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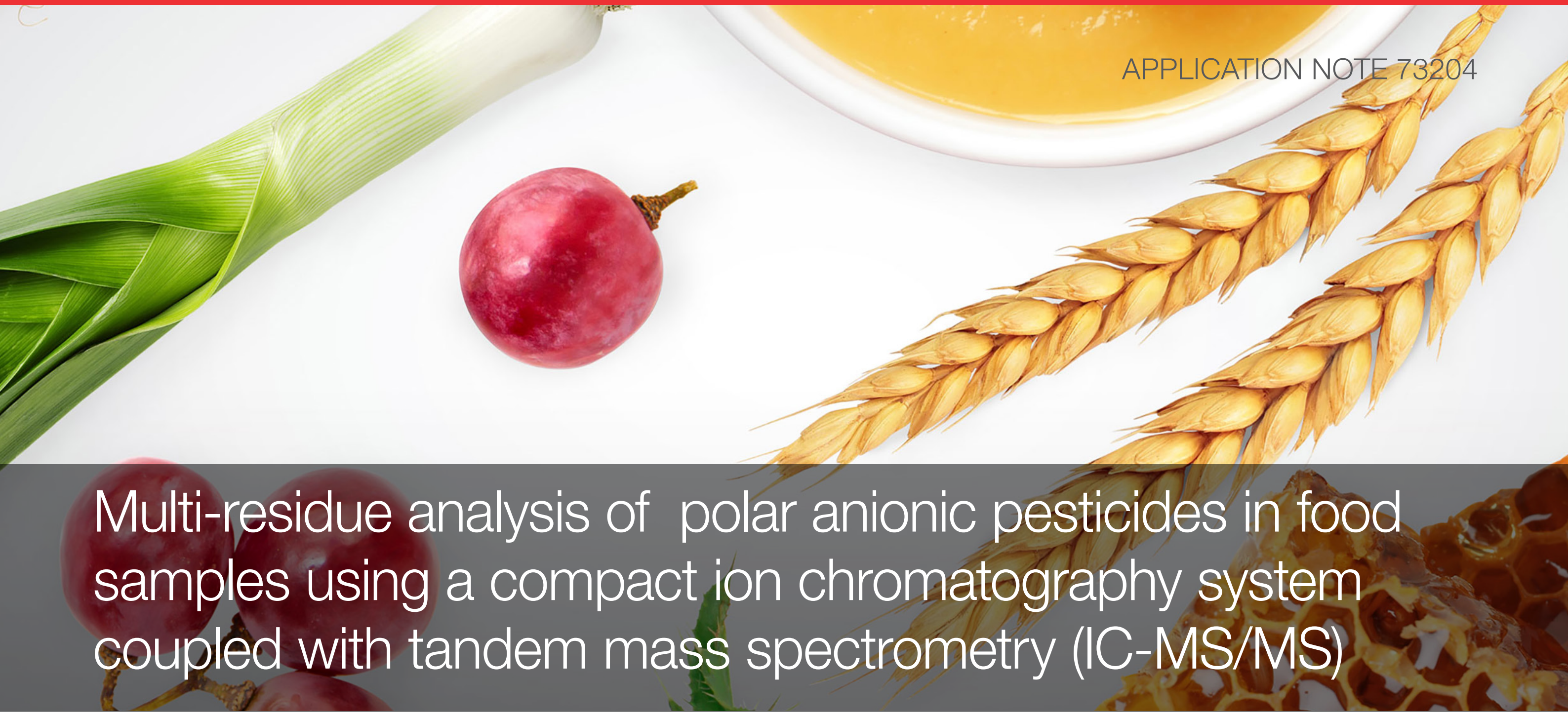

APPLICATION NOTE 73204

Multi-residue analysis of polar anionic pesticides in food samples using a compact ion chromatography system coupled with tandem mass spectrometry (IC-MS/MS)

Authors

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Keywords

Polar pesticides, QuPPe, glyphosate, fosetyl-Al, bialaphos, phosphonic acid, MPPA, glufosinate, chlorate, HEPA, AMPA, *N*-acetyl AMPA, *N*-acetyl-glufosinate, ethephon, cyanuric acid, *N*-acetyl-glyphosate, perchlorate, wheat flour, leek

Goal

To develop and validate an integrated sample-to-result analytical workflow based on ion chromatography (IC) coupled with triple quadrupole mass spectrometry (MS/MS) for the multi-residue determination of polar anionic pesticides and perchlorate in representative food matrices. The performance of this Thermo Scientific™ Anionic Pesticides Explorer workflow must be robust in routine analysis and the results compliant with EU SANTE/11813/2017 method validation and ongoing quality control guideline criteria.[1] Also, the analysis should meet the residue definitions and maximum residue levels (MRLs) or tolerance values applicable in the European Union, United States, Japan, and China.

Introduction

Polar anionic pesticides are widely used in agricultural production with the herbicide glyphosate one of the highest usage pesticides in the world. Residues of glyphosate and other anionic pesticides such as glufosinate, fosetyl, ethephon, and their metabolites, have been detected in vegetables, cereals, and processed foods. Also detected are perchlorate, a contaminant in some fertilizers, and chlorate from the use of biocides in food preparation facilities. Despite the high usage and evidence of residues in food, polar

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pesticides are monitored infrequently, primarily because of the analytical challenges and high costs associated with the analysis. The European Food Safety Authority (EFSA) and the European Commission have highlighted this situation and have requested that the European Reference Laboratories (EURLs) for pesticides develop more effective methods to encourage increased monitoring of polar pesticides in food.

The most popular extraction method for polar pesticides is the Quick Polar Pesticides Extraction (QuPPe) method developed by the European Reference Laboratory for Single Residue Methods (EURL-SRM).[2] The method is based on extraction with methanol/water, without liquid/liquid partition or solid phase extraction clean-up. Consequently, the extracts can contain high levels of co-extractives that can contaminate the chromatographic and detection systems and suppress the MS response.

Furthermore, polar anionic pesticides have poor retention in reversed-phase LC-MS/MS, which is widely used for multi-residue determination of pesticides in food.[3] Pre- or post- column derivatization can increase chromatographic retention and selectivity for glyphosate and glufosinate but is not generally favored because of the limitation on scope (i.e. number of compounds determined), additional labor, and high method variability. Alternatively, more convenient approaches to achieve greater retention of a wider range of polar compounds include the use of ion-pair reversed-phase LC, HILIC, graphitized carbon columns, and IC with or without ion suppression of the mobile phase.

Ion chromatography with electrolytic ion suppression coupled to MS (IC-MS) offers a number of advantages for direct analysis of multi-residue polar anionic pesticides and their metabolites.[4] Ion chromatography provides excellent chromatographic retention and resolution in a wide range of matrices, while triple quadrupole mass spectrometer systems offer high selectivity and therefore low detection limits when operated in the selected reaction monitoring (SRM) mode.

Experimental

Instrumental and method set-up

A Thermo Scientific™ Dionex™ Integrion™ HPIC™ system, fitted with a Thermo Scientific™ Dionex™ electrolytic eluent generator cartridge (EGC) and conductivity cell, was coupled to a Thermo Scientific™ Dionex™ AS-AP Autosampler and Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer. Separation was achieved using a Thermo Scientific™ Dionex™ IonPac™ AG19-4µm Guard column, 2 × 50 mm, coupled to a Thermo Scientific™ Dionex™ IonPac™ AS19-4µm Analytical column, 2 × 250 mm, held at 40 °C with elution of polar anionic analytes using a potassium hydroxide gradient at a flow rate of 0.35 mL/min. Details of the IC experimental conditions are presented in Table 1 (part 1).

