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APPLICATION NOTE 72915

Determination of polar pesticides in grapes using a compact ion chromatography system coupled with tandem mass spectrometry

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Keywords

AMPA, Glyphosate, Integrion, HPIC system, Dionex IonPac AS19-4µm column, TSQ Quantis triple quadrupole mass spectrometer, polar pesticides

Goal

To develop and test a method based on ion chromatography (IC) coupled with a triple quadrupole mass spectrometer (IC-MS/MS) for the determination of polar pesticides and their metabolites in grapes. Method performance should be in compliance with statutory maximum residue levels (MRL)/tolerance levels, residue definitions, and relevant guidelines for method validation and analytical quality control.

Introduction

The group of polar ionic pesticides include some of the most frequently used pesticides worldwide.[1] Although these compounds result in residues in food and have been the subject of recent controversy, they have been monitored infrequently in food-testing programs. In the United States, for example, a report by the Government Accounting Office[2] criticized the responsible government agencies [Environmental Protection Agency (EPA), Food and Drug Administration (FDA), and Department of Agriculture (USDA)] with respect to the lack of testing for glyphosate residues in food. The lack of testing is in part due to the analytical difficulties and higher costs associated with the single-residue methods that were until recently the only options available.

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Polar ionic pesticides are challenging analytes. First, they have very low recovery due to losses in the aqueous fraction when using liquid/liquid partition methods based on QuEChERS, ethyl acetate, and mini-Luke.[3] Second, they have poor retention in reversed-phase LC, which is widely used for the multi-analyte determination of the majority of pesticides.[4] The problem with recovery can be overcome by the use of a more polar water-miscible solvent such as methanol.[3] One approach used to overcome the issue of low retention using reversed-phase chromatography is derivatization of the analyte prior to liquid chromatography–mass spectrometry (LC-MS) analysis.[5] Derivatization can be time consuming and is an extra step that potentially has a negative influence on analysis precision. Alternative, more convenient approaches to achieve greater retention of polar compounds include ion-pair reversed-phase LC, normal-phase chromatography, the use of graphitized carbon columns, and ion chromatography (IC).

Of these, IC coupled to MS (IC-MS) offers a number of advantages for the separation and quantification of polar anionic and cationic pesticides and their polar metabolites. Ion chromatography provides excellent chromatographic resolution in a wide range of matrices, while triple quadrupole mass spectrometer systems offer low detection limits and high selectivity when operated in the selected reaction monitoring (SRM) mode. The IC-MS system robustness allows the routine analysis of food and environmental samples.

The most commonly used approach for the extraction of polar analytes is the quick polar pesticides method (QuPPe) developed by the European Reference Laboratory for Single Residue Methods.[6] Although the method is capable of extracting a wide range of polar analytes, it lacks both a liquid/liquid partition, and a clean-up step, resulting in “dirty extracts” containing high concentrations of matrix-co-extractives. Thus, the separation and accurate quantification of polar pesticides in QuPPe extracts is challenging.

The aim of this work is to develop and validate an IC-MS/MS method for the direct analysis of a total of 16 compounds including 15 polar ionic pesticides and relevant metabolites: glyphosate and metabolites (AMPA and *N*-acetyl glyphosate), bialaphos, chlorate, cyanuric acid, ethephon (and HEPA), fosetyl-aluminium (and phosphoric acid), glufosinate, *N*-acetyl glufosinate, MPPA, maleic hydrazide, *N*-acetyl AMPA, and perchlorate (technically a contaminant). An initial assessment of using the modified QuPPe prior to IC-MS/MS was made using grapes.

Experimental

Equipment and consumables

IC

- Thermo Scientific™ Dionex™ Integrion™ HPIC system including:
  - Eluent Generator
  - Pump
  - Degasser
  - Conductivity Detector (CD)
  - Column Oven Temperature Control
  - Detector-Suppressor Compartment Temperature Control
  - Tablet Control
- Thermo Scientific™ Dionex™ AS-AP Autosampler with Sample Syringe, 250 µL (P/N 074306) and Buffer line, 1.2 mL (P/N 074989)
- Thermo Scientific™ Dionex™ 6-port 2 position valve kit for matrix elimination prior to MS (P/N 22153-62027)
- Two Thermo Scientific™ Dionex™ AXP-MS Auxiliary Pumps for make-up flow and ADRS 600 regeneration (P/N 60684)
- Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column (P/N 088662)
- Thermo Scientific™ Dionex™ ADRS 600 Anion Dynamically Regenerated Suppressor (2 mm), (P/N 088667)
- Thermo Scientific™ Dionex™ IC PEEK Viper™ Fitting Tubing Assembly Kit (P/N 088798)
- IC-MS Installation Kit (P/N 22153-62049)
- Thermo Scientific™ Dionex™ AS-AP Autosampler Vial Kit 10 mL (P/N 074228), 1.5 mL (P/N 079812) or 0.3 mL (P/N 055428)

Mass spectrometer

- Thermo Scientific™ TSQ Quantis™ Triple Quadrupole Mass Spectrometer (P/N TSQ02-10001)

Consumables

- Thermo Scientific™ Dionex™ OnGuard™ II RP Cartridges, 2.5 mL (P/N 057084)
- Thermo Scientific™ Nalgene™ 25 mm Syringe Filters, PES, 0.2 µm (P/N 7252520)
- AirTite™ All-Plastic Norm-Ject™ Syringes, 10 mL, Sterile (Fisher Scientific P/N 14-817-31)
- Thermo Scientific™ Nalgene™ 1000 mL, 0.2 µm Nylon Filter Units (P/N 09-740-46)

Software

Data acquisition

- Thermo Scientific™ Chromeleon™ Chromatography Data System software version 7.2.6 or higher

Or

- Thermo Scientific™ Xcalibur™ 4.1 software with SII for Xcalibur software

Or

- Thermo Scientific™ TraceFinder™ 4.1 software

Data processing

- Thermo Scientific™ TraceFinder™ 4.1 software

Reagents and standards

Reagents

- Deionized (DI) water, Type I reagent grade, with 18 MΩ·cm resistivity or better filtered through a 0.2 µm filter immediately before use
- Thermo Scientific™ Pierce™ Triple Quadrupole Calibration Solution, extended mass range (P/N 88340)
- Methanol, Optima™ LC/MS Grade, Fisher Chemical (P/N A456-1)
- Acetonitrile, Optima™ LC/MS Grade, Fisher Chemical (P/N A955-1)

Standards

- QPP-Lab® Standard Kit 1.3 QuPPe-PO V.9.3 Working Solutions (www.labinstruments.org, P/N CRM3G11L346)
- Maleic hydrazide, 97%, Alfa Aesar™ (Fisher Scientific P/N AAA1253122)
- Glyphosate (Chemical Purity 95%) (13C3, 99%; 15N, 98%) 100 µg/mL in water, (Cambridge Isotope Laboratories, P/N CNLM-6792-1.2)

Samples

- Fresh seedless green grape samples labeled as organic and purchased from a local retail outlet.

Conditions

IC System:	Dionex Integrion HPIC system
MS Detector:	TSQ Quantis triple quadrupole mass spectrometer
Columns:	Thermo Scientific™ Dionex™ IonPac™ AS19-4µm Guard, 2 × 50 mm (P/N 083225) Thermo Scientific™ Dionex™ IonPac™ AS19-4µm Analytical, 2 × 250 mm (P/N 083223)
Eluent Source:	Dionex EGC 500 KOH Eluent Generator Cartridge with Dionex CR-ATC 600
Gradient:	15–20 mM (0–4 min), 20–75 mM (4–10 min), 75 mM (10–18 min), 75–15 mM (18–18.1), 15 mM (18.1–20 min)
Flow Rate:	0.35 mL/min
Injection Volume:	25 µL
Temperature:	30 ºC (column compartment), 20 ºC (detector compartment)
System Backpressure:	~4300 psi (100 psi = 0.6894 MPa)
Detection:	Suppressed Conductivity, Dionex ADRS 600 Suppressor (2 mm) operated in Legacy mode, AutoSuppression, 65 mA, external water mode via AXP-MS Pump, external water flow rate (0.70 mL/min)
Background Conductance:	~0.7 µS/cm
Run Time:	20 min

Conditions for mass spectrometric detection

IC-MS Interface:	Tee union to combine the analyte from conductivity detector via Viper fitting tubing
Post Suppressor Makeup Solution:	Acetonitrile at 0.2 mL/min via AXP-MS pump

Conditions for mass spectrometric detection (continued)

