
Ethephon	7.62
Cyanuric acid	13.31
N-Acetyl-Glyphosate	13.61
Glyphosate	13.87
Perchlorate	17.83

Table 1. The retention times of analytes

RESULTS

In QuPPe method, there is no partitioning stage and no clean-up suggested in the extraction method which will result in a high concentration of co-extractives in the final extract. Wheat flour is a representative sample matrix for dry commodities, like cereals. Figure 4 shows the conductivity detector (CD) signal of wheat flour sample extracts without clean-up and with clean-up stage. A freezing step and a reverse phase cartridge clean-up helps to minimize the matrix effects to some extent. At the same time, many laboratories use isotopically labelled internal standards (ILIS) to correct for matrix effects including low recovery of spiked analytes binding to the matrix during extraction.

Figure 4. CD signals of wheat flour sample extract with or without clean-up procedure.



Native standards of 15 analytes and seven isotopically labelled internal standards (ILIS): AMPA, chlorate, perchlorate, ethephon, glyphosate, glufosinate and MPPA, were added to cleaned-up matrix-extracts and to samples before extraction to correct for matrix effects. Improved recovery results were achieved for some compounds using ILIS compared to matrix-matched standards without ILIS. Since ILIS are not readily available in all laboratories the use of procedural standards (samples spiked with a range of known concentrations of native pesticides before extraction) were evaluated. Table 2 shows the result of recoveries and RSD in wheat flour with matrix-matched standards and procedural standards with or without ILIS. In our preliminary findings, the results between wheat flour samples from different sources spiked with native standards (no ILIS) can be variable and therefore laboratories need to be aware of the possibility of less accurate results for incurred residues.

Table 2. The results of recovery and repeatability, obtained using different calibration approaches for anionic pesticides spiked at 10 ng/g in wheat flour samples

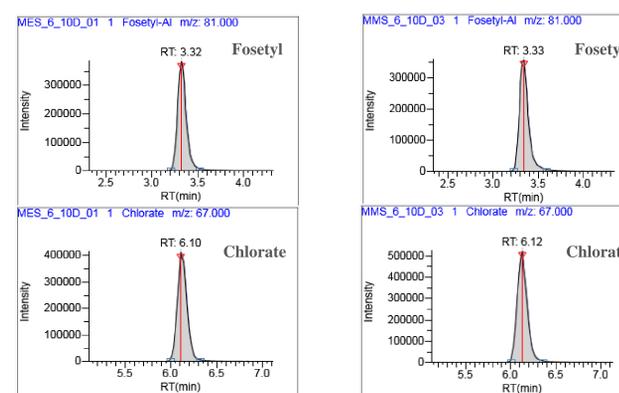
Analytes	Spiked level =10 ng/g (n=5)				Spiked level 10 ng/g (n=3) Procedural calib (different sample matrices) No ILIS Av App Rec%
	Matrix-Matched Calibration (same matrix)		Procedural Calibration (same sample matrix)		
	No ILIS RSD%	+ILIS Rec.%	No ILIS RSD%	+ ILIS App Rec.%	
AMPA	65 (6.6)	115 (6.1)	104 (6.5)	108 (6.5)	68-85
Chlorate	77 (2.2)	96 (1.7)	100 (2.3)	98 (1.7)	91-109
Ethephon	79 (1.4)	97 (2.1)	100 (1.4)	97 (2.2)	83-89
Glufosinate	85 (12)	92 (8.6)	87 (12.4)	98 (8.7)	41-87
Glyphosate	40 (4.5)	111 (2.2)	104 (5.4)	102 (2.4)	44-143
MPPA	71 (1.0)	96 (1.4)	95 (1.1)	96 (1.4)	62-89
Perchlorate	66 (4.2)	100 (1.0)	90 (5.2)	86 (1.0)	92-133
Cyanuric acid	87 (12)	ILIS NA	95 (12.2)	ILIS NA	67 - 69
Bialophos	96 (6.4)	ILIS NA	95 (5.7)	ILIS NA	58 - 68
Fosetyl-AI	93 (2.7)	ILIS NA	96 (2.7)	ILIS NA	49 - 85
HEPA	86 (2.4)	ILIS NA	96 (2.6)	ILIS NA	80 - 87
N-acetyl- AMPA	85 (1.0)	ILIS NA	98 (1.1)	ILIS NA	79 - 95
N-Acetyl-Glufosinate	79 (2.4)	ILIS NA	87 (2.8)	ILIS NA	68 -94
N-Acetyl-Glyphosate	60 (4.2)	ILIS NA	100 (3.0)	ILIS NA	59 - 87
Phosphonic acid	36 (25)*	ILIS NA	84 (14)	ILIS NA	79 -93

Note: 1) ILIS NA = ILIS not available in the lab conducting validation. 2) poor precision due to contribution from blank

Robustness of IC-MS/MS System

After about 80 injections of wheat flour extracts, the peak shapes, retention time and analyte responses remained stable and the mass spectrometer source remained clean demonstrating the within-batch robustness of the system. Figure 6 show the peak shape and retention time of Fosetyl-AI and chlorate. The biggest change in retention time for the 16 analytes is about 0.09 min.

Figure 6. Stability of peak shape and retention time after a sequence of 89 injections of wheat flour extracts



CONCLUSIONS

The results demonstrate that a workflow based on IC-MS/MS can overcome many of the issues experienced with previous methods reported for the analysis of polar pesticides. The Dionex HPIC is metal free so no problem with metal chelation that has been reported using conventional HPLC systems. Overall the workflow described can increase productivity by aggregating more polar anionic compounds into a single method, and provide greater confidence in the results by full compliance with the EU SANTE/11813/2017 method performance criteria. The workflow is robust and has also been validated for leek and baby food matrices.

REFERENCES

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- 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 (accessed Mar 12, 2019).

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