A Thermo Scientific™ Dionex™ ADRS 600 Anion Dynamically Regenerated Suppressor (2 mm) was operated in external water mode using DI water delivered at 0.7 mL/min by an auxiliary Thermo Scientific™ Dionex™ AXP pump. The Dionex ADRS device, installed after the column, converted the KOH eluent to water before it flowed through the conductivity detector and mass spectrometer connected in series. Acetonitrile was delivered at a flow rate of 0.2 mL/min by an auxiliary Dionex AXP-MS pump, via a tee junction between the conductivity cell and mass spectrometer. This addition of acetonitrile assists electrospray aerosol desolvation and increases the response of most analytes by three- to four-fold. The injection volume was 25 µL. The system control, data acquisition, and data processing were done using Thermo Scientific™ Chromeleon™ Chromatography Data System software, version 7.2.9, or Thermo Scientific™ TraceFinder™ software, version 4.1. The MS instrument settings are summarized in Table 1 (part 2) and the IC-MS/MS configuration is illustrated in Figures 1A and 1B.

Table 1 (part 1). Summary of experimental conditions and settings

Conditions for ion chromatography	
IC system:	Dionex Integrion HPIC system
Conductivity monitor:	Conductivity detector
Columns:	IonPac AG19-4µm Guard, 2 × 50 mm (P/N 083225) IonPac AS19-4µm Analytical, 2 × 250 mm (P/N 083223)
Eluent source:	Dionex EGC 500 KOH Eluent Generator Cartridge with Dionex CR-ATC 600
KOH gradient:	20–30 mM (0–2 min) 30 mM (2–8 min) 45-55 mM (8–12 min) 80 mM (12–14 min) 85 mM (14–19 min) 20 mM (19–21 min)
Flow rate:	0.35 mL/min
Injection volume:	25 µL
Temperature:	40 ºC (column oven) 20 ºC (compartment temperature) 35 ºC (conductivity detector cell)
System backpressure:	~3900 psi (100 psi = 0.6895 MPa)
Suppressor:	Suppressed Conductivity, Dionex ADRS 600 Suppressor (2 mm) operated in constant current mode, AutoSuppression, 74 mA, external water mode via Dionex AXP pump, external water flow rate (0.70 mL/min)
Background conductance:	~0.3 µS/cm
Run time:	21 min
IC-MS interface:	Tee union (PEEK, P/N 00101-18204) to combine the analyte from conductivity detector via Thermo Scientific™ Viper™ fitting tubing
Post-suppressor makeup solution:	Acetonitrile at 0.2 mL/min via Dionex AXP-MS pump

Table 1 (part 2). Summary of experimental conditions and settings

Conditions for mass spectrometric detection	
Ion source settings	
Ion source type:	HESI
Spray voltage:	Static
Negative ion: Positive ion:	3250 V 3500 V
Sheath gas:	60 Arbitrary units (Arb)
Aux gas:	13 Arb
Sweep gas:	1 Arb
Ion transfer tube temp.:	350 °C
Vaporizer temp.:	250 °C
MS global settings	
Start time:	0 min
End time:	21 min
Master scan	
Scan mode:	SRM
Polarity:	Defined in Table 2
Use cycle time:	True
Cycle time:	1.0 s
Q1 resolution (FWHM):	0.7
Q3 resolution (FWHM):	1.2
CID gas:	2.0 mTorr
Source fragmentation:	0 V
Chromatographic peak width:	6 s
Transition conditions:	Optimized for each compound using TSQ Altis mass spectrometer (Table 2)