Ion Source	
Ion Source Type:	HESI
Spray Voltage:	Static
Negative Ion:	3800 V
Sheath Gas:	42 Arbitrary units (Arb)
Aux Gas:	12 Arb
Sweep Gas:	1 Arb
Ion Transfer Tube Temp:	300 °C
Vaporizer Temp:	300 °C
MS Global Settings	
Start Time:	0 min
End Time:	20 min
Master Scan	
Scan Mode:	SRM
Polarity:	Negative
Use Cycle Time:	True
Cycle Time:	1.25 s
Use Calibrated RF Lens:	False
Q1 Resolution (FWHM):	0.7
Q3 Resolution (FWHM):	1.2
CID Gas:	1.5 mTorr
Source Fragmentation:	0 V
Chromatographic Peak Width:	25 s
Use Chromatographic Filter:	True
Use Retention Time Reference:	False
Display Retention Time:	True
Use Quan Ion:	False
Show Visualization:	False
Transitions	
Transition conditions:	Optimized for each compound using TSQ Quantis mass spectrometer (Table 1, next page)

Preparation of solutions and reagents

Deionized water was used for eluent preparation, standard preparation, and rehydrating samples. Individual stock standard solutions of 1000 mg/L were prepared gravimetrically from the reagents and DI water. QPP-Lab Standard Kit 1.3 QuPPe-PO V.9.3 Working Solutions include AMPA, bialaphos, chlorate, cyanuric acid, ethephon, fosetyl-aluminium, glufosinate, glyphosate, HEPA, MPPA, maleic hydrazide, *N*-acetyl AMPA, *N*-acetyl glufosinate, *N*-acetyl glyphosate, perchlorate, and phosphonic acid, each with a concentration of 10 mg/L. A mixed calibration standard solution was prepared by diluting the individual stock standard solutions or QuPPe-PO V.9.3 Working Solutions into 10 or 1.5 mL plastic vials with a methanol and DI water mixture (50:50). The concentrations for calibration standards in 50% methanol were 1, 2, 5, 10, 20, 30, and 50 µg/L.

The internal standard (ISTD) solution of $^{13}C_3{}^{15}N$ glyphosate at 1 mg/L was prepared by dissolving the stock standard in a methanol and DI water mixture (50:50). The internal standard concentration in the sample was 2 µg/kg, equivalent to 1 µg/L in the matrix blank.

Sample preparation

Grapes were selected for the validation of the method. Grapes represent group 2 (high acid and high water content) of the SANTE/11813/2017 guidelines on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed.[7]

Modified QuPPe extraction method

Fresh grape samples were homogenized using a kitchen blender, and the homogenized grape samples (10 ± 0.1 g each) were weighed into 50 mL polypropylene centrifuge tubes. Matrix-extracted calibration standards were prepared by spiking with standards and 20 µL of 1 mg/mL $^{13}C_3{}^{15}N$ glyphosate solution as appropriate. The spiked samples were left to stand for 10 min. DI water was added to fill 2 mL of total volume, followed by 10 mL of non-acidified methanol. The sample was then placed on a rotary shaker for 20 min. Afterwards, the samples were centrifuged at 4500 rpm for 5 min. Supernatant was filtered through a PES syringe filter (0.2 µm), followed by treatment with a Dionex OnGuard II RP cartridge.

Table 1. IC-MS/MS parameters for selected reaction monitoring transitions in negative mode

Compound	Retention Time (min)	RT Window (min)	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)
Fosetyl-Al	4.21	2	109	63	29.49	95
Fosetyl-Al	4.21	2	109	81	10.45	95
Maleic hydrazide	6.50	4	111	42	40.55	113
Maleic hydrazide	6.50	4	111	82	18.18	113
Bialaphos	7.50	4	322	172	22.32	209
Bialaphos	7.50	4	322	216	18.45	209
Bialaphos	7.50	4	322	233	17.96	209
AMPA	7.80	4	110	63	19.55	116
AMPA	7.80	4	110	79	22.74	116
AMPA	7.80	4	110	81	12.27	116
Glufosinate	7.80	3	180	95	16.82	141
Glufosinate	7.80	3	180	136	16.29	141
Chlorate	7.73	2	83	51	28.12	125
Chlorate	7.73	2	83	67	20.50	125
Chlorate	7.73	2	85	69	20.84	122
N-acetyl glufosinate	8.00	2	222	136	21.68	140
N-acetyl glufosinate	8.00	2	222	180	16.82	140
HEPA	8.10	2	125	79	21.07	110
HEPA	8.10	2	125	95	13.11	110
N-acetyl AMPA	8.40	2	152	63	25.43	123
N-acetyl AMPA	8.40	2	152	79	42.34	123
N-acetyl AMPA	8.40	2	152	110	12.50	123
Ethephon	8.93	3	143	79	17.96	75
Ethephon	8.93	3	143	107	10.23	75
MPPA	8.50	2	151	107	15.91	112
MPPA	8.50	2	151	133	12.69	112
Phosphonic acid	9.00	2	81	63	26.76	96
Phosphonic acid	9.00	2	81	79	14.28	96
Cyanuric acid	12.50	4	128	42	14.47	90
Cyanuric acid	12.50	4	128	85	10.23	90
N-Acetyl glyphosate	12.20	2	210	150	13.07	123
N-Acetyl glyphosate	12.20	2	210	192	10.23	123
Glyphosate	12.30	2	168	63	22.62	110
Glyphosate	12.30	2	168	79	38.85	110
Glyphosate ISTD	12.30	2	172	63	25.00	110
Perchlorate	17.80	3	99	83	26.19	152
Perchlorate	17.80	3	101	85	26.30	152

ISTD: Internal Standard

The cartridge was equilibrated by flushing with 10 mL methanol followed by 15 mL DI water. Sample filtrate was passed through the cartridge, and at least 1 mL of the sample was discarded. The final extracts were transferred to plastic autosampler vials and ready for IC-MS/MS analysis. The concentrations of Matrix Extracted Standards (MES) calibration standards were equivalent to 10, 20, 40, 60, and 100 µg/kg (5, 10, 20, 30, and 50 µg/L) in the sample except for maleic hydrazide, which were equivalent to 40, 60, 100, 200, and 400 µg/kg (20, 30, 50, 100, and 200 µg/L) in the sample. Plasticware was used throughout to avoid adsorption of the analytes onto glass surfaces.

Matrix-matched calibration standards (MMS) were prepared by making the highest concentration calibration standard in the matrix blank (only spiked with internal standard before extraction) and then performing serial dilution with blank that had been spiked with $^{13}C_3{}^{15}N$ glyphosate before extraction. Glyphosate labeled with $^{13}C^{15}N$ was used to control the final volume of the extract. The following concentrations were used to construct the calibration curve: 1, 2, 5, 10, 20, 30, 50, and 100 µg/L, though maleic hydrazide used the following concentrations: 10, 20, 30, 50, 100, and 200 µg/L.

Instrument setup and installation

The instrument system comprised a metal-free Dionex Integrion ion chromatograph and a Dionex AS-AP autosampler coupled to a TSQ Quantis mass spectrometer (Figure 1).

The Dionex AS-AP Autosampler was set-up in Push Mode by following the instructions in the Dionex AS-AP Autosampler Operator's Manual (Document No. 065361).[8] The syringe inlet was checked to make sure no air bubbles were present.

The Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 continuously regenerating anion trap column, and Dionex ADRS 600 suppressor were set up according to the product manual instructions.[9-11] Note: The system pressure needs to be above 2000 psi for effective degassing of the eluent generator produced KOH. The TSQ Quantis mass spectrometer is installed according to the TSQ documents (Document numbers 80111-97046, 80111-97047, and 80111-97048).[12-14]

The IC-MS/MS flow path is described as follows: Deionized water from the pump enters the EGC, which generates the eluent. Eluent exits the Dionex EGC and passes through the Dionex CR-TC (which traps anionic

