APPLICATION NOTE

Fast routine analysis of polar pesticides in foods by suppressed ion chromatography and mass spectrometry

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Key Words

Glyphosate, AMPA, polar pesticides, pesticide residues, IC-MS, TSQ Endura, Integrion

Goal

To develop and test an IC-MS/MS multi-residue method that can be applied for high-throughput screening and quantitation of polar pesticide residues and their metabolites in food matrices below the current legislative requirements.

Introduction

The presence of very polar ionic pesticides in surface and drinking water, as well as food and beverages, has become a controversial issue in recent years. The development of genetically modified crops tolerant to glyphosate and glufosinate, for example, promoted the use of these broad spectrum herbicides.

In addition, glyphosate is used as a crop desiccant to suppress weeds in parks and at roadsides. Consequently, these pesticides often occur in foods as residues and in the environment as contaminants of surface waters. There are concerns about their potential adverse effects on human health, such as their potential carcinogenicity,[1] although the latest toxicological assessments do not predict risks for humans under normal conditions or environmental exposures.[2] Current regulations offset maximum residue levels (MRLs) of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) at 100 ng/L in drinking water. In food and beverage samples, higher MRLs typically apply, ranging generally from 10 µg/kg for food intended for consumption by children up to hundreds of mg/kg in other matrices.[3]

The analysis of glyphosate and other polar compounds presents a difficult analytical challenge. Their polarity does not allow the direct analysis by reversed-phase HPLC, so alternative methods need to be applied. Derivatization of glyphosate prior to analysis[4] or application of specific chromatographic columns, such as the Thermo Scientific™ Hypercarb™ column, are the common approaches.[5]

With both of these approaches, poor method robustness and questionable results are often reported in laboratories, especially when the method is applied in routine high-throughput analysis of samples with rather complex matrix composition.

Recent developments in ion chromatography and mass spectrometry offer many advantages for the analysis of very polar substances. Ion chromatography is the preferred separation technique for polar ionic analytes, such as anions, cations or small polar analytes (metabolites), and sugars. Mass spectrometry, namely in triple quadrupole MS/MS systems, offers very low detection limits and high detection selectivity when operated in selected reaction monitoring (SRM) mode. The system robustness allows the analysis of food and environmental samples.

The aim of this work is to develop and validate an IC-MS/MS method for direct analysis of polar ionic pesticides in food samples and to assess its applicability under routine conditions.

Experimental

Sample preparation

For sample preparation, an optimized method was used that was developed by the EU Reference Laboratory for Residues of Pesticides in Stuttgart, Germany.[5] Since a different chromatographic technique was used, a small change improving the efficiency was implemented. A different volume of non-acidified cold methanol was used for sample extraction.

Homogenized samples of lettuce, oranges, and wheat flour were extracted first with water and then with cold methanol. The sample extracts were then centrifuged and, after filtration through syringe filters, were injected into the IC-MS/MS system (Figure 1). Plasticware was used instead of glassware to minimize losses of polar pesticides by adsorption onto glass.

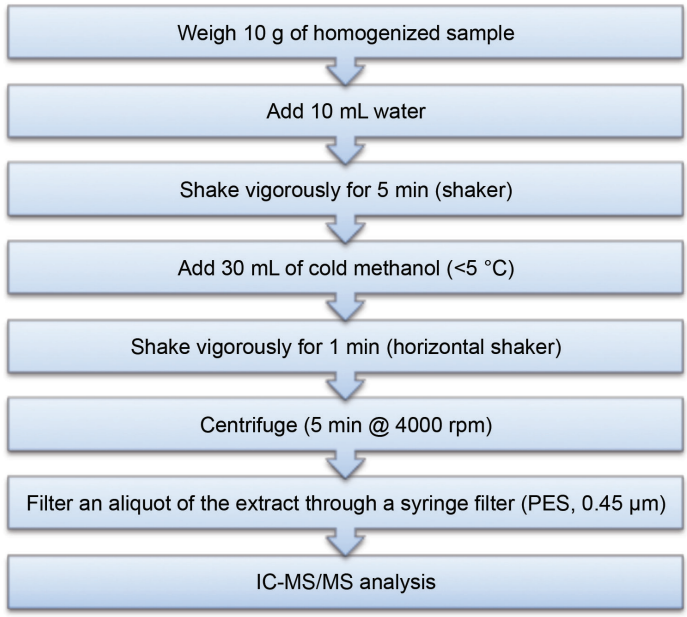

Figure 1. Schematic of method.