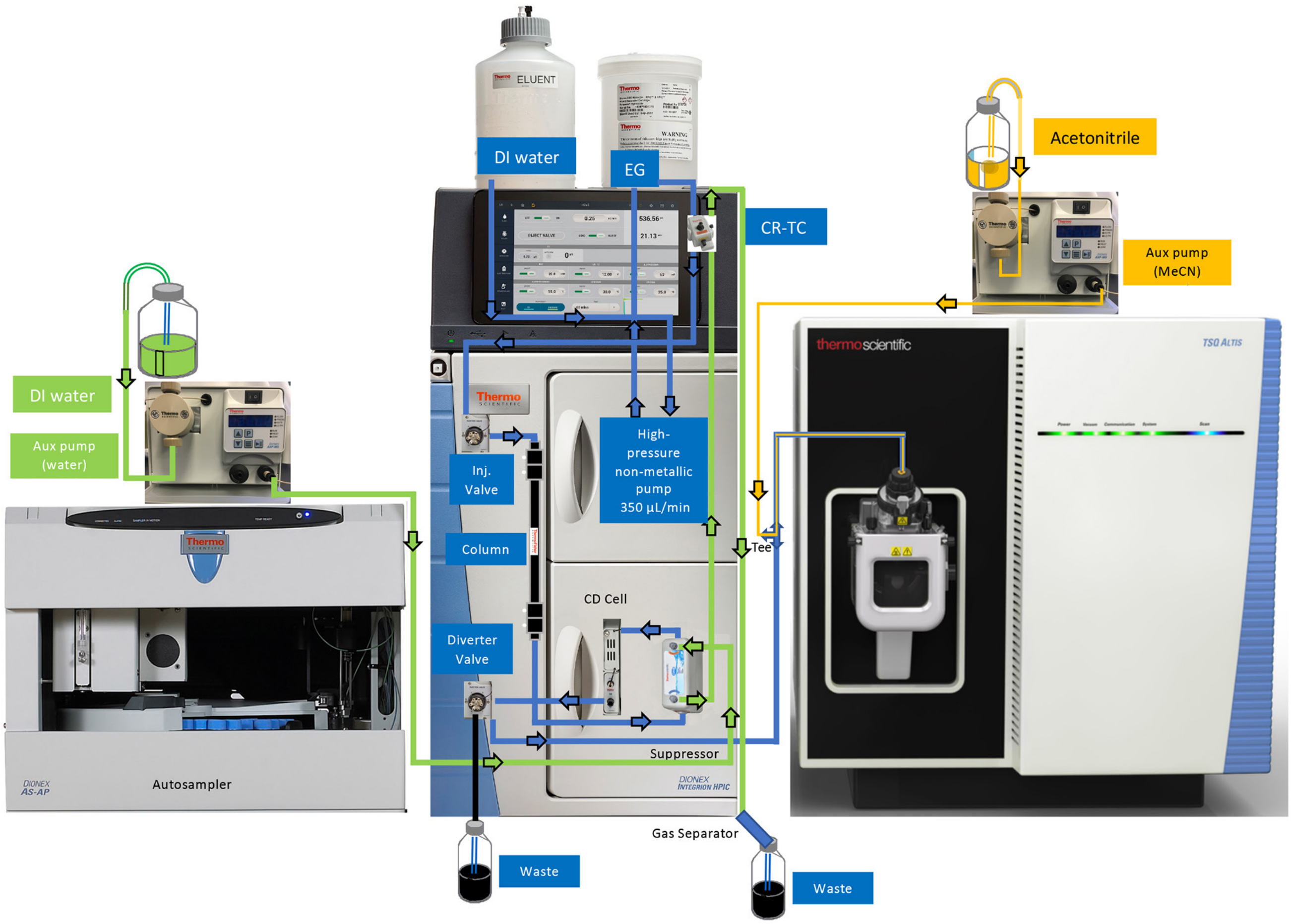


Figure 1A. Configuration of the fully integrated IC- MS/MS system

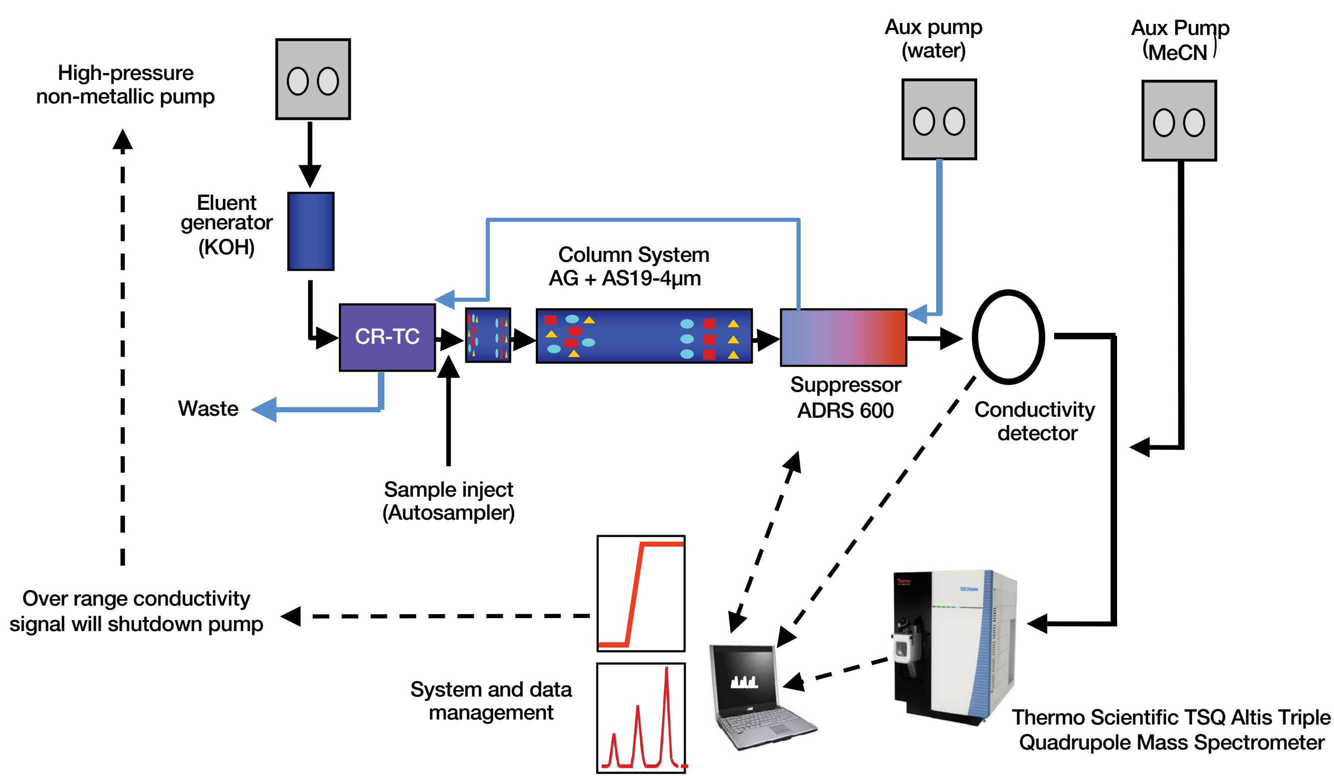


Figure 1B. Configuration of the fully integrated IC- MS/MS system

Deionized water delivered by the pump enters the Dionex EGC cartridge,[5] which automatically generates the eluent, which then exits the cartridge and passes through the Thermo Scientific™ Dionex™ Continuously Regenerated Trap Column (CR-TC)[6] to remove any impurities. The eluent then passes through the EG degas tubing to remove the hydrogen gas produced during KOH generation and then into the injection valve. The sample is loaded into the sample loop and the injection valve is toggled to the *Inject* position to allow eluent to pass through the loop. The pump pushes the eluent and sample through the guard and analytical columns, and then through the suppressor,[7] where the cations from both the eluent and the sample are replaced with hydronium ions, effectively neutralizing the high pH eluent and making it compatible with the mass spectrometer. From the suppressor, the eluent flows into the conductivity detector to monitor the background conductivity, which is typically below 1.5 µS/cm before injection of a sample or standard. A Dionex AXP-MS pump was used to add acetonitrile (0.2 mL/min) after the conductivity detector and before the electrospray interface to increase analyte signal intensity.

The make-up flow rate of acetonitrile was 0.2 mL/min, giving a total flow into the source of 0.55 mL/min, which was within the accepted flow rate range of the TSQ Altis mass spectrometer. The backpressure on the suppressor was below the recommended maximum value of 150 psi.[7,8]

Mass spectrometer

Data acquisition was performed in selected reaction monitoring mode (SRM). The product ions were individually tuned for each target analyte using TSQ Altis 3.1 Tune software by infusing the corresponding standard solution (1 mg/L). The mass spectrometer parameters including the precursor-product ion transitions monitored are shown in Table 2. Data was acquired using Chromeleon CDS 7.2.9 or Thermo Scientific™ Xcalibur™ 4.1 software with SII for Xcalibur and processed using TraceFinder 4.1 software, which allow easy creation of the acquisition and processing methods for high-throughput quantitative analysis along with efficient data review and reporting.

Because the target analytes are small molecules with low mass-to-charge (*m/z*) product ions, the mass spectrometer was calibrated using the Thermo Scientific™ Pierce™ Triple Quadrupole Extended Mass Range Calibration Solution (P/N 88340), which contains 14 components (mass range from 69 *m/z* to 2800 *m/z*) for calibration in both positive and negative ionization modes. This solution improves mass accuracy and transmission compared to conventional polytyrosine mass calibration solution, especially in the low *m/z* range.

Chemicals and consumables

Deionized (DI) water, ASTM Type I reagent grade, with 18 MΩ·cm resistivity or better, was filtered through a 0.2 µm filter immediately before use. Fisher Chemical Methanol, Optima™ LC/MS grade, (P/N A456-1) and acetonitrile, Optima™ LC/MS grade (P/N A955-1), were used.

In addition to calibration of the mass spectrometer, satisfactory performance of instrument modules was tested using the QPP-Lab® Standard Kit QuPPe EURL v.10-1.3 Method Compliance stock solution, obtained from Lab Instruments, Italy (Code: KIT4AC3L016).

Isotopically labeled standards were obtained from various sources: glyphosate-$^{13}C_2$,^{15}N, glufosinate-d_3 hydrochloride and 3-methylphosphinicopropionic acid-d_3 sodium salt from Trc-Canada; potassium chlorate $^{18}O_3$, aminomethylphosphonic acid-^{13}C, ^{15}N, ^{2}D and perchloric acid sodium salt ($^{18}O_4$) from Cambridge Isotope Laboratories Inc.; and ethephon-d_4 from A ChemTek, Inc.,

Sample extraction and clean-up

Samples of wheat flour and of leeks were purchased from local retail outlets in Beijing. Wheat flour samples were thoroughly mixed before taking test portions, while leek samples were homogenized using a blender.