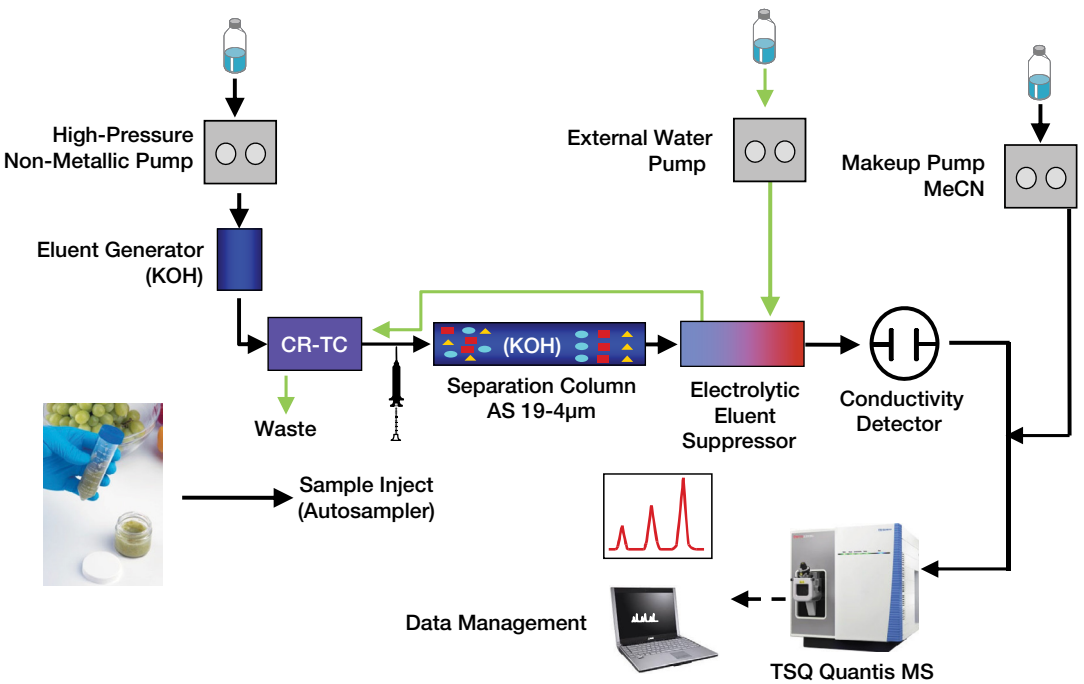

Figure 1. IC-MS/MS system configuration

contaminants), through the EG degas tubing to remove the hydrogen gas produced during KOH generation, and then into the injection valve. After the sample is loaded into the sample loop and the injection valve is toggled to the Inject position, eluent passes through the loop. The pump pushes the eluent and sample through the guard and analytical columns, and then through the suppressor, where the cations from both the eluent and the sample are replaced with hydronium ions, effectively neutralizing the high pH eluent and rendering it compatible with a mass spectrometer. The Dionex ADRS 600 suppressor runs in external mode using DI water delivered by an AXP-MS pump for the regenerant. From the suppressor, the flow goes into the conductivity detector to monitor the background as in this application analyte concentrations are typically too low to be detected by suppressed conductivity. The background is typically below 1.5 µS/cm before injection of a sample or standard. A second 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.

Mass spectrometer conditions

Data acquisition was performed in selected reaction monitoring mode (SRM). All SRM traces (precursor, quantifier, and qualifier ions) were individually tuned for each target analyte using TSQ Quantis 3.1 Tune software by infusing the corresponding standard solution (10 mg/L). The mass spectrometer conditions are shown in "Conditions" and SRM parameters for analyzing targeted analytes are shown in Table 1. Data was acquired using Chromeleon CDS 7.2.6 or Xcalibur 4.1 software with SII for Xcalibur software and processed using TraceFinder 4.1 software, which allow easy creation of the acquisition and processing methods for high-throughput quantitative analysis along with data reviewing and reporting.

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

Because the target analytes are small molecules with low mass-to-charge (*m/z*) product ions, calibrate the mass spectrometer with the Pierce Triple Quadrupole Extended Mass Range Calibration Solution. It consists of 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 where many of the polar pesticides are found.

Results and discussion

IC-MS/MS separation

In this study, a fast IC-MS/MS method was developed to separate 16 polar pesticides on the Dionex IonPac AS19-4µm column set at 30 °C. The IC eluent flow rate was 0.35 mL/min with a gradient from 15 mM KOH to 20 mM KOH at 4 min, then to 75 mM KOH at 15 min, held at 75 mM KOH until 18 min to elute perchlorate, and back to 15 mM KOH at 18 min to re-equilibrate the column prior to the next injection. The total run time was 20 min. The KOH eluent was neutralized using a Dionex ADRS 600 2 mm dynamically regenerated suppressor. The injection volume was 25 µL for grape samples, which caused less distortion of peak shape compared to 100 µL.

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 on the TSQ Quantis mass spectrometer (max flow rate 3 mL/min). The backpressure on the suppressor was checked and found to be satisfactory below 150 psi.[11,15]

A good IC-MS/MS separation was achieved to resolve 16 analytes in different SRM channels. (Figure 2). Fosetyl tends to degrade into phosphonic acid both in solution and in the IC-MS/MS via in-source fragmentation. Phosphonic acid and fosetyl were fully separated on the Dionex IonPac AS19-4µm column with retention times of 8.92 min and 3.56 min, respectively (Figure 2).

Ion extracted chromatograms for SRM transitions are shown in Figure 3. Peak shape and sensitivity were good for the majority of polar pesticides at 10 µg/L in grape matrix (equivalent to 20 µg/kg in sample). Acceptable peak shape were obtained for AMPA (10 µg/L), bialaphos (10 µg/L), and maleic hydrazide (20 µg/L) in grape matrix.

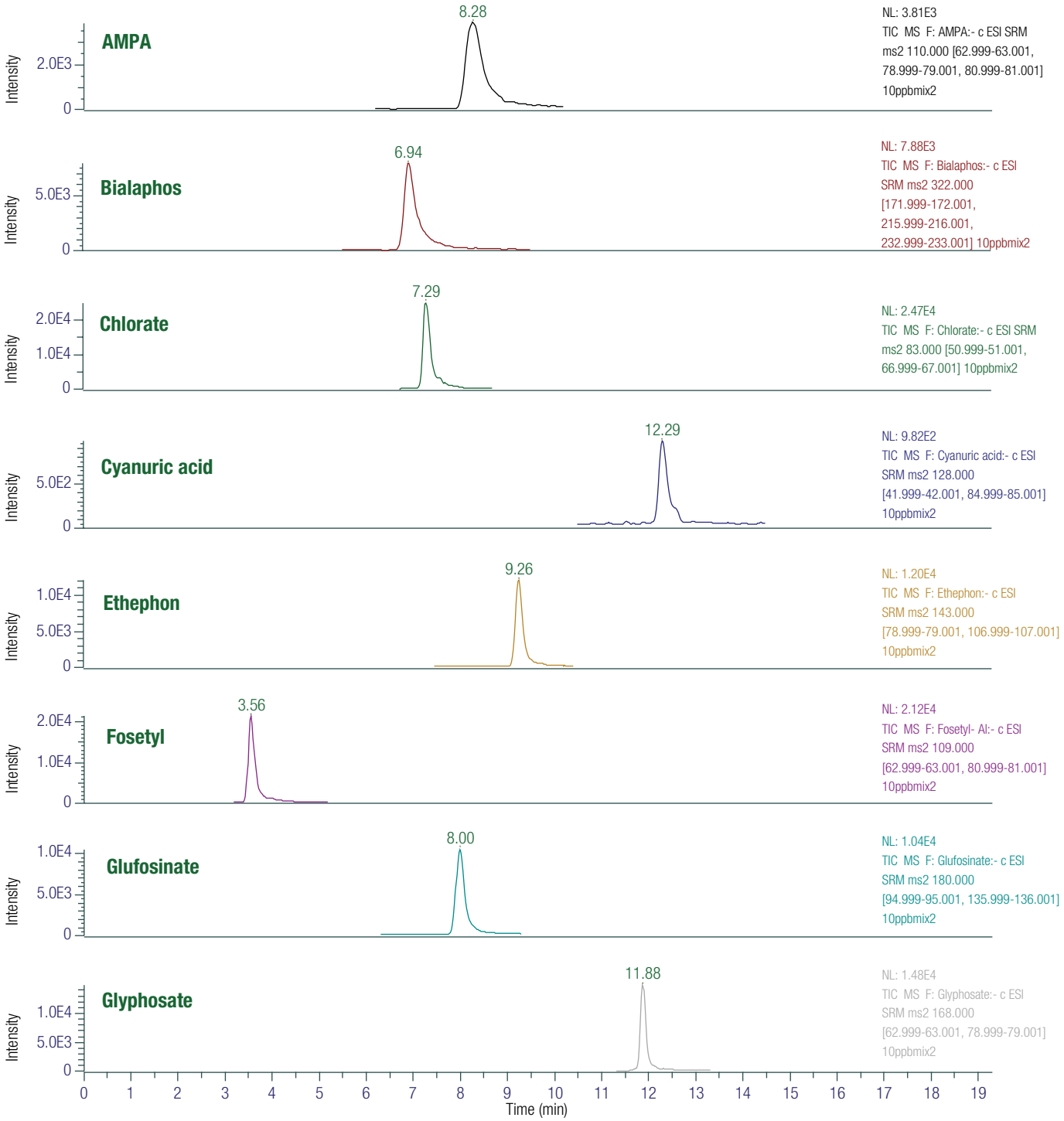

Figure 2 (part 1). SRM chromatograms of polar pesticides (first 8 of 16, 10 µg/L each)

