APPLICATION NOTE

LC-MS/MS analysis of polar pesticides in honey with QuPPe extraction

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Key Words
AMPA, Glyphosate, Hypercarb, polar pesticides, QuPPe, TSQ Quantiva

Goal

To analyze polar pesticide residues in food samples with complex matrices using QuPPe extraction and LC-MS/MS.

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, 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 some of the polar pesticides.[1]

Current regulations set maximum residue limits (MRLs) for polar pesticides in food and beverage samples to range from 10 µg/kg for food intended for consumption by children to hundreds of mg/kg in other matrices.[2] Their polarity does not allow direct analysis by reverse-phase HPLC, so alternative methods and workflows are necessary for successful analysis and sensitive quantitation of polar pesticides.

The aim of this work is to develop a sensitive, robust, fast method to determine and quantify residues of polar pesticides in food samples in complex matrices using an LC-MS/MS-based method employing separation on the Thermo Scientific™ Hypercarb™ column.

Experimental

Sample preparation

Samples of honey were extracted using the QuPPe procedure.[2]

LC-MS conditions

LC-MS analysis was performed using a Thermo Scientific™ UltiMate™ 3400 RSLC system in combination with a Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer. The system is shown in Figure 1. The LC conditions and gradient are shown in Tables 1 and 2. The mass spectrometer settings are provided in Table 3 and the SRM transitions are listed in Table 4.

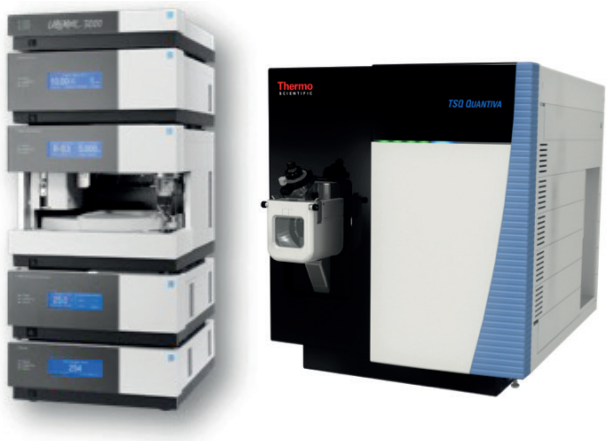

Figure 1. UHPLC-MS/MS system.

Table 1. LC conditions.

Column	Hypercarb 2.1 x 100 mm, 5 µm
Guard Column	Hypercarb 2.1 x 10 mm, 5 µm
Eluent	A: 1% acetic acid in water + 5% methanol B: 1% acetic acid in methanol
Injection volume	5 µL
Column Temperature	40 °C

Table 2. Gradient conditions.

%A	Flow (mL/min)	Time (min)
100	0.2	0
70	0.2	10
70	0.4	11
70	0.4	18
10	0.4	19
10	0.4	22
100	0.2	22.1
100	0.2	30

Table 3. Mass spectrometer settings.

Ionization mode	Heated electrospray (H-ESI)
Scan type	SRM
Polarity	Negative ion mode
Spray voltage	2750 V
Sheath gas pressure	55 arb
Aux gas pressure	8 arb
Ion sweep gas pressure	2 arb
Capillary temperature	325 °C
Vaporizer temperature	450 °C
Cycle time	0.8 s
Q1/Q3 resolution (FWHM)	0.7
Collision gas pressure (CID)	1.5 mTorr
Chrom filter	3 s

Table 4. SRM transitions used in the method.

Compound	Polarity	Precursor (*m/z*)	Product (*m/z*)	CE	RF lens (V)
AMPA	Neg	110.1	63	25	70
	Neg	110.1	79	27	70
	Neg	110.1	81	14	70
Chlorate	Neg	83	67	21	80
	Neg	85	69	21	80
Ethephon	Neg	143.1	79	17	40
	Neg	143.1	107	8	40
	Neg	145.1	107	10	40
Fosetyl Aluminium	Neg	109.1	63	28	55
	Neg	109.1	81	13	55
Glufosinate	Neg	180.1	63	40	85
	Neg	180.1	85	20	85
	Neg	180.1	95	18	85
	Neg	180.1	136	18	85
Glyphosate	Neg	168	63	24	70
	Neg	168	81	17	70
	Neg	168	124	12	70
	Neg	168	150	10	70
Maleic Hydrazide	Neg	111.1	42	43	65
	Neg	111.1	55	16	65
	Neg	111.1	82	18	65
	Neg	111.1	83	12	65
MPPA	Neg	151.1	63	35	70
	Neg	151.1	107	17	70
	Neg	151.1	133	14	70
Perchlorate	Neg	99	83	25	90
	Neg	101	85	25	90
Phosphonic Acid	Neg	81	63	25	60
	Neg	81	79	15	60

Mass calibration - Extended Mass Range solution (EMRS)

Because the target analytes are small molecules with product ions after fragmentation < 100 Da, it is recommended that the mass spectrometer be calibrated with Thermo Scientific™ Pierce™ triple quadrupole, EMR calibration solution. This calibration solution consists of 14 compounds (mass range from 69 *m/z* to 2800 *m/z*) that are suitable for calibration in both positive and negative ionization modes.This solution improves mass accuracy compared to conventional calibration solutions containing only three components (polytyrosines) in the lower segment of the mass range (181 *m/z* to 996 *m/z*).