Table 2. IC-MS/MS parameters for selected reaction monitoring transitions

Compound	Start time (min)	End time (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	Min dwell time (ms)	RF lens (V)
Fosetyl-Al	2.5	5	Negative	109	63*	29	81.984	33
Fosetyl-Al	2.5	5	Negative	109	79**	22	81.984	33
Fosetyl-Al	2.5	5	Negative	109	81	12	81.984	33
Bialaphos	5	8	Positive	324	136**	24	18.142	47
Bialaphos	5	8	Positive	324	207*	18	18.142	47
Phosphonic acid	5	9	Negative	81	63**	27	18.142	41
Phosphonic acid	5	9	Negative	81	79*	15	18.142	41
MPPA	5	8.5	Negative	151	63**	34	18.142	36
MPPA	5	8.5	Negative	151	107	16	18.142	36
MPPA	5	8.5	Negative	151	133*	13	18.142	36
MPPA_IS	5	8.5	Negative	154	136	14	18.142	36
Glufosinate	5	7.8	Negative	180	95*	17	18.142	45
Glufosinate	5	7.8	Negative	180	134**	16	18.142	45
Glufosinate	5	7.8	Negative	180	136	17	18.142	45
Glufosinate_IS	5	7.8	Negative	183	98	18	18.142	45
Chlorate	5.2	7.8	Negative	83	67*	21	18.142	66
Chlorate	5.2	7.8	Negative	85	69**	21	18.142	66
Chlorate_IS	5.2	7.8	Negative	89	71	22	18.142	66
HEPA	5.2	7.5	Negative	125	79*	21	18.142	42
HEPA	5.2	7.5	Negative	125	89	7	18.142	42
HEPA	5.2	7.5	Negative	125	95**	14	18.142	42
AMPA	5.5	9	Negative	110	63**	20	18.142	52
AMPA	5.5	9	Negative	110	79*	29	18.142	52
AMPA_IS	5.5	9	Negative	114	79*	29	18.142	52
AMPA_IS	5.5	9	Negative	114	81	14	18.142	52
N-acetyl AMPA	5.5	7.5	Negative	152	63**	25	18.142	45
N-acetyl AMPA	5.5	7.5	Negative	152	110*	13	18.142	45
N-acetyl glufosinate	5.5	8	Negative	222	134**	20	18.142	47
N-acetyl-glufosinate	5.5	8	Negative	222	136*	22	18.142	47
N-acetyl-glufosinate	5.5	8	Negative	222	178	15	18.142	47
Ethephon	6	9.5	Negative	143	35	20	18.142	30
Ethephon	6	9.5	Negative	143	63	55	18.142	30
Ethephon	6	9.5	Negative	143	79**	18	18.142	30
Ethephon	6	9.5	Negative	143	107*	8	18.142	30
Ethephon_IS	6	9.5	Negative	147	111	8	18.142	30
Cyanuric acid	10	15	Negative	128	42*	16	44.055	34
Cyanuric acid	10	15	Negative	128	85**	10	44.055	34
Glyphosate	11	17	Negative	168	63*	22	44.055	35
Glyphosate	11	17	Negative	168	79**	40	44.055	35
Glyphosate	11	17	Negative	168	81	16	44.055	35
Glyphosate	11	17	Negative	168	124	12	44.055	35
Glyphosate_IS	11	17	Negative	171	63*	23	44.055	35
N-acetyl-glyphosate	11	17	Negative	210	124*	19	44.055	40
N-acetyl-glyphosate	11	17	Negative	210	148**	16	44.055	40
N-acetyl-glyphosate	11	17	Negative	210	150	13	44.055	40
Perchlorate	13	19	Negative	99	83*	26	44.055	70
Perchlorate	13	19	Negative	101	85**	26	44.055	70
Perchlorate_IS	13	19	Negative	107	89*	28	44.055	70
Perchlorate_IS	13	19	Negative	109	91	28	44.055	70

Note: * = quantifier ion and ** = qualifier ion

Extraction of the samples was based on a modification of the QuPPe Method as illustrated for wheat flour in Figure 2.

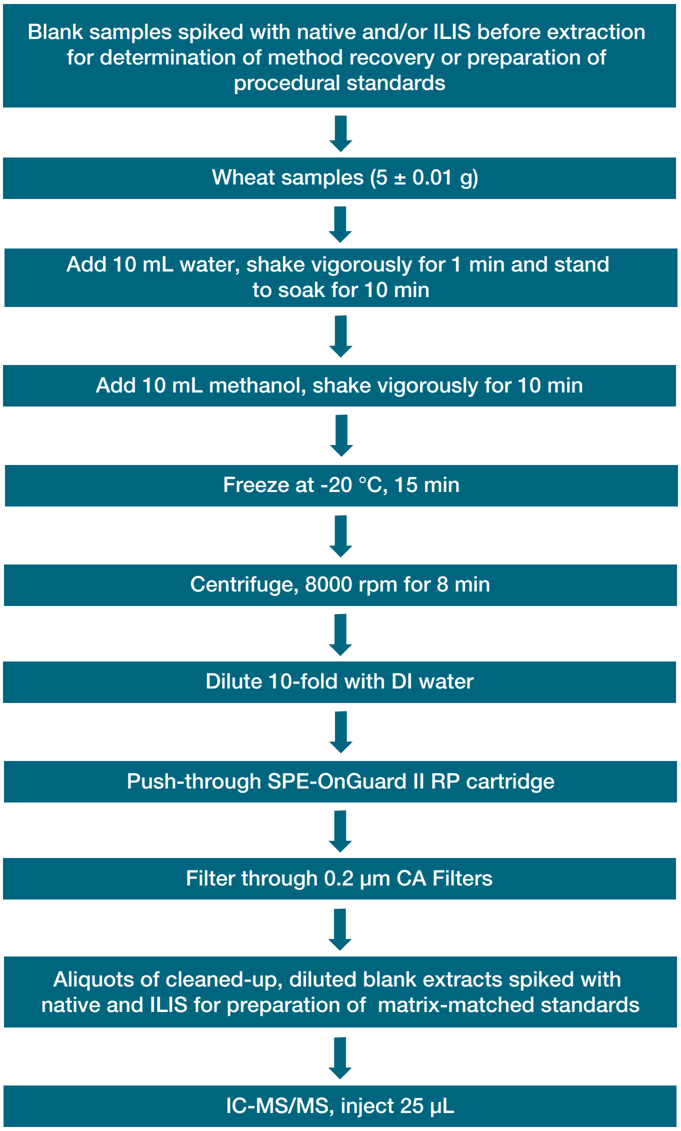


Figure 2. Flow diagram of the Modified QuPPe Extraction Method

Sub-samples of homogenized leek (10 ± 0.01 g) or wheat flour (5 ± 0.01 g) were weighed into 50 mL polypropylene centrifuge tubes (P/N 339653). DI water (1.5 mL for leek, 10 mL for wheat flour) was added to adjust the water content to 10 mL (the wheat flour samples were allowed to soak for 10 min) followed by addition of methanol (10 mL). The hydrated samples were mixed vigorously for 10 min using a vortex mixer. The extract was placed in a freezer for 15 min and then centrifuged (8000 rpm, for 8 min at 5 °C). The supernatant was diluted 10-fold with DI water and an aliquot (5 mL) placed in a syringe (5 mL) and pushed through a Thermo Scientific™ Dionex™ OnGuard™ II RP cartridge (P/N 057083) coupled to a Thermo Scientific™ Titan3™ CA Membrane Syringe Filter (0.2 µm, P/N 42213-CA) connected in series. The Dionex OnGuard II RP cartridge was preconditioned by flushing with 5 mL methanol followed by 10 mL DI water. The first 3 mL of filtrate were discarded, and 1.5 mL collected in a plastic vial (P/N 079812) for IC-MS/MS determination. Plasticware was used throughout to avoid adsorption of the analytes onto glass surfaces.

Matrix-matched standards (MMS) were prepared by spiking the diluted and cleaned-up extract with native standards and ILIS, while procedural standards (PS) were prepared by spiking samples with native standards and ILIS before extraction.

Results and discussion

Wheat flour was selected as a representative of dry commodities (group 5) and leek as a representative of green vegetables (group 1) in the SANTE guidelines.[1]

Selectivity and sensitivity

A combination of chromatographic resolution and mass resolution provided satisfactory separation for the 15 pesticides including metabolites of interest in 18 min as shown in Figure 3. The total cycle time was 21 min.