Chemicals (Fisher Scientific product numbers valid for Germany)	
Deionized water (Thermo Scientific™ Barnstead™ EASYpure™ II water system)	11337021
Methanol (99.9% purity, LC/MS grade, Fisher Chemical™, Optima™)	10767665
Methanol (99.9 % purity, HPLC grade, Fisher Chemical)	10675112
Thermo Scientific™ Pierce™ Triple Quadrupole Calibration Solution, ext. mass range	88340

Apparatus	
ULTRA-TURRAX® High speed blender	13326309
ULTRA-TURRAX Rotor/Stator - Dispensing tool	10400253
ULTRA-TURRAX Plug-in coupling (dispersing element)	10748201
Waring® laboratory blender with timer	11972919
Fisherbrand™ Compact balance	15335103
Sartorius™ analytical balance	15294638

Instruments	
Thermo Scientific™ TSQ Endura™ Triple Quadrupole MS	TSQ-50003
Thermo Scientific™ Dionex™ Integrion™ HPIC™ System	22153-60208
Thermo Scientific™ Dionex™ EGC KOH Eluent Generator	075778
Thermo Scientific™ Dionex™ AERS™ 500 Anion Electrolytically Regenerated Suppressor 500 – 2 mm	082541
Thermo Scientific™ Dionex™ AS-AP Autosampler	074926
Thermo Scientific™ Dionex™ CR-ATC 600	088662
Thermo Scientific™ Dionex™ AXP-MS Auxiliary pump (make-up flow)	60684
Thermo Scientific™ Dionex™ AXP-MS Auxiliary pump (AERS regeneration)	60684
Thermo Scientific™ Heraeus™ Multifuge™ X3 Centrifuge	10254304

Automatic Pipettes	
Thermo Scientific™ Finnpipette™ Novus Electronic 1–10 µL	11770715
Thermo Scientific Finnpipette Novus Electronic 10–100 µL	11766914
Thermo Scientific Finnpipette Novus Electronic 30–300 µL	11776914
Thermo Scientific Finnpipette Novus Electronic 100–1000 µL	11786914
Thermo Scientific Finnpipette Novus Electronic 0.5–5 mL	11786914
Thermo Scientific Finnpipette Novus Electronic 100–1000 µL	11796914
Thermo Scientific™ Finnpipette™ F1 1000–10,000 µL	11837401

Consumables	
Thermo Scientific™ Dionex™ IonPac™ AS24 Analytical column (2 x 250 mm)	064153
Thermo Scientific Dionex IonPac AG24 Guard column (2 x 50 mm)	064151

Pesticide standard	
Ethephon	Sigma-Aldrich®; P/N: 45473
HEPA (2-hydroxyethylphosphonic acid)	LGC Standards®; P/N: CA13230200
Glufosinate	Sigma-Aldrich®; P/N: 45520
N-Acetyl-glufosinate	LGC Standards®; P/N: CA14031500
MPPA (3-Methylphosphinicopropionic acid)	LGC Standards®; P/N: XA15141200AL
Glyphosate	Sigma-Aldrich®; P/N: 45521
AMPA (aminomethylphosphonic acid)	Sigma-Aldrich®; P/N: 324817
Phosphonic acid (phosphorous acid)	Sigma-Aldrich®; P/N: 389025000
N-Acetyl-AMPA (N-Acetyl-aminomethylphosphonic acid)	LGC Standards®; P/N: DRE-C10205150
Fosetyl-Al (Fosetyl-aluminium)	LGC Standards®; P/N: CA13940000

Proficiency test material	
T19186 red grape purée	FAPAS®; P/N: T19186QC

Preparation of standards

Stock standard solutions

Stock standard solutions were prepared individually by dissolving the analytes in methanol, acidified methanol, water, acetonitrile, or mixtures. Table 1 shows the used solvents and concentrations in the stock solutions. Plastic flasks and stoppers were used for preparation of stock solutions of glyphosate and AMPA, compounds that tend to interact with glass surfaces. Solutions were stored at -20 °C. The frozen solution was allowed to thaw at room temperature before further dilutions were made.

Table 1. Stock standard solutions.

Standard	Solvent	c (µg/mL)
Ethephon	CH_3OH + 1% formic acid	1000
HEPA	CH_3OH	1000
Glufosinate	H_2O/CH_3OH (2:1)	1000
N-Acetyl-Glufosinate	CH_3OH	1000
MPPA*	ACN	10
Glyphosate	H_2O	1000
AMPA	H_2O	100
Phosphonic acid	H_2O	1000
N-Acetyl-AMPA	H_2O	100
Fosetyl-Al	H_2O/CH_3OH (3:1)	100

*MPPA was purchased as the ready standard solution in acetonitrile with concentration 10 µg/mL.

Working standard solution

The working standard solution of 10 compounds (c = 1 mg/L) was prepared by diluting individual stock standard solutions into water. The solution should be prepared freshly every time before use. The solution stored in a plastic tube was used for the spiking of samples in recovery experiments and for the preparation of calibration standards.

Procedure

Sample preparation

Homogenization

A representative amount (100–150 g) of the sample (lettuce, orange, or flour) was homogenized in the blender. For fruits and vegetables, cryogenic milling (e.g. using dry ice) is preferred to minimize degradation, reduce particle size, and to improve homogeneity as well as residue accessibility. However, the classic blender was used in our work because no adverse effects were observed. For dry commodities (e.g. flour), fine grinding is recommended (e.g. particle size < 500 µm).