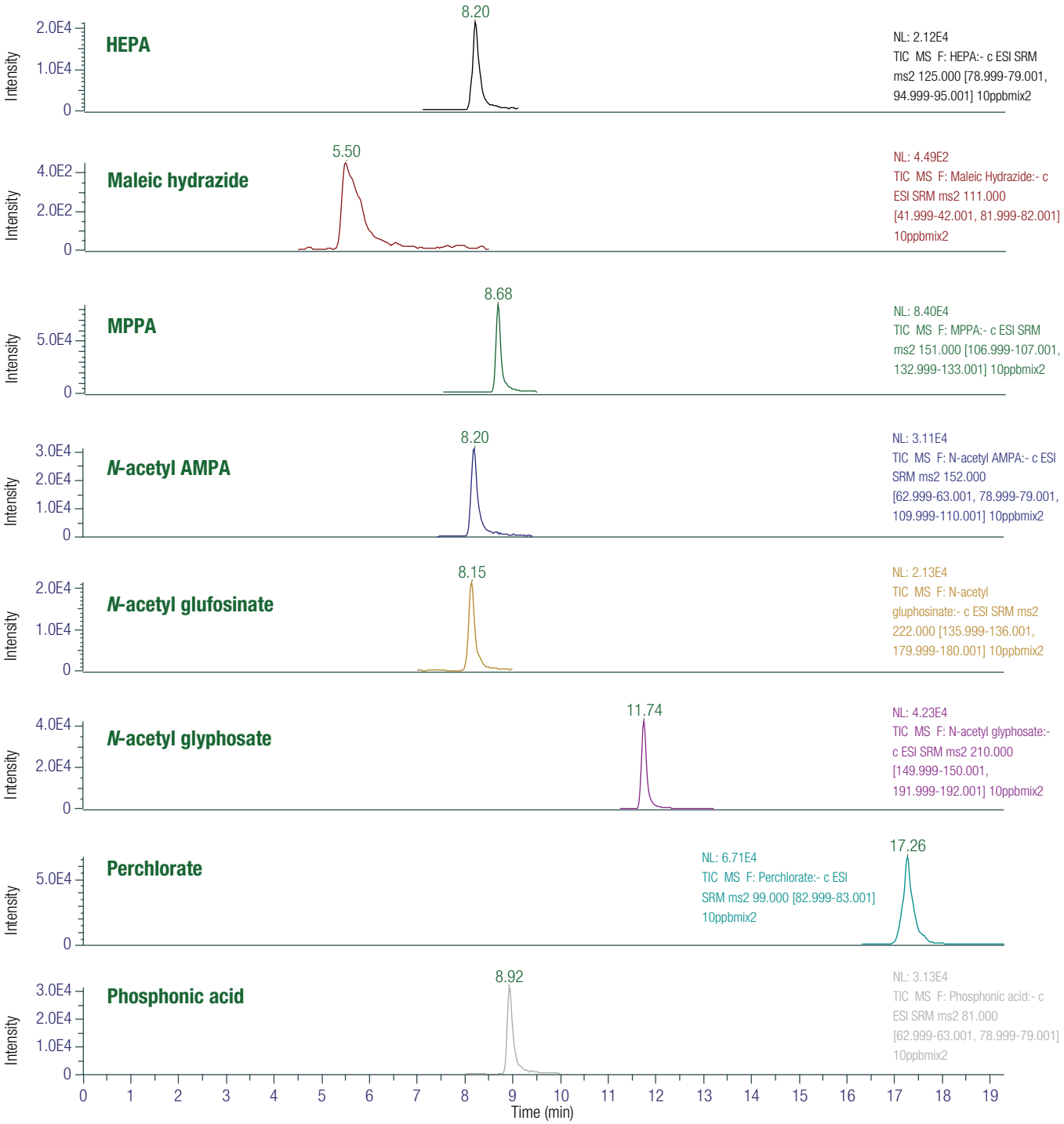

Figure 2 (part 2). SRM chromatograms of polar pesticides (second 8 of 16, 10 µg/L each)

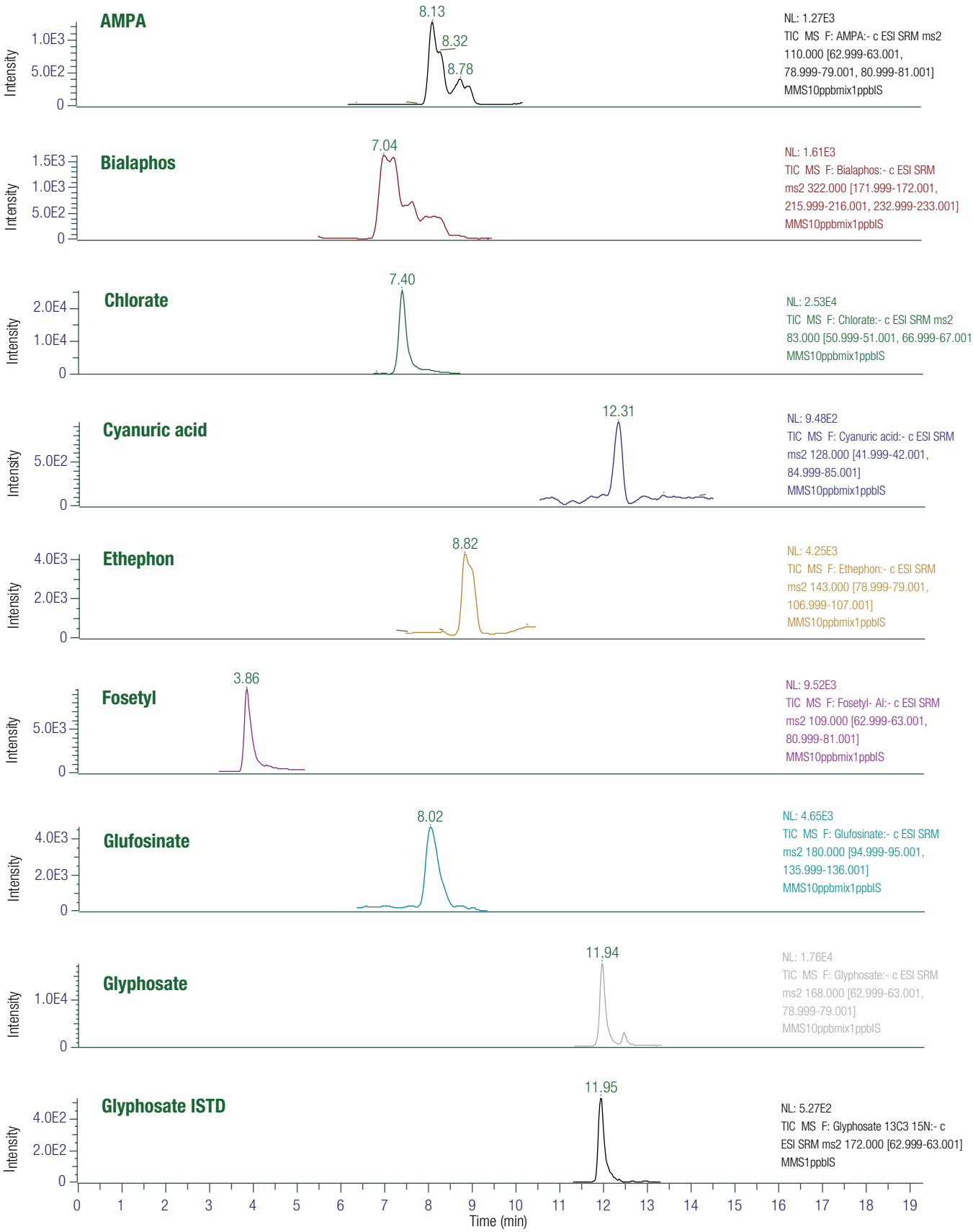

Figure 3 (part 1). SRM chromatograms of the first 8 of 16 polar pesticides in spiked grape matrix at 10 µg/L with the exception of the 1 µg/L spike concentration for the glyphosate ISTD (chromatogram 9)