Figure 4 shows the peak shape and sensitivity were satisfactory for most of the anionic polar pesticides at 0.25 ng/mL in wheat flour extract (equivalent to 10 ng/g in sample). Data for leek are shown in Figure 5.

Analyte identification was confirmed based on the presence of the transition ions (quantifier and qualifier) at the retention times corresponding to those of the respective pesticides. Qualifier/quantifier ratios were within ±30% (relative) of average of calibration standards from the same sequence. Ion ratios in wheat flour and leek matrix are shown in Tables 3 and 4.

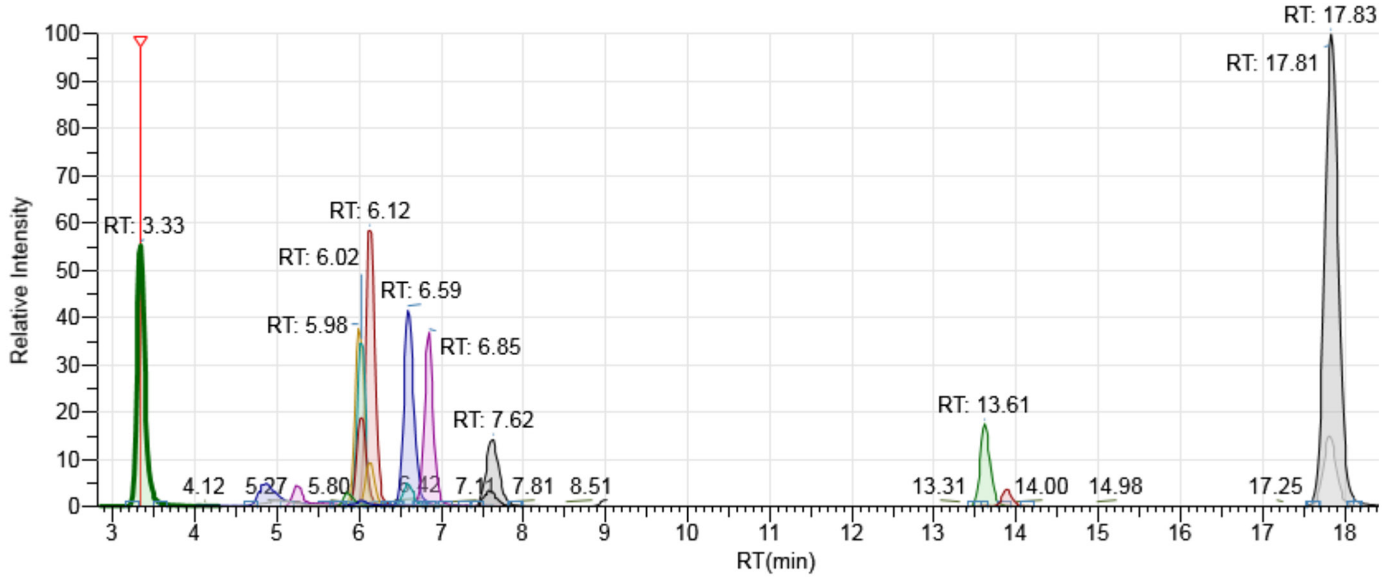


Figure 3. TIC reconstructed ion chromatogram of SRM transitions for 15 analytes at 200 ng/g in wheat flour

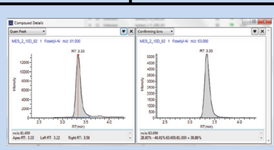
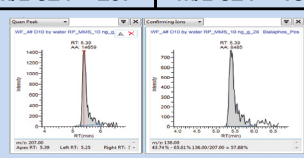
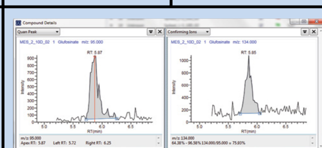
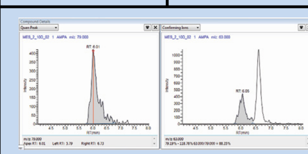
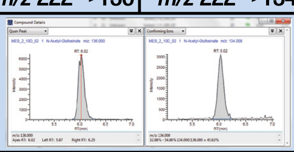
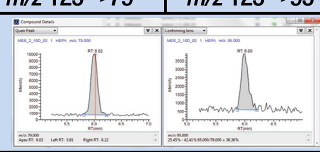
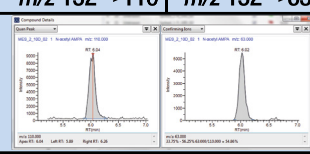
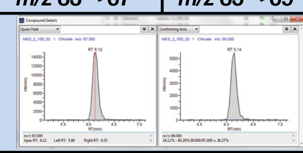
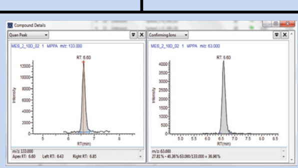
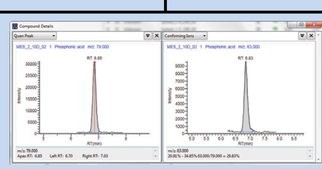
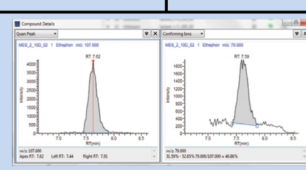
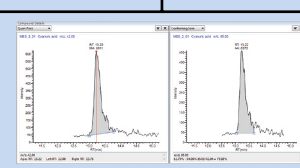
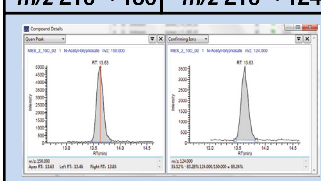
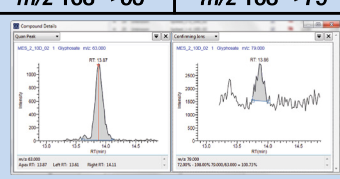
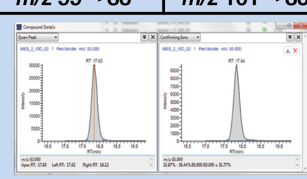
Fosetyl (Rt 3.33 min)		Bialaphos (Rt 5.24 min)		Glufosinate (Rt 5.87 min)		AMPA (Rt 6.01 min)	
m/z 109→81	*m/z* 109→63	*m/z* 324→207	*m/z* 324→136	*m/z* 180→95	*m/z* 180→134	*m/z* 110→79	*m/z* 110→63
							
N-acetyl-glufosinate (Rt 6.02 min)		HEPA (Rt 6.02 min)		*N*-acetyl-AMPA (Rt 6.04 min)		Chlorate (Rt 6.12 min)	
m/z 222→136	*m/z* 222→134	*m/z* 125→79	*m/z* 125→95	*m/z* 152→110	*m/z* 152→63	*m/z* 83→67	*m/z* 85→69
							
MPPA (Rt 6.60 min)		Phosphonic acid (Rt 6.85 min)		Ethephon (Rt 7.62 min)		Cyanuric acid (Rt 13.22 min)	
m/z 151→133	*m/z* 151→63	*m/z* 81→79	*m/z* 81→63	*m/z* 143→107	*m/z* 143→79	*m/z* 128→42	*m/z* 128→85
							
N-acetyl-glyphosate (Rt 13.63 min)		Glyphosate (Rt 13.87 min)		Perchlorate (Rt 17.83 min)			
m/z 210→150	*m/z* 210→124	*m/z* 168→63	*m/z* 168→79	*m/z* 99→83	*m/z* 101→85		
							

