Determination of Polar Pesticides in Grapes Using an IC-MS/MS System

Gemma Ellison, Beibei Huang, Michael Volny, Claudia Martins, Alexander Semyonov, and Jeffrey Rohrer, Thermo Fisher Scientific, Sunnyvale, CA , USA, 94085

ABSTRACT

Purpose: To develop and test a method based on ion chromatography (IC) coupled with a triple quadrupole MS/MS (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.

Methods: We introduced a new workflow based on a modified Quick Polar Pesticides Method (QuPPe Method) and IC-MS/MS that supports simultaneous multi-residue analysis of grape samples for polar pesticides. The IC-MS/MS method was developed using a Thermo Scientific™ Dionex™ IonPac™ AS19 4-µm column set and a compact IC system coupled to a Thermo Scientific™ TSQ Quantis™ Triple Quadrupole Mass Spectrometer.

Results: A good IC-MS/MS separation was achieved to resolve 16 analytes in different SRM channels. 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 shapes were obtained for AMPA (10 µg/L), bialaphos (10 µg/L), and maleic hydrazide (20 µg/L). The results showed that the sensitivity, linearity, retention time precision, and recovery comply with the SANTE/11813/2017 method performance criteria[1]. The method provides lower LOQs than EU maximum residue limits (MRLs). Overall, this workflow supported simultaneous multiresidue analysis of polar pesticides in the grape samples using the modified QuPPe method.

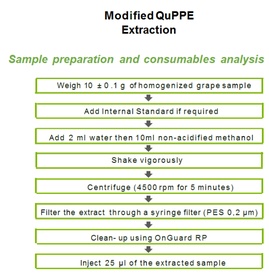
INTRODUCTION

Polar pesticides in food and beverages have become an area of interest in recent years. Two well-known representatives of this group are the broad-spectrum systematic herbicide glyphosate and its metabolite AMPA. Pesticides are used in vineyards worldwide, and this can lead to pesticide residues on grapes and in the final wine product. Other foods and beverages may also contain pesticide residues. This has led to much controversy as reported in the media and scrutiny from governing bodies such as the World Health Organization (WHO) and the European Food Safety Authority (EFSA), due to the potential adverse health effects of pesticides. There is increased demand to test for these compounds.

Analyzing polar pesticides is challenging, as they can have low recovery when using liquid/liquid partition methods based on QuEChERS, and poor retention in reversed-phase liquid chromatography. Ion chromatography (IC) is a technique designed for polar analytes and provides excellent chromatographic resolution in a wide range of samples. Combining IC with the power of a highly selective and sensitive mass spectrometer (MS) has led to the development of an IC-MS/MS method for the direct analysis of 16 pesticides and related compounds: glyphosate and metabolites (AMPA and N-acetyl glyphosate), bialaphos, chlorate, cyanuric acid, ethephon (and HEPA), fosetyl-aluminium (and phosphonic acid), glufosinate, N-acetyl glufosinate, MPPA, maleic hydrazide, N-acetyl AMPA, and perchlorate (classified as a contaminant). Using grapes as the sample, this method was developed with a run time of 20 min and detection limits below those required to meet EU MRLs.

MATERIALS AND METHODS

Sample Preparation



Test Method(s)

IC conditions	
IC system:	Thermo Scientific™ Dionex™ Integrion™ HPIC™ system
Mobile Phase:	KOH, Source: EGC 500 KOH
Column:	Dionex IonPac AS19-4 µm Guard 2X50 mm Dionex IonPac AS19-4 µm Analytical 2X250 mm
Gradient:	15–20 mM (0–4 min), 20–75 mM (4–10 min), 75–75 mM (10–18 min), 75–15 mM (18),15 mM (18–20 min)
Suppressor:	Dionex ADRS 600 Suppressor (2 mm)
Pump Flow:	0.35 mL/min
Injection Volume:	25 µL
Column Temp:	30˚ C
Detector Comp. Temp:	20˚ C
Suppressor Current:	65 mA external water mode via AXP-MS Pump, external water flow rate (0.70 mL/min)
IC-MS Interface: post suppressor Makeup solution:	Tee union to combine the analyte from conductivity detector via viper fitting tubing, and acetonitrile at 0.2 mL/min via Thermo Scientific™ AXP-MS Pump.
Triple quadrupole MS/MS detection	
Ionization Mode:	Heated Electrospray (H-ESI)
Scan Type:	SRM
Polarity:	Negative
Spray Voltage:	3800 V
Sheath Gas Pressure:	42 Arb
Aux Gas Pressure:	12 Arb
Ion Sweep Gas Pressure:	1 Arb
Ion Transfer Tube Temp:	300˚ C
Vaporizer Temp:	300˚ C
Cycle Time:	1.25 s
Q1 Resolution:	0.7
Q3 Resolution:	1.2
Source Fragmentation:	0 V
Use Calibrated RF Lens:	each component was optimized