Extraction and filtration

Homogenized sample (10 g) was accurately (+/- 0.01 g) weighed into a 50 mL plastic centrifuge tube. Then, 10 mL of water was added and the tube was shaken vigorously for 5 minutes on the horizontal shaker. Afterwards, 30 mL of cold methanol (~5 °C) was added, and the sample was again agitated for 1 minute on the shaker. The samples were then centrifuged for 5 minutes at 4000 rpm and 5 °C, filtered (PES, 45 µm), and injected into the IC-MS/MS system. The 2 mL plastic vials were used to avoid possible analyte adsorption to the surface of glass walls, hence improving analyte recovery. To improve recovery in wheat flour samples, respective extraction times may need to be extended (up to 15–20 min with water and 5–10 min with methanol).

Instrument and method setup

The instrument system comprised a metal-free Dionex Integrion ion chromatograph and a Dionex AS–AP autosampler coupled to a TSQ Endura mass spectrometer (Figure 2). The chromatographic separation was carried out using a polymer-based Dionex IonPac AS24 column with guard in the 2 mm format. Instrument parameters and settings are shown in Table 2. The hydroxide eluent was prepared in-situ using an eluent generator, the Dionex EluGen KOH cartridge and a Dionex CR-ATC II, preventing the use of external chemicals.

After separation, the eluent passed the electrochemically regenerated AERS suppressor, where the cations from both the eluent and the sample were replaced with hydronium ions, effectively neutralizing the high pH eluent and rendering it compatible with a mass spectrometer. No external chemical regenerants were needed, as an external pump delivered water feeding the electrolytic process to continuously regenerate the suppressor membranes. In order to improve desolvation, a second pump added methanol as a make-up solvent at a low flow rate before entering the mass spectrometer (Figure 2).

Table 2. IC conditions.

Parameter	Setting
Mobile Phase:	KOH (Gradient conditions, Table 3a)
Eluent Source:	Eluent Generator
Analytical Column:	Dionex IonPac AS24 (2 x 250 mm) with guard column
Suppressor:	Dionex AERS 500–2mm (External water mode, Table 3b)
Flow Pump 1 (AERS Regeneration):	1.2 mL/min
Make-Up Solvent:	CH_3OH
Flow Pump 2:	0.1 mL/min
Injection Volume:	10 µL
Column Temperature:	21 °C
Flow Rate:	0.3 mL/min

Table 3a. Gradient conditions.

Time (min)	Concentration of KOH in Eluent (mM)
0	25
0.2	25
11	80
11.1	100
12.5	100
12.6	25
17	25

Table 3b. Suppressor conditions.

Time (min)	Suppressor Current (mA)
0	32
8	60
15.4	60
15.5	32
17	32

Mass spectrometer conditions

Data acquisition was performed in selected reaction monitoring mode (SRM). All SRM traces (parent, quantifier, and qualifier ions) were individually tuned for each target analyte injecting the corresponding standard solution (10 mg/L). The mass spectrometer conditions are shown in Table 4 and SRM parameters for analyzing targeted analytes are shown in Table 5. Data was acquired and processed using Thermo Scientific™ TraceFinder™ 4.0 software allowing easy building of the acquisition and processing methods for high-throughput quantitative analysis with improved data reviewing and reporting.

Mass spectrometer calibration - extended mass range (EMRS) versus classic (with polytyrosine)

Since the target analytes are small molecules with product ions after fragmentation < 100 Daltons, it is recommended to calibrate the mass spectrometer with the Thermo Scientific™ Pierce™ triple quadrupole, EMRS, calibration solution. It consists of 14 components (mass range from 69 *m/z* to 2800 *m/z*) for the calibration in both positive and negative ionization modes. This solution improves mass accuracy and transmission compared to conventional polytyrosine tune solutions, especially in the low *m/z* range where many of the polar pesticides are found.

Table 4. Mass spectrometer conditions.

Parameter	Setting
Ionization Mode:	Heated Electrospray (H-ESI)
Scan Type:	SRM
Polarity:	Negative ion mode
Spray Voltage:	2500 V
Sheath Gas Pressure:	20 Arb
Aux Gas Pressure:	5 Arb
Ion Sweep Gas Pressure:	0 Arb
Capillary Temperature:	329 °C
Vaporizer Temperature:	400 °C
Dwell Time:	10 ms
Q1/Q3 Resolution:	0.7
Collision Gas Pressure (CID) Gas:	1.5 mTorr
Source Fragmentation:	0 V
Use Calibrated RF Lens:	Yes