Figure 4. Response for quantification and qualifier product ions for individual anionic pesticides equivalent to 10 ng/g in wheat flour

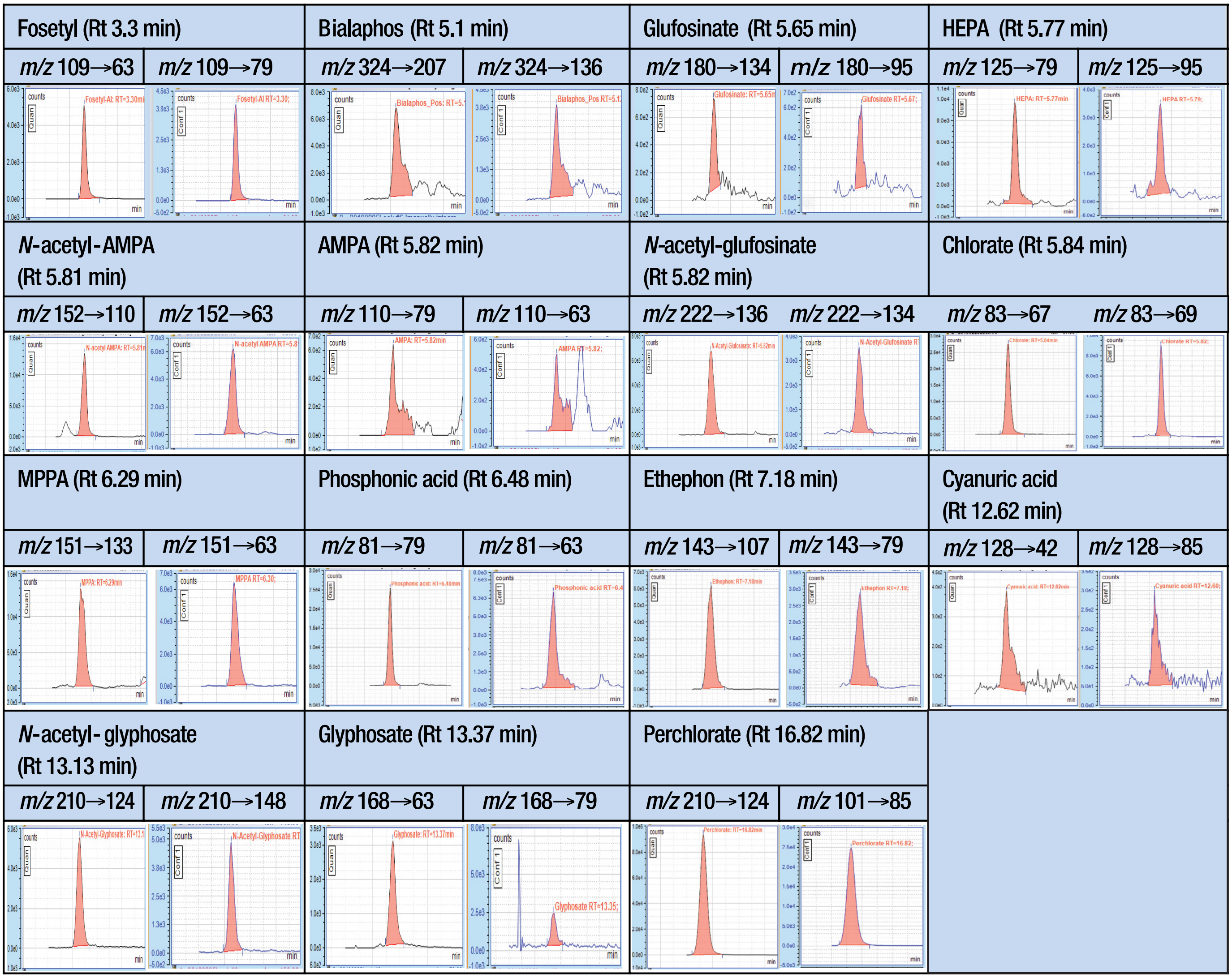


Figure 5. Response for quantification and qualifier product ions for individual anionic pesticides equivalent to 10 ng/g in leek

Table 3. Ion ratios of analytes at 10 ng/g in wheat flour matrix

Wheat flour	Quantifier ion	Qualifier ion	Ion ratios	Range
Fosetyl-Al	81	63	38.88	within ±30%
Bialaphos	207	136	39.42	within ±30%
Glufosinate	95	134	75.93	within ±30%
HEPA	79	95	36.36	within ±30%
N-acetyl AMPA	110	63	54.86	within ±30%
N-acetyl-glufosinate	136	134	45.63	within ±30%
AMPA	79	63	88.35	within ±30%
Chlorate	67	69	36.27	within ±30%
MPPA	133	63	36.96	within ±30%
Phosphonic acid	79	63	29.83	within ±30%
Ethephon	107	79	46.86	within ±30%
Cyanuric acid	42	85	73.96	within ±30%
N-acetyl-glyphosate	150	124	69.24	within ±30%
Glyphosate	63	79	100.73	within ±30%
Perchlorate	83	85	31.77	within ±30%

Table 4. Ion ratios of analytes at 10 ng/g in leek matrix

Leek	Quantifier ion	Qualifier ion	Ion ratios	Range
Fosetyl-Al	63	79	78.12	within ±30%
Bialaphos	207	136	54.68	within ±30%
Glufosinate	134	95	114.44	within ±30%
HEPA	79	95	28.85	within ±30%
N-acetyl AMPA	110	63	47.38	within ±30%
N-acetyl-glufosinate	136	134	48.82	within ±30%
AMPA	79	63	71.27	within ±30%
Chlorate	67	69	31.28	within ±30%
MPPA	133	63	42.33	within ±30%
Phosphonic acid	79	63	32.82	within ±30%
Ethephon	107	79	52.49	within ±30%
Cyanuric acid	42	85	69.39	within ±30%
N-acetyl-glyphosate	124	148	84.24	within ±30%
Glyphosate	63	79	68.85	within ±30%
Perchlorate	83	85	27.37	within ±30%

Calibration

Matrix-matched calibration curves were linear over the concentration range equivalent to 4–100 ng/g in wheat flour and leek matrices. Residuals (or back-calculated concentrations) were compliant with SANTE guidelines.[1] The matrix-matched external calibration graphs for wheat are shown in Figure 6.