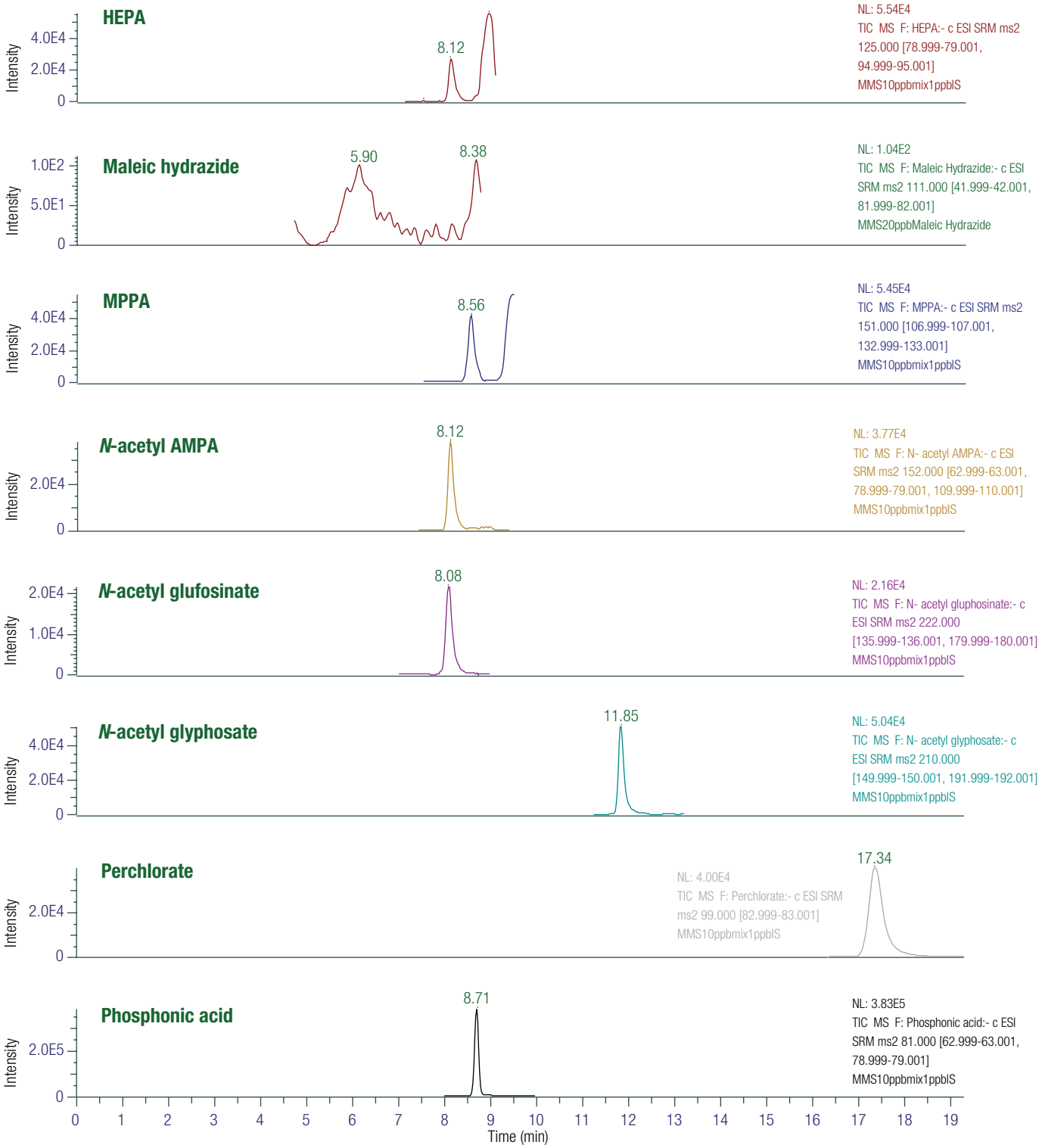

Figure 3 (part 2). SRM chromatograms of the second 8 of 16 polar pesticides in spiked grape matrix at 10 µg/L with the exception of the 20 µg/L spike concentration for maleic hydrazide

Calibration and linearity

Three calibration curves were constructed using standards in neat solvents, MMS, and MES, respectively. Table 2 shows the quantifier transitions, calibration ranges, and the coefficients of determination (r^2) for each analyte in the three different matrices; coefficients of determination obtained ranged 0.9953–0.9999. Coefficient of determinations (r^2) of the calibration curves that were internally and externally standardized range from 0.9961 to 0.9999. The method provides better LOQs than EU maximum residue levels (MRLs) (Figures 4–9). The EU residue definition is for ethephon (parent only) and the MRL is set at 1 mg/kg, glyphosate (parent only) and the MRL is set at 0.5 mg/kg, perchlorate only and the MRL is set at 0.1 mg/kg, chlorate only and the MRL is set at 0.01 mg/kg, and fosetyl as sum of fosetyl, phosphonic acid, and their salts and the MRL is set at 100 mg/kg in table grapes, as well as glufosinate as the sum of glufosinate, *N*-acetyl glufosinate, MPPA, and their salts, and the MRL is set at 0.15 mg/kg in wine grapes.[16]

Table 2. Method calibrations for 16 polar pesticides using neat standards, MMS, and MES

Analyte	Quantifier Transition	Standards in MeOH/ DI water (50:50)		MMS		MES	
		Range (µg/L)	Coefficient of Determination* (r^2)	Range (µg/L)	Coefficient of Determination *(r^2)	Range (µg/L)	Coefficient of Determination* (r^2)
AMPA	110→63	1–50	0.9989	1–100	0.9985	5–50	0.9973
Bialaphos	322→216	1–50	0.9999	1–100	0.9997	5–50	0.9993
Chlorate	83→67	1–50	0.9994	1–100	0.9984	5–50	0.9982
Cyanuric acid	128→85	2–50	0.9992	10–100	0.9994	10–50	0.9918
Ethephon	143→107	1–50	0.9997	1–100	0.9995	5–50	0.9987
Fosetyl	109→81	1–50	0.9991	1–100	0.9997	5–50	0.9991
Glufosinate	180→136	1–50	0.9993	1–100	0.9996	5–50	0.9991
Glyphosate	168→63	1–50	0.9990	1–100	0.9996 0.9995**	5–50	0.9975 0.9992**
HEPA	125→79	1–50	0.9991	1–100	0.9999	5–50	0.9961
Maleic hydrazide	111→82	2–50	0.9994	10–200	0.9995	20–200	0.9992
MPPA	151→133	1–50	0.9985	1–100	0.9995	5–50	0.9986
N-acetyl AMPA	152→110	1–50	0.9988	1–100	0.9997	5–50	0.9985
N-acetyl glufosinate	222→136	1–50	0.9995	1–100	0.9995	5–50	0.9973
N-acetyl glyphosate	210→150	1–50	0.9996	1–100	0.9998	5–50	0.9980
Perchlorate	99→83	1–50	0.9995	1–100	0.9998	5–50	0.9971
Phosphonic acid	81→79	1–50	0.9995	1–100	0.9980	5–50	0.9985

*External standard calibration, quadratic fitting
**Internal standard calibration, quadratic fitting

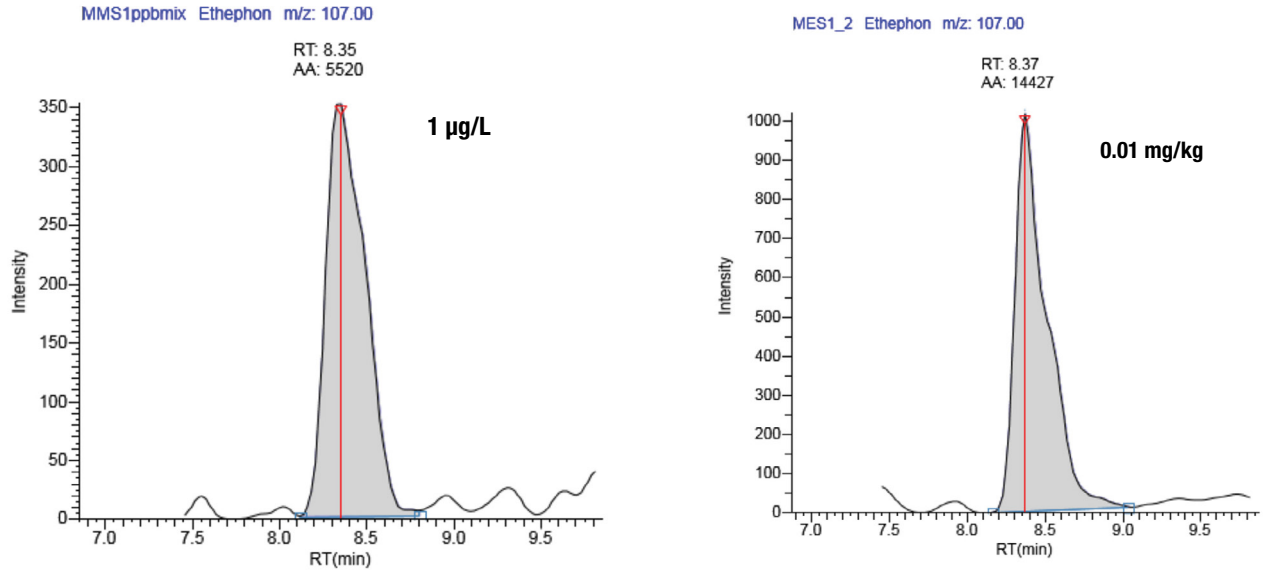

Figure 4. SRM chromatograms of ethephon MMS (1 µg/L) and MES (0.01 mg/kg) in table grapes. The EU residue definition for ethephon is ethephon only and the MRL is set at 1 mg/kg in table grapes.