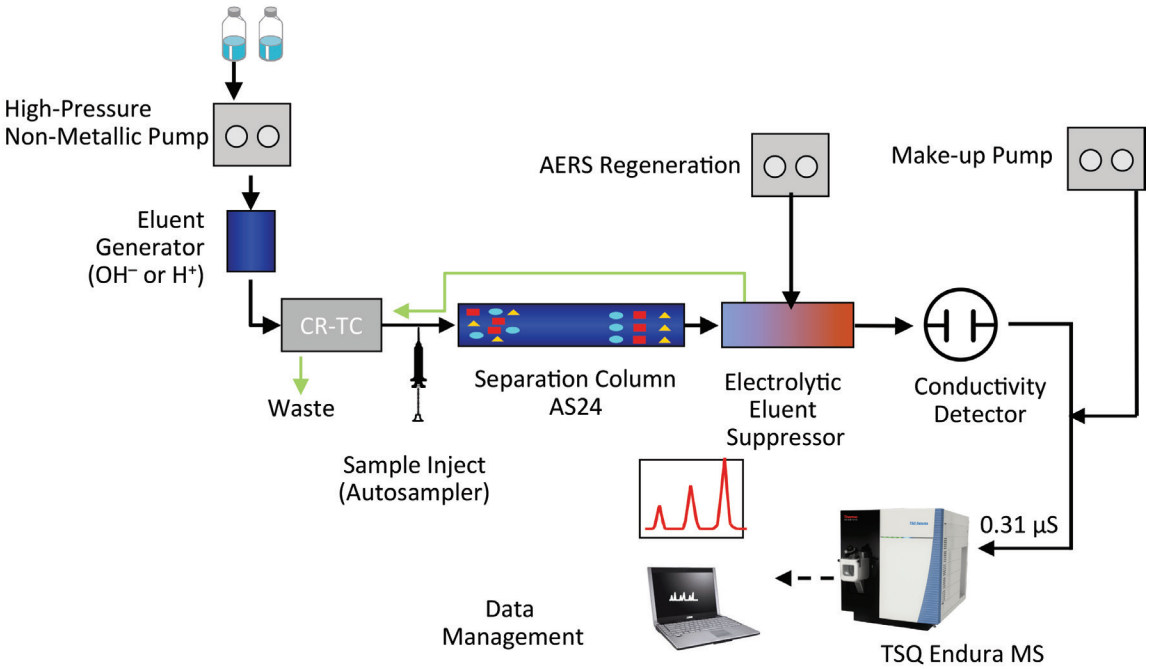

Figure 2. IC-MS/MS system.

Table 5. IC-MS/MS parameters for selected reaction monitoring transitions.

Compound	R.T. (min)	Polarity	Transition Type	Precursor (*m/z*)	Product (*m/z*)	Collision Energy
AMPA	5.70	Neg	Quantifier	110.1	79.2	27
			Qualifier 1	110.1	63.3	20
			Qualifier 2	110.1	81.3	13
Glyphosate	9.95	Neg	Quantifier	168.1	63.3	23
			Qualifier 1	168.1	79.2	34
			Qualifier 2	168.1	81.2	15
			Qualifier 3	168.1	94.2	24
Ethephon	7.35	Neg	Quantifier	143.0	107.2	5
			Qualifier 1	143.0	79.2	17
			Qualifier 2	145.0	107.1	5
Fosetyl-Al	3.95	Neg	Quantifier	109.1	81.2	12
			Qualifier 1	109.1	79.2	19
			Qualifier 2	109.1	63.2	21
Glufosinate	5.51	Neg	Quantifier	180.1	95.2	18
			Qualifier 1	180.1	78.2	25
			Qualifier 2	180.1	85.3	19
			Qualifier 3	180.1	102.1	18
MPPA	6.40	Neg	Quantifier	151.1	133.0	13
			Qualifier 1	151.1	107.2	16
			Qualifier 2	151.1	63.3	37
			Qualifier 3	151.1	79.2	22
Phosphonic Acid	3.84	Neg	Quantifier	81.2	79.2	16
			Qualifier 1	81.2	63.3	31
			Qualifier 2	81.2	81.0	5
HEPA	5.72	Neg	Quantifier	125.1	79.2	20
			Qualifier 1	125.1	95.2	14
			Qualifier 2	125.1	63.3	58
N-acetyl-AMPA	5.76	Neg	Quantifier	152.0	110.0	15
			Qualifier 1	152.0	63.0	25
			Qualifier 2	152.0	79.0	20
N-acetyl-glufosinate	5.67	Neg	Quantifier	222.3	136.1	25
			Qualifier 1	222.3	63.3	51
			Qualifier 2	222.3	59.2	17

Note: RF lens values are not optimized for the individual transitions (calibrated RF lens value is used).

Calculations

Identification and quantification

Identification of the pesticides was indicated by the presence of three or four transition ions measured in SRM mode corresponding to the retention times (±2.5%) of the corresponding standards. The measured peak area ratios for qualifier and quantifier ions must be in close agreement with ratios of the standards[6] as shown in Table 7. The quantifier and qualifier ions were selected among the product ions produced by the fragmentation of the selected precursor ion on the basis of the intensity and selectivity. Matrix-matched calibration was utilized for the quantification of the target pesticides in the samples. A calibration curve was plotted as the peak area is a linear function of the concentration of the analyte.

Results and discussion

The objective of this study was to evaluate the possibility of IC-MS/MS application for fast routine analysis of polar pesticides and their metabolites in food extracts. Various analytical parameters were assessed and the results of these experiments are described.