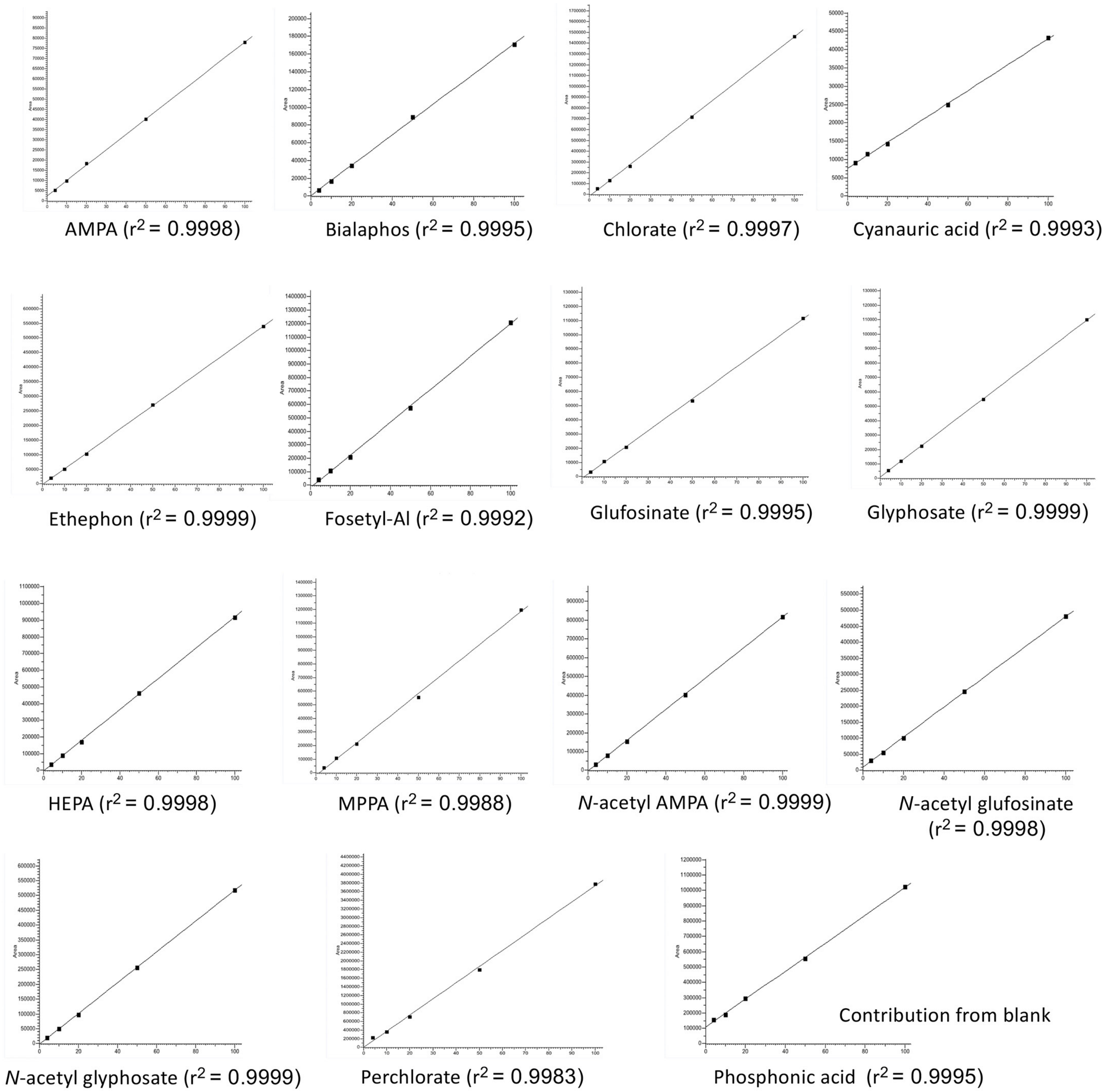


Figure 6. Matrix-matched calibration curves in wheat flour matrix

Recovery and precision

The optimized system provided excellent results for analytes in both matrices when using matrix-matched standards in combination with ILIS. Results for wheat flour are shown in Table 5. As has been widely reported for some compounds, the use of ILIS did substantially improve the recovery compared to matrix-matched calibration without ILIS as shown in Table 5. For example, the recovery for glyphosate improved from 40% to 111% and for perchlorate from 60% to 100%.

The use of ILIS is effective, but it is also costly and the appropriate labeled standards are not always readily available in some parts of the world. The precision of results for all analytes was excellent, with or without ILIS with RSDs of 0.9–9% and 1–12%, respectively, at 10 ng/g, an indication that the use of procedural standards could be an acceptable alternative.

Therefore, the use of procedural standards (prepared by spiking sub-samples with analytes over a range of known concentrations) was evaluated. In theory these samples will be subject to losses similar to samples and thus correct for recovery losses, and it is an approach permitted in the EU SANTE guidance document.[1]

Using procedural standards without ILIS resulted in excellent recoveries for all analytes, in wheat, in the range 84% to 104% with associated RSDs in the range 0.9% to 7%. However, when procedural standards were applied to different individual samples the results were more variable, and not quantitative for all analytes, as shown in Table 6. Although the semi-quantitative results may be considered sufficient for screening, improved accuracy for quantitation will require the use of ILIS or standard addition. When applied to leek, the results were more consistent (Table 7) and the use of ILIS was not necessary, because of lower matrix effects in leek compared to wheat flour.

Table 5. Summary of results for recovery and precision using different calibration approaches for wheat flour

	Spiked 10 ng/g (n=5)								Spiked 50 ng/g (n=5)							
	MMS no ILIS		MMS + ILIS		PS no ILIS		PS + ILIS		MMS no ILIS		MMS + ILIS		PS no ILIS		PS + ILIS	
	Rec. %	RSD %	Rec. %	RSD %	Rec. %	RSD %	Rec. %	RSD %	Rec. %	RSD %	Rec. %	RSD %	Rec. %	RSD %	Rec. %	RSD %
Fosetyl-Al	93	2.7	-	-	96	2.7	-	-	89	1.9	-	-	94.6	1.08	-	-
Bialphos	96	6.4	-	-	95	5.7	-	-	90	3.2	-	-	76	9.5	-	-
Glufosinate	85	12	92	8.6	87	12	92	8.6	76	4.5	94	3.0	95	6.0	111	4.8
AMPA	65	6.6	115	6.1	104	6.5	115	6.1	61	4.9	108	9.0	98	1.5	98	6.1
HEPA	86	2.4	-	-	96	2.6	-	-	80	0.7	-	-	79	2.5	-	-
N-acetyl AMPA	85	1.0	-	-	98	1.1	-	-	81	0.6	-	-	89	1.0	-	-
N-acetyl glufosinate	79	2.4	-	-	87	2.8	-	-	72	2.9	-	-	83	2.4	-	-
Chlorate	77	2.2	96	1.7	100	2.3	96	1.7	73	2.0	92	0.8	89	2.3	96	1.5
MPPA	71	1.0	96	1.4	95	1.1	96	1.4	63	2.5	94	1.9	101	2.3	101	2.0
Phosphonic acid	36	25*	-	-	84	14	-	-	69	3.4	-	-	85	3.5	-	-
Ethephon	79	1.4	97	2.1	100	1.4	97	2.1	74	0.9	92	0.3	99	0.4	99	2.0
Cyanauric acid	87	12	-	-	95	12	-	-	89	1.8	-	-	87	5.0	-	-
N-acetyl-glyphosate	60	2.9	-	-	100	3.0	-	-	53	1.7	-	-	98	1.2	-	-
Glyphosate	40	4.5	111	2.2	104	5.4	111	2.2	34	2.0	100	1.5	100	2.2	101	2.0
Perchlorate	66	4.2	100	0.9	90	5.2	100	0.9	63	3.0	96	0.7	101	0.6	100	0.3

Note: PS = procedural standards
* Poor precision to phosphonic acid contribution from blank

Table 6. Wheat flour data summary- procedural standards

Sample No. 7 used as calibration curve matrix	PS Curve	Spiked level (10 ng/g)							
		Sample No. 7 (n=5)		Sample No. 4 (n=3)		Sample No. 6 (n=3)		Sample No. 9 (n=3)	
		Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
AMPA	ISTD	108	6.5	114	7.6	86	6.5	111	4.1
Chlorate	ISTD	98	1.7	84	2.0	87	2.4	77	0.8
Ethephon	ISTD	97	2.2	103	3.7	100	7.6	103	1.5
Glufosinate	ISTD	98	8.7	88	8.7	95	5.5	100	7.3
Glyphosate	ISTD	101	2.4	90	4.1	93	9.9	99	7.3
MPPA	ISTD	96	1.4	102	3.1	116	2.2	97	1.7
Perchlorate	ISTD	86	1.0	95	1.1	88	4.0	77	2.0
Bialaphos	No ILIS	95	5.7	67	0.3	58	4.5	68	2.0
Fosetyl-Al	No ILIS	96	2.7	85	1.8	75	2.0	49	1.3
HEPA	No ILIS	95	2.6	85	2.6	80	6.5	87	4.6
N-acetyl AMPA	No ILIS	97	1.1	95	4.0	79	1.0	91	0.9
N-acetyl-glufosinate	No ILIS	87	2.8	94	0.9	68	2.4	92	1.6
N-acetyl-glyphosate	No ILIS	100	3.0	68	3.7	59	2.7	87	2.3
Phosphonic acid	No ILIS	84	14	87	1.7	79	1.9	93	2.2