Data analysis software

Thermo Scientific™ TraceFinder™ software was used for data analysis.

Results and discussion

The objective of this study was to develop a sensitive, robust LC-MS/MS-based approach for fast routine analysis of polar pesticides in food extracts. The results are summarized below.

Limits of detection (LOD) and quantification (LOQ)

Limits of quantification were determined as the lowest calibration level with < 20% RSD obtained. Limits of detection were estimated as the lowest concentration level with peak detectable with signal-to-noise ratio S/N > 3. As shown in Table 5, reported LOQs in neat solvent (methanol) allow sub ppb determination of most of the analyzed compounds.

However, one major drawback of the QuPPe method is the presence of high amounts of matrix co-extractives and poor retention of some compounds on the Hypercarb column. As a result, higher LOQs are observed in matrix samples when compared to neat solvent standards due to ion suppression. Despite this limitation, the method complies with currently valid pesticide MRLs defined by the EU, and allows the use of the method in routine food monitoring for the tested pesticides and matrices (Table 6).

Figure 2 shows the linear calibration range obtained in the working range from 5 to 500 µg/kg in honey extracts. Figure 3 then demonstrates the chromatographic separation of glyphosate and its main metabolite AMPA, allowing the acquisition of both quantifier and qualifier ion transitions.

Table 5. Instrumental detection and quantitation limits for neat solvent.

Compound	LOD (pg on column)	LOQ (pg on column)	LOD (µg/L)	LOQ (µg/L)
AMPA	100	250	0.2	0.5
Chlorate	50	100	0.1	0.2
Ethephon	100	250	0.2	0.5
Fosetyl Aluminium	5000	25,000	10	50
Glufosinate	1000	2500	2	5
Glyphosate	2500	5000	5	10
Maleic Hydrazide	5000	25,000	10	50
MPPA	2500	5000	5	10
Perchlorate	100	250	0.2	0.5
Phosphonic Acid	100	250	0.2	0.5

Table 6. Limits of detection and quantification of the method (LOD and LOQ) obtained for spiked honey extracts.

Compound	LOD (pg on column)	LOQ (pg on column)	LOD (µg/kg)	LOQ (µg/kg)
AMPA	25	50	5	10
Chlorate	25	50	5	10
Ethephon	50	250	10	50
Fosetyl Aluminium	50	50	10	10
Glufosinate	10	25	2	5
Glyphosate	10	25	2	5
Maleic Hydrazide	250	500	50	100
MPPA	500	2500	100	500
Perchlorate	Not spiked in sample			
Phosphonic Acid	25	25	5	5