Samples and quality control materials

For recovery and repeatability experiments, blank matrices of lettuce, orange and flour were used. The absence of the target analytes was checked by repeated measurements of the food products purchased in local food stores. After homogenization the blank food samples were weighed and fortified at desired levels with working standard solution. Sample preparation was performed as described above. In addition, the method's accuracy was assessed using a FAPAS T19186 test sample of red grape containing a known amount of ethephon.

Matrix effect

Strong sample matrix effects were identified comparing calibration curves obtained using matrix-matched calibration standards with those derived from the use of matrix free standards. The significant difference for both slopes and intercepts (> 20%) obtained for all analytes-calibrations and all investigated matrices strongly suggested the use of matrix matched calibrations.
The influence of matrix can be observed by comparing chromatograms of spiked orange sample (Figure 3 and Figure 4) and the related chromatograms of standard mixture (Figure 5).

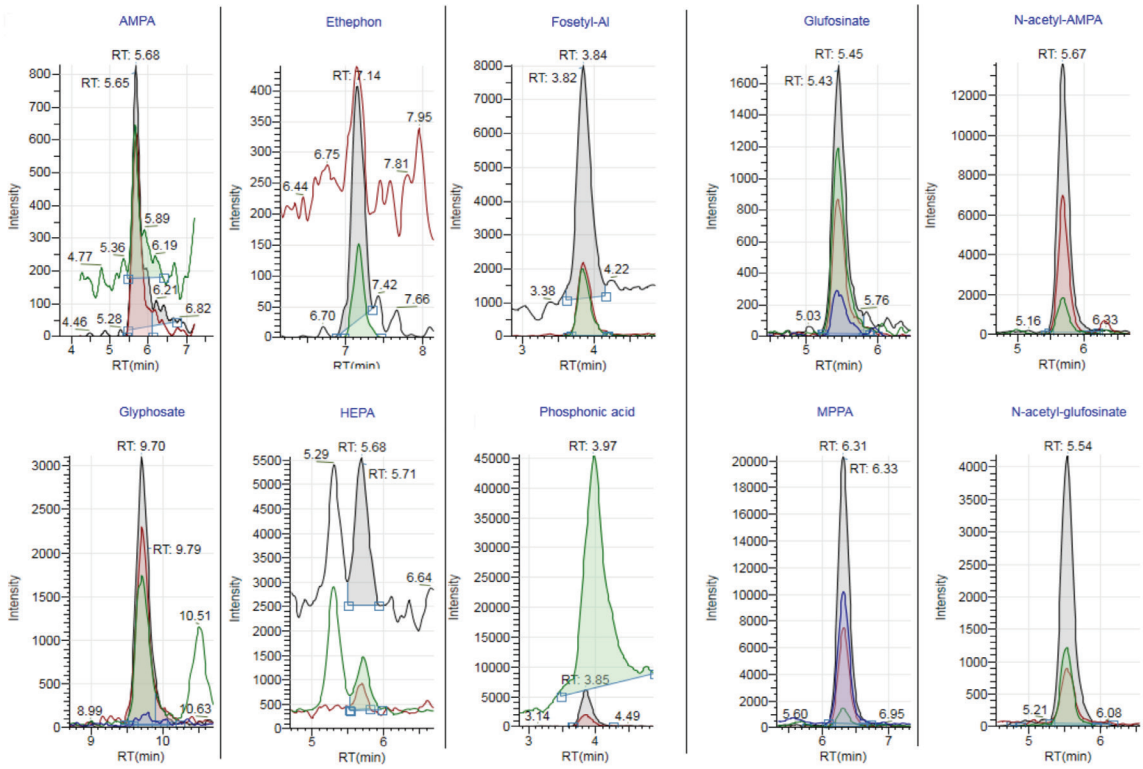

Figure 3. SRM chromatograms of multiple transitions in orange sample spiked with 10 pesticides at level 100 µg/kg.