Over-spiking/or standard addition is the only option without availability of ILIS

Table 7. Summary of results for recovery and precision using different calibration approaches for leek

	Spiked 10 ng/g (n=5)								Spiked 50 ng/g (n=5)							
	MMS no ILIS		MMS + ILIS		PS no ILIS		PS + ILIS		MMS no ILIS		MMS + ILIS		PS no ILIS		PS + ILIS	
	Rec. %	RSD %	Rec. %	RSD %	Rec. %	RSD %	Rec. %	RSD %	Rec. %	RSD %	Rec. %	RSD %	Rec. %	RSD %	Rec. %	RSD %
Fosetyl-Al	97	0.8	-	-	93	0.8	-	-	96	1.1	-	-	95	1.1	-	-
Bialphos	122	8.3	-	-	82	9.0	-	-	98	8.2	-	-	76	9.5	-	-
Glufosinate	90	2.9	94	7.1	96	7.5	119	8.0	92	2.9	85	5.1	95	6.0	111	4.8
AMPA	100	7.6	111	8.4	96	8.4	94	9.6	95	2.5	104	6.1	98	1.5	98	6.1
HEPA	99	7.5	-	-	87	6.1	-	-	100	2.4	-	-	79	2.5	-	-
N-acetyl AMPA	91	1.0	-	-	100	0.7	-	-	101	1.0	-	-	89	1.0	-	-
N-acetyl glufosinate	102	1.9	-	-	88	1.6	-	-	105	2.3	-	-	83	2.4	-	-
Chlorate	93	2.6	86	2.1	86	2.6	105	1.8	97	2.2	90	1.5	89	2.3	96	1.5
MPPA	94	1.9	89	1.7	96	1.9	92	1.8	100	2.3	95	2.0	101	2.3	101	2.0
Phosphonic acid*	64	12	-	-	85	8.1	-	-	87	3.6	-	-	85	3.5	-	-
Ethephon	97	2.5	104	2.7	97	2.7	102	2.8	96	0.4	98	1.9	99	0.4	99	2.0
Cyanauric acid	118	4.6	-	-	104	4.4	-	-	100	4.9	-	-	87	5.0	-	-
N-acetyl-glyphosate	93	0.9	-	-	103	0.8	-	-	96	1.2	-	-	98	1.2	-	-
Glyphosate	91	1.6	90	1.5	95	1.6	94	1.6	95	2.2	94	2.0	100	2.2	101	2.0
Perchlorate	93	0.6	89	0.4	95	0.6	98	0.4	96	0.6	90	0.3	101	0.6	100	0.3

*Recovery and precision are less accurate for phosphonic acid because of an incurred residue in the blank.

Robustness of IC-MS/MS system

The inclusion of the Dionex OnGuard II RP cartridge clean-up substantially improved the robustness of the workflow compared to analysis of samples with no clean-up. After 500 injections of matrix extracts, the retention times and peak shapes remained stable as shown in Figure 7 for fosetyl-Al (first peak) and perchlorate (last peak). From the first to the 500th injection there was no change in the retention time for fosetyl and 0.14 min difference for perchlorate. Also, the column and the mass spectrometer source remained clean with no required maintenance, while pressure in the suppressor was consistent.

Conclusion

The new integrated workflow based on the modified QuPPe method and IC-MS/MS supports simultaneous multi-residue analysis for anionic pesticides. All of the chromatographic and mass spectrometer parameters have been carefully optimized so the workflow provides excellent sensitivity to meet EU Maximum Residue Levels and quantitative analysis of parent and metabolite pesticides to meet the EU residue definitions. The excellent precision and accuracy provides results compliant with the EU SANTE guidelines for method validation and ongoing quality control for pesticides. The excellent precision is due to the inert peek flowpath in the IC system, which negates contamination of the columns or chelation of analytes from metal ions that can leach from stainless steel LC systems. For the analysis of wheat flour, the use of ILIS provides improved accuracy and precision compared to the use of matrix-matched standards but does not correct for any deficiencies in the extraction of incurred residues. In cases where ILIS are not available, the standard addition approach provides accurate quantitation of the residue concentrations in extracts, while the procedural standard approach may be acceptable for screening. Overall, this workflow, which is compliant with EU SANTE guidelines, will supply a sensitive and reliable method for simultaneous multi-residue analysis of polar anionic pesticides in complex samples. Extensive testing over several months and more than 1500 sample injections has demonstrated the Anionic Pesticides Explorer to be reliable, reproducible, and robust and hence suitable for routine analysis.

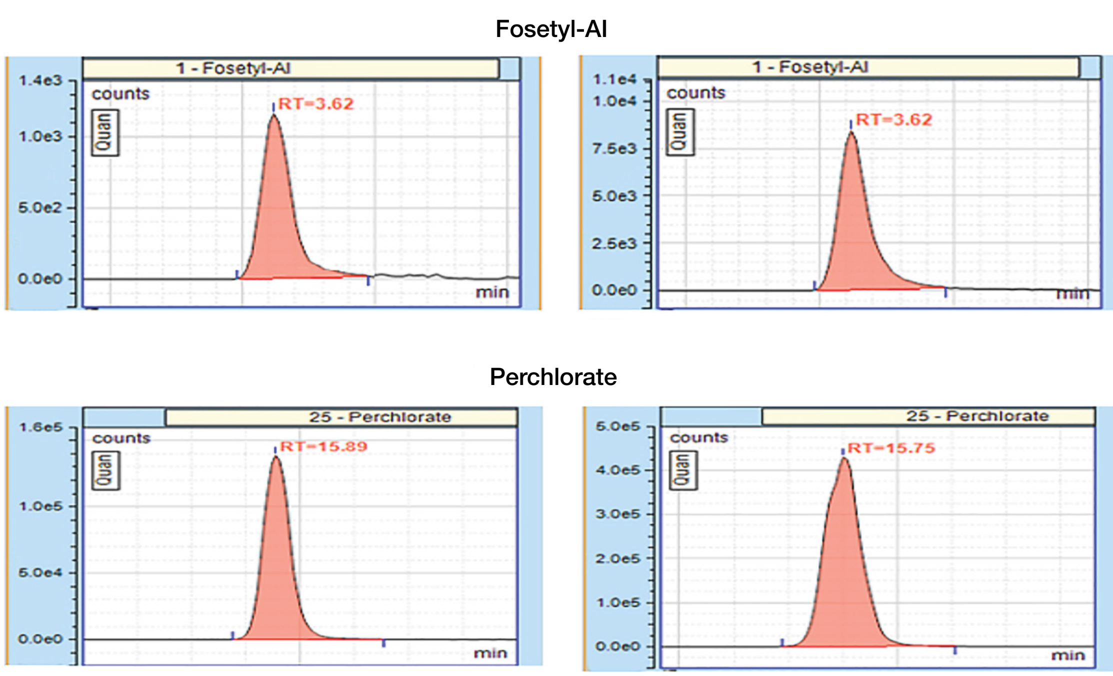


Figure 7. Peak shapes comparison of fosetyl-Al and perchlorate after 500 injections of matrix

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