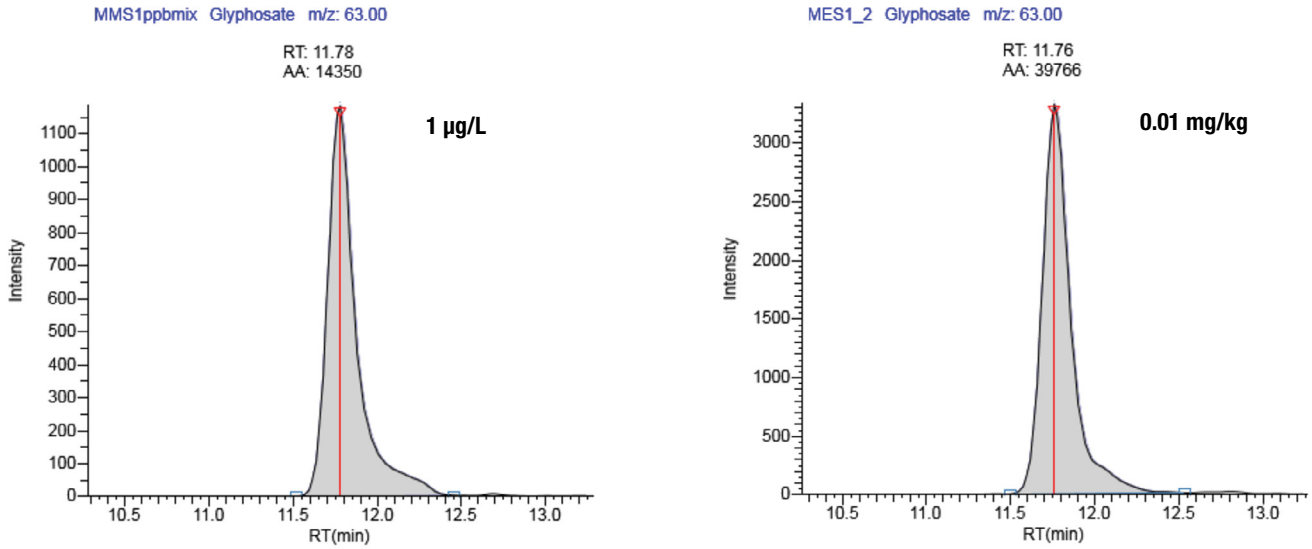

Figure 5. SRM chromatograms of glyphosate MMS (1 µg/L) and MES (0.01 mg/kg) in table grapes. The EU residue definition for glyphosate is glyphosate only and the MRL is set at 0.5 mg/kg in table grapes.

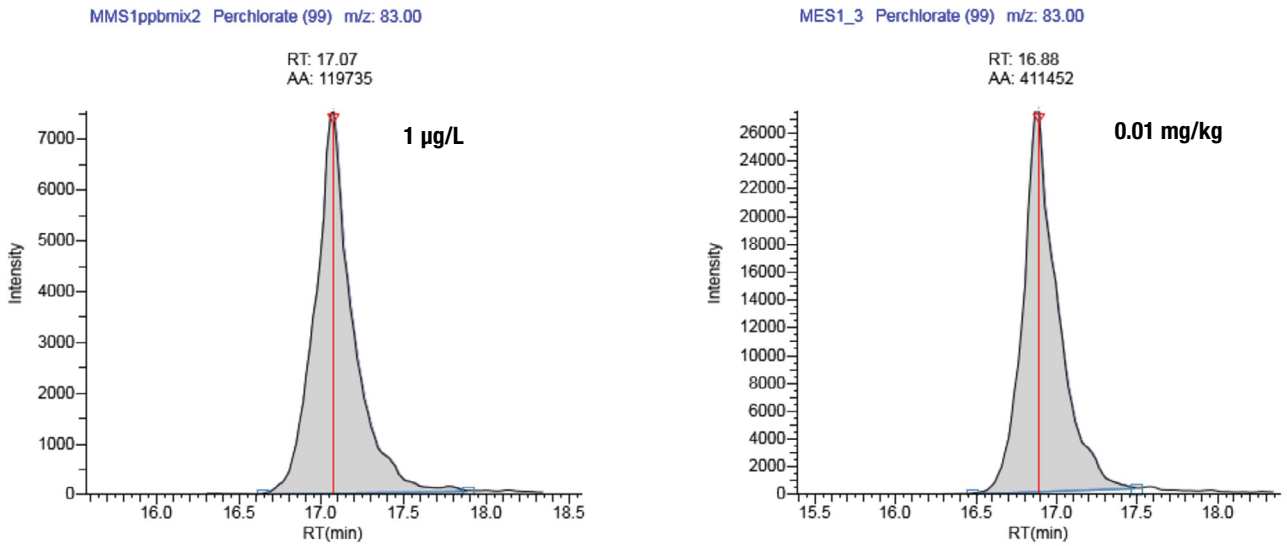

Figure 6. SRM chromatograms of perchlorate MMS (1 µg/L) and MES (0.01 mg/kg) in table grapes. The EU residue definition for perchlorate is perchlorate only and the MRL is set at 0.1 mg/kg in table grapes.

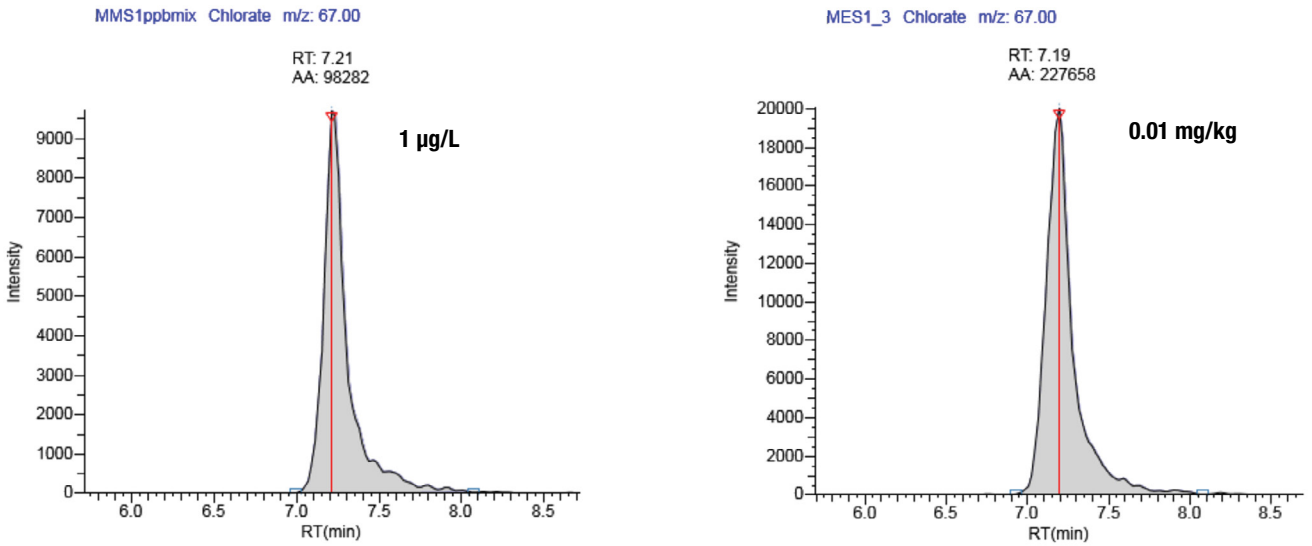

Figure 7. SRM chromatograms of chlorate MMS (1 µg/L) and MES (0.01 mg/kg) in table grapes. The EU residue definition for chlorate is chlorate only and the MRL is set at 0.01 mg/kg in table grapes.