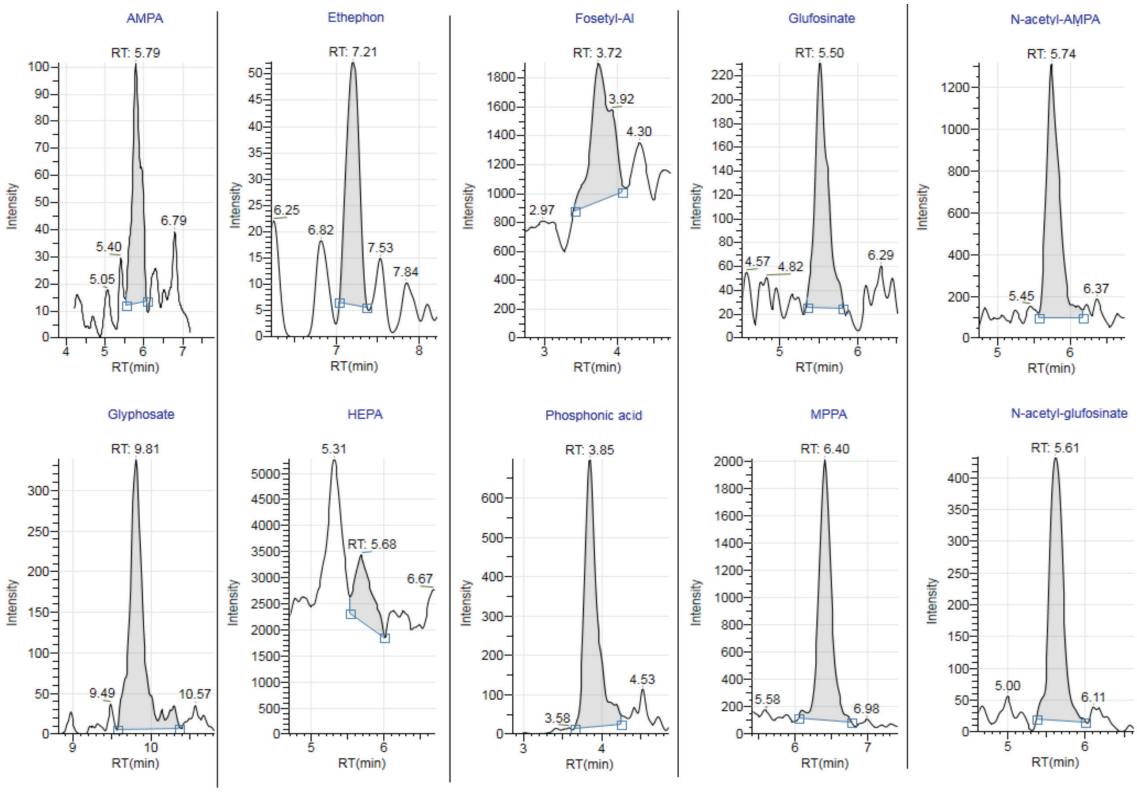

Figure 4. SRM chromatograms of orange sample spiked with 10 pesticides at level 10 µg/kg.

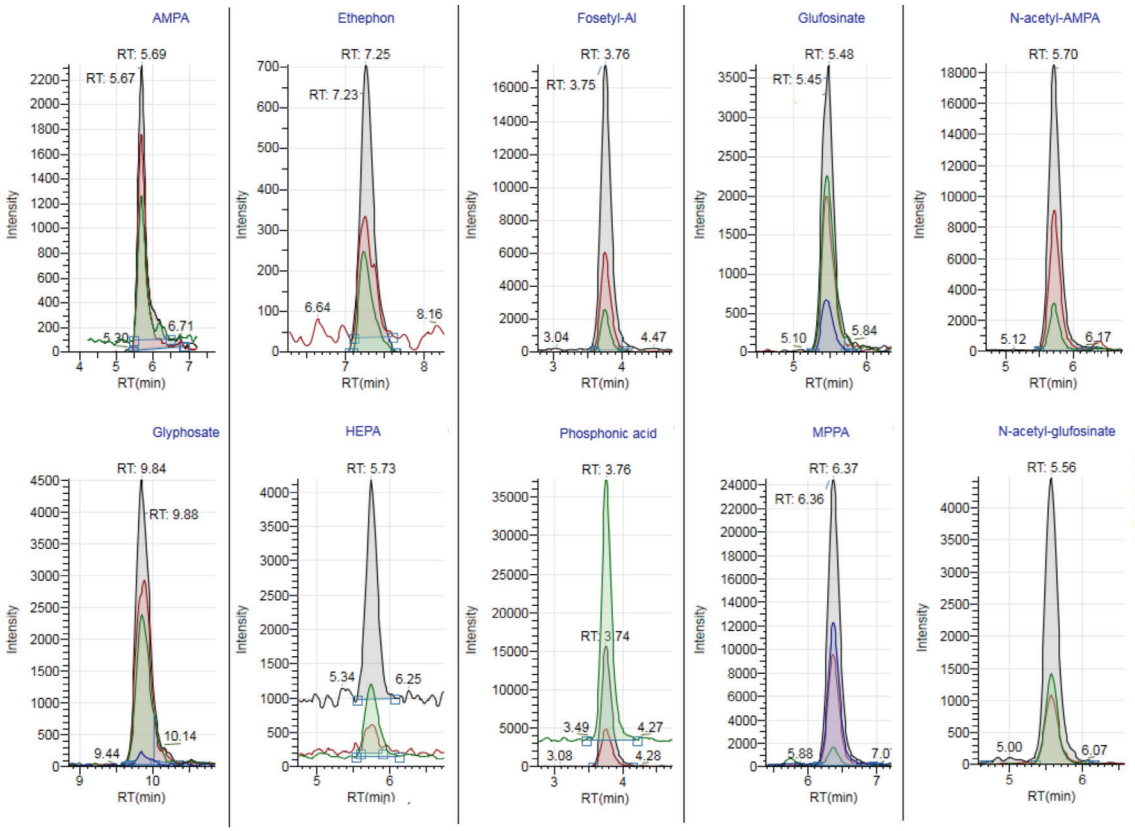

Figure 5. SRM chromatograms of standard mixture in solvent (water) with 10 pesticides at concentration 100 µg/L.