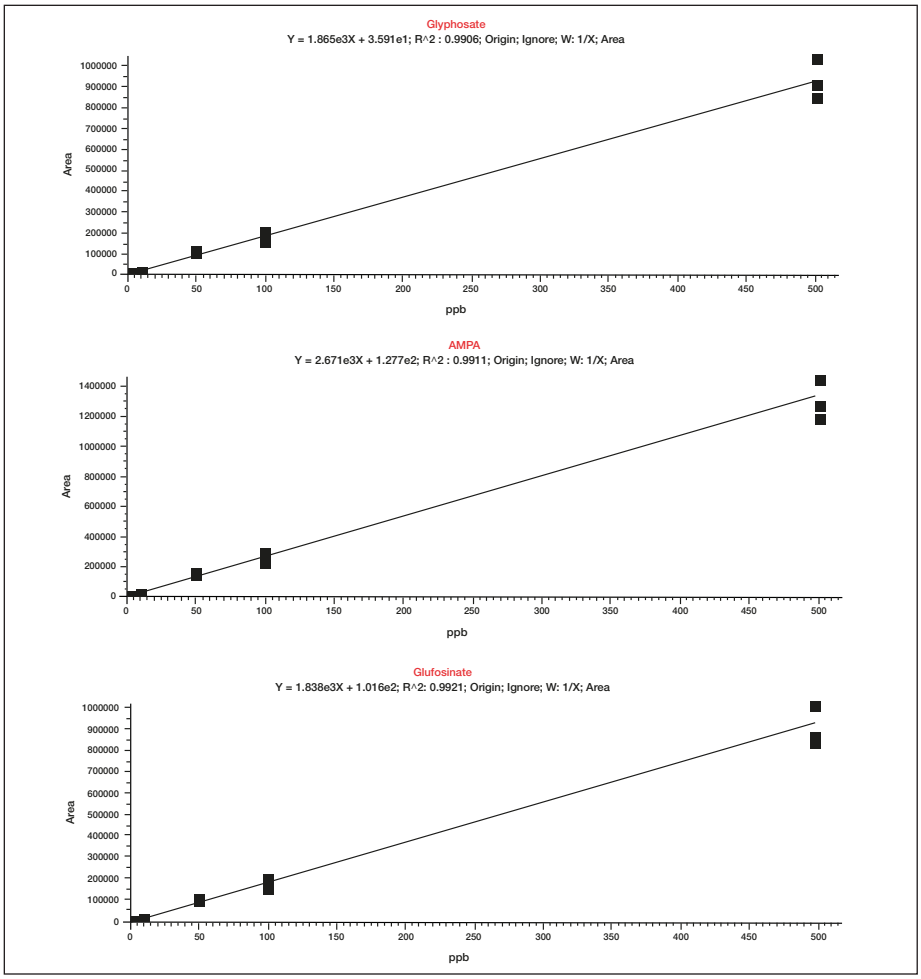

Figure 2. Calibration curve of glyphosate, AMPA, and glufosinate in honey matrix.

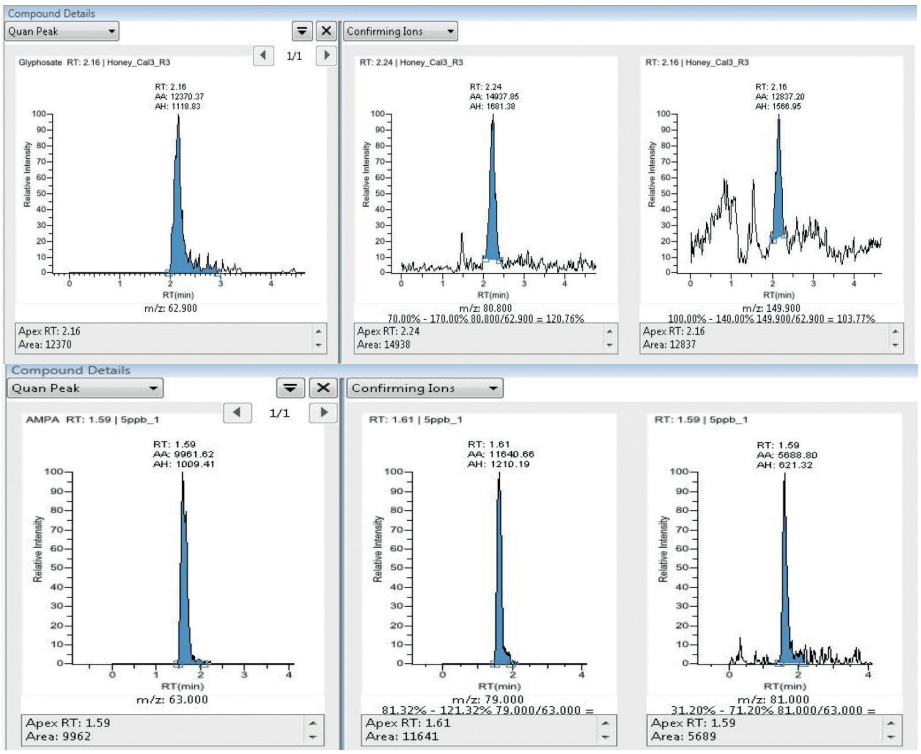

Figure 3. Quantitative and confirming ions of glyphosate (top) and AMPA (bottom) at concentration levels of 5 µg/kg in honey matrix.

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Robustness of the system

The robustness of the TSQ Quantiva LC-MS/MS system was tested by analyzing 500 honey samples in a sequence without any interruption. Figure 4 shows the contamination of the sweep cone of the MS system after analysis of 500 samples and after cleaning the cone by flushing with hot water and sonication with water/methanol (1:1) for 10 min. It is important to mention that even after 500 injections, the ion transfer tube capillary was not clogged and the system performance met all expectations. Cleaning the ion introduction system can be performed without breaking the vacuum of the system, which saves time.

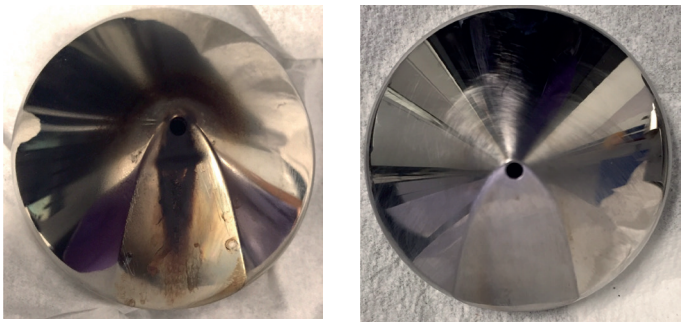

Figure 4. Contamination of sweep cone after 500 honey samples injected (left) and after cleaning procedure (right).

Conclusion

This study reported has demonstrated the applicability of the TSQ Quantiva LC-MS/MS system to analyze the QuPPe method extracts in complex matrices, such as honey, at the required regulatory levels. In addition to the easily achieved LOQs, the study demonstrated robustness of the system, allowing uninterrupted analysis of up to 500 honey samples and easy cleaning of the ion introduction system.

References

1. http://quppe.eu/ (Accessed November 2016)
2. Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC Text with EEA relevance. http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN (Accessed November 2016)

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