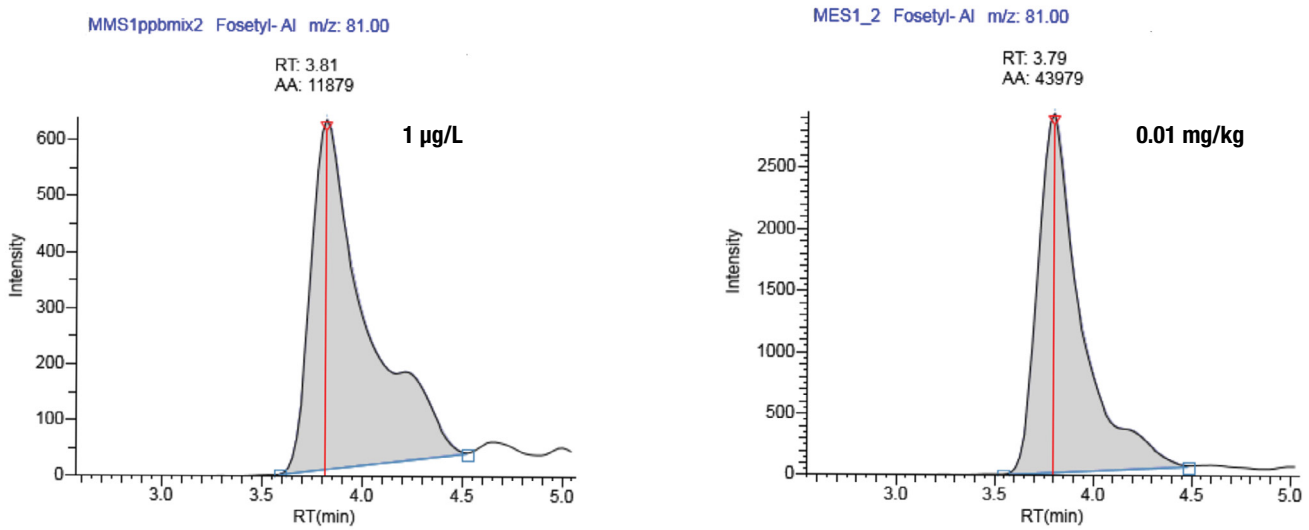

Figure 8. SRM chromatograms of fosetyl MMS (1 µg/L) and MES (0.01 mg/kg) in table grapes. The EU residue definition for fosetyl is the sum of fosetyl, phosphonic acid, and their salts and the MRL is set at 100 mg/kg in table grapes.

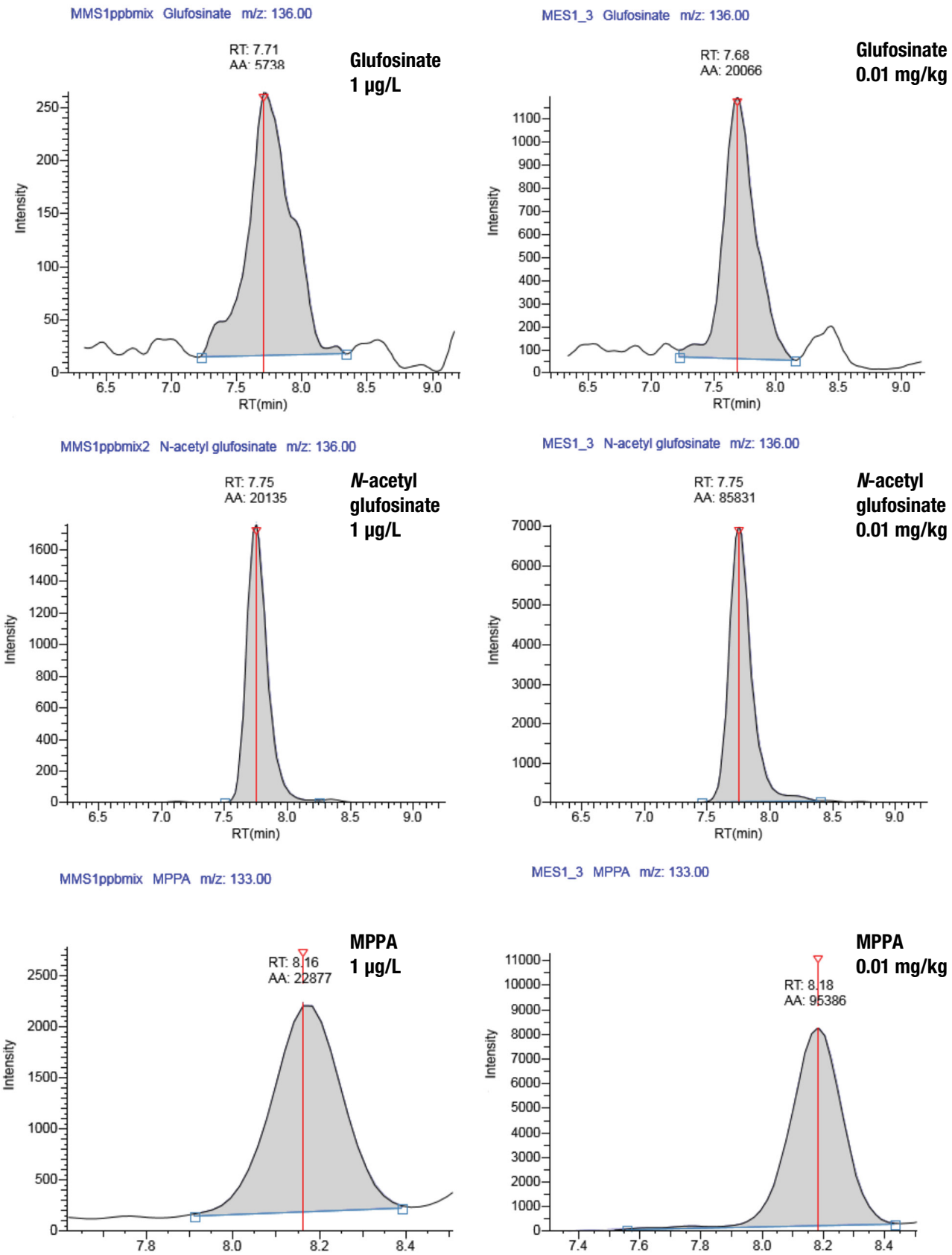

Figure 9. SRM chromatograms of glufosinate MMS (1 µg/L) and MES (0.01 mg/kg), *N*-acetyl glufosinate MMS (1 µg/L) and MES (0.01 mg/kg), and MPPA MMS (1 µg/L) and MES (0.01 mg/kg) in table grapes. The EU residue definition for glufosinate is the sum of glufosinate, *N*-acetyl glufosinate, MPPA and their salts, and the MRL is set at 0.15 mg/kg in wine grapes.

Retention time stability

In our study, retention time stability was determined by five replicates of MMS in spiked grape matrix at 10 µg/L. Typically, the longest retention times were observed in neat solvent and shorter retention times were observed in matrixes. Differences in the retention times between solvent and matrixes may be related to the column capacity and the amount of matrix compounds present in the extract. In matrix blank conditions, there was more competition for the active sites of the stationary phase, and in pure solvent, the entire column capacity was available for pesticides. In matrixes, however, a great number of active sites are occupied by the matrix compounds, and with this decrease in column capacity available for pesticides, the retention times were shortened. Acceptable retention time deviation is <±0.1 min.[7] In grape matrix, our results showed good retention time stability within ±0.1 min (Table 3).

Selectivity

By using the SRM mode, analyte selectivity was confirmed based on the presence of the transition ions (quantifier and qualifier) at the retention times corresponding to those of the respective pesticides. The measured peak area ratios of qualifier/quantifier should be within ±30% (relative) of average of calibration standards from the same sequence, defined in Reference 7 when compared to the standards (Table 4).

Recovery

The recoveries were checked at two spiking levels, 20 and 100 µg/kg (10 and 50 µg/L), except for maleic hydrazide at 40 and 100 µg/kg (20 and 50 µg/L). Samples in triplicate were extracted with a modified QuPPe method. Glyphosate labeled with $^{13}C^{15}N$ was used to control the final volume of the extract. Recoveries against MMS calibration curves were in the acceptable range (70–120%) (Table 5). Grape blank matrix contains phosphonic acid, so its recovery was calculated using blank subtracted calibration.

Conclusion

We introduced a new workflow based on the modified QuPPe method and IC-MS/MS that supports simultaneous multiresidue analysis for polar pesticides in grape samples. The IC-MS/MS method was developed using a Dionex IonPac AS19-4µm column set and a compact IC system coupled to a TSQ Quantis triple quadrupole mass spectrometer. The modified QuPPe method used pure methanol instead of default acidified methanol to extract the analytes and a Dionex OnGuard II RP cartridge as the clean-up step. To increase sensitivity, electrospray ionization was improved by post-column introduction of acetonitrile into the eluents. Matrix-matched calibration was used to compensate for matrix effects. The results showed that the sensitivity, linearity, retention time precision, and recovery align with the SANTE/11813/2017 method performance criteria. The method provides lower LOQs than EU MRLs. Overall, this workflow supported simultaneous multiresidue analysis of polar pesticides in the grape samples using the modified QuPPe method.