Matrix matched calibration & linearity

Matrix-matched calibration standards were prepared from blank extracts (Table 6). It is recommended to add an aliquot volume of a working standard to fresh blank extract and use this solution for the preparation of matrix-matched calibration. The linearity of the calibration curves for all target compounds was demonstrated for the concentration range from 0–600 µg/kg; correlation coefficients obtained ranged from 0.985–0.990.

Selectivity

Due to the SRM mode used for the measurements, the selectivity was confirmed based on the presence of the transition ions (quantifier and two qualifiers) at the retention times corresponding to those of the respective pesticides. The measured peak area ratios of qualifier/quantifier are within ±30% (relative) of average of calibration standards from the same sequence, defined in Reference 6 when compared to the standards (Table 7).

Table 6. Preparation of matrix-matched calibration standards. Final volume is 1 mL; working standard solution – c = 1000 µg/L.

	Cal. Standard 1	Cal. Standard 2	Cal. Standard 3	Cal. Standard 4	Cal. Standard 5	Cal. Standard 6	Cal. Standard 7
c (µg/kg) in Matrix	0	10	50	100	200	500	600
c (µg/L) in Vial	0	2.5	12.5	25	50	125	150
V (µL) of Working Standard Solution	0	2.5	12.5	25	50	125	150
V (µL) of Blank Extract	1000	997.5	987.5	975	950	875	850

Table 7. Ion ratios (Quan/Qual1 and Quan/Qual2) in matrix and standard mixture at level 10 µg/kg (µg/L).

Compound	Ion Ratio							
	Standard Mix – Quan / Qual1	Standard Mix – Quan / Qual2	Lettuce – Quan / Qual1	Lettuce – Quan / Qual2	Oranges – Quan / Qual1	Oranges – Quan / Qual2	Flour – Quan / Qual1	Flour – Quan / Qual2
AMPA	0.73	0.43	0.76	0.47	0.70	0.48	0.72	0.48
Ethephon	0.50	0.33	0.53	0.38	0.50	0.33	*	*
Fosetyl-Al	0.38	0.13	0.30	0.27	0.29	0.28	0.28	0.19
Glufosinate	0.46	0.65	0.53	0.56	0.47	0.62	0.47	0.58
Glyphosate	0.70	0.49	0.74	0.46	0.74	0.45	0.77	0.48
HEPA	0.13	0.31	0.12	0.40	0.11	0.34	0.14	0.36
MPPA	0.40	0.06	0.34	0.08	0.36	0.06	0.43	0.09
N-acetyl-AMPA	0.48	0.14	0.49	0.16	0.50	0.15	0.47	0.18
N-acetyl-glufosinate	0.26	0.23	0.21	0.27	0.21	0.26	0.25	0.29
Phosphonic acid	0.31	0.49	0.14	0.43	0.33	**	0.33	**

The agreement between ion ratios should be within the permitted tolerance, which is defined in SANTE 11945/2015.[6]
Note:*[1]Ethephon was not detectable in the wheat flour samples, **Ion Qual2 is coeluting with interference of the same *m/z*.

Precision and accuracy

The precision and accuracy of the method was determined by analyzing fortified blank samples of lettuce, oranges, and wheat flour. The samples were fortified by addition of a calculated amount of working solution to the homogenized food matrix. Six replicates at three different concentration levels were prepared, after the sample was left 30 minutes to allow soaking of the standard into the matrix. The intermediate precision was determined by the analysis of two other sample sets, with six replicates prepared only at one concentration level (middle level) and measured over a period of two days.

The results are shown in Table 8 to Table 10. Additional accuracy was established for ethephon by analyzing FAPAS T19186 proficiency test material. The matrix was red grape purée and the obtained results were in the satisfactory range (Table 11). The most demanding sample matrix during this work was wheat flour, as low recovery was achieved in flour matrix for glyphosate and AMPA, and ethephon couldn't be analyzed at all. One possible way to improve the recovery for this troublesome matrix is to use labeled internal standards.

Table 8. Results of method precision (expressed as relative standard deviation – RSD (%)) at three different spike levels (n=6).

Compound	Spiking Levels (µg/kg)			RSD (%)								
	All Three Matrices			Lettuce			Oranges			Flour		
	I	II	III	I	II	III	I	II	III	I	II	III
AMPA	50	200	500	14	8	3	13	5	4	19	11	10
Ethephon	50	200	500	10	5	12	13	19	10	-	-	-
Fosetyl-Al	50	200	500	16	12	3	5	6	3	12	11	7
Glufosinate	50	200	500	4	8	3	10	4	1	18	11	13
Glyphosate	50	200	500	12	10	3	6	6	3	10	17	8
HEPA	50	200	500	7	9	4	19	11	4	18	9	16
MPPA	50	200	500	4	8	3	6	5	5	9	5	14
N-acetyl-AMPA	50	200	500	8	9	2	3	4	4	7	9	14
N-acetyl-glufosinate	50	200	500	8	8	2	4	5	4	8	10	12
Phosphonic acid	50	200	500	11	11	3	14	12	7	10	18	17

Table 9. Results of method accuracy (expressed as recovery) at three different levels (n=6).