Table 3 (part 1). Retention time stability

Analyte	RT (min)	Average RT (min)	RT RSD
AMPA	8.47 8.49 8.47 8.55 8.57	8.51	0.551
Bialaphos	7.79 7.79 7.65 7.69 7.69	7.72	0.831
Chlorate	7.84 7.79 7.73 7.73 7.73	7.76	0.641
Cyanuric acid	12.77 12.77 12.65 12.69 12.69	12.7	0.422
Ethephon	9.26 9.26 9.16 9.18 9.18	9.21	0.523
Fosetyl	4.29 4.23 4.15 4.17 4.17	4.2	1.37
Glufosinate	8.64 8.66 8.44 8.46 8.52	8.54	1.19
Glyphosate	12.4 12.4 12.3 12.32 12.34	12.35	0.373

Table 3 (part 2). Retention time stability

Analyte	RT (min)	Average RT (min)	RT RSD
HEPA	8.55 8.59 8.47 8.49 8.49	8.52	0.589
Maleic hydrazide	5.46 5.46 5.44 5.44 5.50	5.46	0.449
MPPA	8.97 9.01 8.89 8.91 8.91	8.94	0.562
N-acetyl AMPA	8.54 8.56 8.47 8.50 8.47	8.51	0.480
N-acetyl glufosinate	8.50 8.52 8.42 8.44 8.44	8.46	0.512
N-acetyl glyphosate	12.29 12.33 12.20 12.22 12.22	12.25	0.452
Perchlorate	17.80 17.83 17.72 17.74 17.72	17.76	0.283
Phosphonic acid	9.11 9.13 9.05 9.07 9.05	9.08	0.400

Table 4. Ion ratios (Qual/Quan) in neat standard, MMS and MES at level 10 and 50 µg/L except for maleic hydrazide at 20 and 50 µg/L

Analyte	Quantifier	Qualifier	Ion Ratio at 10 µg/L (Maleic Hydrazide, 20 µg/L)			Ion Ratio at 50 µg/L		
			Neat Standards-Qual/Quan	MMS-Qual/Quan	MES-Qual/Quan	Neat Standards-Qual/Quan	MMS-Qual/Quan	MES-Qual/Quan
AMPA	63	79	0.83	0.71	0.70	0.81	0.81	0.80
Bialaphos	216	172	0.35	0.33	0.32	0.34	0.32	0.31
Chlorate	67	51	0.16	0.16	0.16	0.16	0.16	0.16
Cyanuric acid	85	42	0.92	1.02	0.87	0.93	0.95	0.86
Ethephon	107	79	0.48	**	**	0.47	**	**
Fosetyl	81	63	0.43	0.42	0.42	0.43	0.42	0.42
Glufosinate	136	95	0.86	0.79	0.77	0.86	0.88	0.86
Glyphosate	63	79	0.81	0.83	0.84	0.79	0.81	0.77
HEPA	79	95	0.41	0.39	0.39	0.40	0.41	0.41
Maleic hydrazide	82	42	0.12	0.17	0.16	0.12	0.13	0.14
MPPA	133	107	0.49	0.51	0.50	0.50	0.49	0.50
N-acetyl AMPA	110	63	0.40	0.42	0.38	0.42	0.40	0.40
N-acetyl glufosinate	136	180	0.38	0.37	0.39	0.37	0.37	0.37
N-acetyl glyphosate	150	192	0.82	0.87	0.89	0.81	0.82	0.83
Perchlorate	83	85	0.33	0.33	0.31	0.31	0.32	0.31
Phosphonic acid	79	63	0.31	0.33	0.31	0.31	0.31	0.31

Note: **Ion Qual is coeluting with interference of the same *m/z*.

Table 5. Recovery at 20 and 100 µg/kg (10 and 50 µg/L) except for maleic hydrazide at 40 and 100 µg/kg (20 and 50 µg/L)

Analyte	At 10 µg/L Spiking Level			At 50 µg/L Spiking Level		
	Calculated Amount	Recovery (%)	RSD	Calculated Amount	Recovery (%)	RSD
AMPA	9.42	94	5.4	39.2	78	2.2
Bialaphos	10.3	103	8.7	49.4	99	2.5
Chlorate	7.89	79	5.2	40.0	80	0.9
Cyanuric acid	9.58	96	9.7	41.2	82	9.2
Ethephon	8.71	87	4.2	42.2	84	2.8
Fosetyl	8.35	84	0.9	40.1	80	0.2
Glufosinate	9.01	90	3.0	41.2	82	1.3
Glyphosate	8.25 8.62 (IS)	83 86 (IS)	2.4	39.9 40.1 (IS)	80 80 (IS)	2.5
HEPA	8.31	83	0.8	36.8	74	1.2
Maleic hydrazide	18.5	93	10.9	37.5	75	4.1
MPPA	9.32	93	3.5	45.2	90	2.6
N-acetyl AMPA	8.86	89	3.2	38.3	77	0.5
N-acetyl glufosinate	8.05	81	2.4	38.3	77	1.3
N-acetyl glyphosate	8.48	85	0.1	40.5	81	1.4
Perchlorate	7.93	79	2.2	39.4	79	3.5
Phosphonic acid	9.99	100	4.8	57.9	116	3.1

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References

1. Adams, S.; Guest, J.; Dickinson, M.; Fussell, R.J.; Beck, J.; Schoutsen, F. J. *Agric. Food Chem.* **2017,** *65,* 7294–7304. doi: 10.1021/acs.jafc.7b00476.
2. GAO-15-38, Report to the Ranking Member, Subcommittee on Environment and the Economy, Committee on Energy and Commerce, House of Representatives; http://www.gao.gov/assets/670/666408.pdf (accessed Dec 29, 2018).
3. Mol, H.G.; van Dam, R.C. *Anal. Bioanal. Chem.* **2014,** *406,* 6817–6825. doi:10.1007/s00216-014-7644-8
4. Vass, A.; Robles-Molina, J.; Perez-Ortega, P.; Gilbert-Lopez, B.; Dernovics, M.; Molina-Diaz, A.; Garcia-Reyes, J.F. *Anal. Bioanal. Chem.* **2016,** *408,* 4857–4869. doi:10.1007/s00216-016-9589-6
5. Poiger, T.; Buerge, I.J.; B¨achli, A.; M¨uller, M.D.; Balmer, M.E. *Environ. Sci. Pollut. Res. Int.* **2017,** *24,* 1588–1596.
6. Anastassiades, M.; Kolberg, D. I.; Benkenstein, A.; Eichhorn, E.; Zechmann, S.; Mack, D.; Wildgrube, C.; Sigalov, I.; Dork, D.; Barth, A. Quick method for the analysis of numerous highly polar pesticides in foods of plant origin via LC-MS/MS involving simultaneous extraction with methanol (QuPPe-method), version 9.3; http://www.eurl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPe-PO_EurlSRM.pdf (accessed Dec 29, 2018).
7. 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 Dec 29, 2018).
8. Thermo Fisher Scientific. Dionex AS-AP Autosampler Operator's Manual. Doc No. 065361 Revision 07, 2012.
9. Thermo Fisher Scientific. Product Manual for Eluent Generator Cartridges. Doc. No 065018, Revision 05, Sunnyvale, CA, 2014.
10. Thermo Fisher Scientific. Product Manual for Continuously Regenerated Trap Columns (CR-TC). Doc. No 031910, Sunnyvale, CA, 2010.
11. Thermo Fisher Scientific. Product Manual for Dionex Suppressor. Doc No. 031956 Revision 13, 2018.
12. Thermo Fisher Scientific. TSQ Altis, TSQ Quantis, and TSQ Fortis Getting Connected Guide Doc No. 80111-97046 Revision A, 2018.
13. Thermo Fisher Scientific. TSQ Altis, TSQ Quantis, and TSQ Fortis Hardware Manual Doc No. 80111-97047 Revision A, 2018.
14. Thermo Fisher Scientific. TSQ Altis, TSQ Quantis, and TSQ Fortis Preinstallation Requirements Guide Doc No. 80111-97048 Revision A, 2018.
15. Thermo Scientific Technical Note 72611: Configuring and optimizing an IC-MS system using a compact IC and a single quadrupole mass spectrometer. Sunnyvale, CA, 2018. https://assets.thermofisher.com/TFS-Assets/CMD/Technical-Notes/tn-72611-ic-ms-system-configuring-optimizing-tn72611-en.pdf
16. Liaison MRL database; https://secure.fera.defra.gov.uk/liaison/secure/ (accessed Dec 29, 2018).

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