Compound	Recovery (%)								
	Lettuce			Oranges			Flour		
	I	II	III	I	II	III	I	II	III
AMPA	84	85	80	87	83	80	76	84	68
Ethephon	120	88	92	74	80	76	-	-	-
Fosetyl-Al	98	97	82	96	78	72	91	111	91
Glufosinate	101	93	86	89	83	77	81	98	76
Glyphosate	88	83	81	79	79	76	54	54	56
HEPA	118	93	81	86	78	77	89	94	85
MPPA	116	98	81	83	85	79	70	81	70
N-acetyl-AMPA	95	89	79	79	79	76	80	87	76
N-acetyl-glufosinate	93	91	84	86	84	79	87	92	79
Phosphonic acid	115	99	81	95	97	72	93	122	86

Table 10. Method intermediate precision expressed as RSD (%).

Compound	Intermediate Precision at Level II (%)							
	Lettuce			Oranges			Flour	
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2
AMPA	8	4	8	13	4	7	11	13
Ethephon	5	10	8	13	3	11	10	30
Fosetyl-Al	12	6	7	5	2	1	11	29
Glufosinate	8	5	5	10	8	4	11	16
Glyphosate	10	4	4	6	4	2	17	5
HEPA	9	5	7	19	6	9	9	13
MPPA	8	3	6	6	3	1	5	8
N-acetyl-AMPA	9	4	7	3	4	2	9	15
N-acetyl-glufosinate	8	4	7	4	2	2	10	9
Phosphonic acid	11	5	6	14	11	12	18	10

Note: Six sample replicates were prepared for each set at one level and measured during three days.
Flour samples were measured in only two days due to time constraints.

Table 11. Results of FAPAS proficiency test material – red grape purée T19186.

Compound	Assigned Value (µg/kg)	Average (µg/kg) RSD (%)	RSD (%)	REC (%)
Ethephon	629 ± 216	553	7	88

Limits of detection (LOD) and quantification (LOQ)

Limits of detection and quantification were estimated following the IUPAC approach, which consists of analyzing the blank sample to establish noise levels and then estimating LODs and LOQs for signal/noise at 3 and 10, respectively. In addition, the sample injections' repeatability at the LOQ level has to be below 20% (expressed as RSD; n = 3). The LODs and LOQs are shown in Table 12. Finally, as shown in Table 13, reported LOQs comply with currently valid pesticide maximum residue limits (MRL) defined by the EU and allow the use of the method in routine food control for the tested pesticides and matrices. In Figure 4 is shown the chromatogram of spiked orange sample at level 10 µg/kg, since this value is for most of the target pesticides LOD or LOQ.

Table 12. Limits of detection and quantification of the method (LOD and LOQ) for lettuce, oranges, and wheat flour.

Compound	Lettuce		Oranges		Flour	
	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)
AMPA	10	20	10	20	10	20
Ethephon	10	20	20	50	Not detected	Not detected
Fosetyl-Al	10	20	20	50	20	50
Glufosinate	1	10	10	20	10	20
Glyphosate	5	10	10	20	10	20
HEPA	10	20	20	50	20	50
MPPA	1	10	1	10	1	10
N-acetyl-AMPA	1	10	1	10	1	10
N-acetyl-glufosinate	3	10	3	10	1	10
Phosphonic acid	1	10	1	10	1	10

Table 13. Comparison of method detection limits and maximum residue limits defined by EC 396/2005.[2]

Compound	Lettuce		Oranges		Flour	
	MRL (µg/kg)	LOQ (µg/kg)	MRL (µg/kg)	LOQ (µg/kg)	MRL (µg/kg)	LOQ (µg/kg)
AMPA	n.r.	20	n.d	20	n.d	20
Ethephon	50	20	50	50	1000	Not detected
Fosetyl-Al[1]	75,000	20	75,000	50	2000	50
Glufosinate[2]	500	10	100	20	100	20
Glyphosate	100	10	500	20	10,000	20
HEPA	n.r.	20	n.r.	50	n.r.	50
MPPA	n.r.	10	n.r.	10	n.r.	10
N-acetyl-AMPA	n.r.	10	n.r.	10	n.r.	10
N-acetyl-glufosinate	n.r.	10	n.r.	10	n.r.	10
Phosphonic acid[1]	75,000	10	75,000	10	75,000	10

Note: n.r.= not required

[1]Fosetyl-Al = sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl

[2]Glufosinate ammonium = sum of glufosinate, its salts, MPPA, and NAG, expressed as glufosinate equivalents.

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Conclusion

The reported in-house validated method enables the quantification of ten polar ionic compounds or four ionic pesticides and their metabolites in different food matrices by coupling ion chromatography to a triple quadrupole mass spectrometer. In contrast to methods described in the literature, sample preparation was simplified and the use of ion chromatography speeds up the separation. This method can be recommended as a reliable and cost-effective solution for any routine lab dealing with the determination of polar pesticides and their metabolites in food samples.

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