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
Maleic Hydrazide	6.50	4	111	55	16.14	113
Maleic Hydrazide	6.50	4	111	83	13.34	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	20.5	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	12.5	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	12.5	4	128	42	14.47	90
Cyanuric acid	12.5	4	128	85	10.23	90
***N*-Acetyl glyphosate**	12.2	2	210	150	13.07	123
***N*-Acetyl glyphosate**	12.2	2	210	192	10.23	123
glyphosate	12.3	2	168	63	22.62	110
glyphosate	12.3	2	168	79	38.85	110
glyphosate ISTD	12.3	2	172	63	25	110
Perchlorate	17.8	3	99	83	26.19	152
Perchlorate	17.8	3	101	85	26.3	152

ISTD: Internal Standard

Data Analysis

Data Acquisition:

- Thermo Scientific™ Chromeleon™ Chromatography Data System software version 7.2.6 or higher
- Thermo Scientific™ Xcalibur™ 4.1 software with SII for Xcalibur software
- Thermo Scientific™ TraceFinder™ 4.1 software

Data Processing:

- Thermo Scientific TraceFinder 4.1 software

IC-MS/MS System Configuration

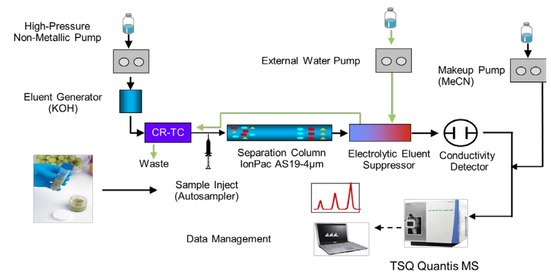


Figure 1. IC-MS/MS workflow.

RESULTS

IC-MS/MS Separation

An good IC-MS/MS separation was achieved to resolve 16 analytes in different SRM channels. (Figure 2). SRM chromatograms in grape samples are shown in Figure 3.

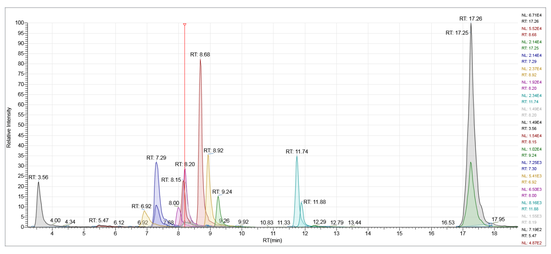


Figure 2. SRM chromatograms of 16 polar pesticides (10 µg/L each).

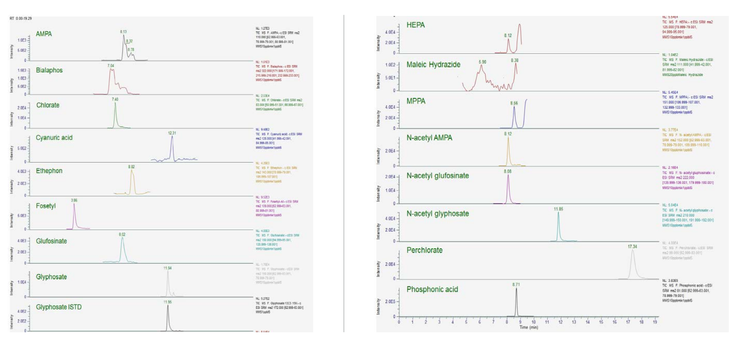


Figure 3. SRM chromatograms of 16 polar pesticides in spiked grape matrix at 10 µg/L with the exception of the 20 µg/L spike concentration for maleic hydrazide and 1 µg/L spike concentration for the glyphosate ISTD.

Method calibrations

Three calibration curves are constructed using standards in neat solvents, matrix-matched calibration standards (MMS), and Matrix Extracted Standards (MES) respectively. Table 1 shows the quantitation ions, calibration ranges, calibration method, and the coefficients of determination (r^2) ; coefficients of determination obtained ranged from 0.9953–0.9999. The method provides better LOQs than EU MRLs (Figures 4-9).

Table 1. 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

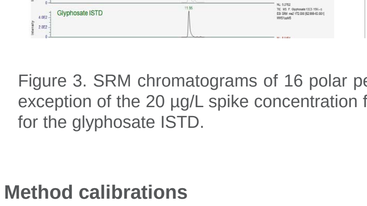


Figure 4 SRM chromatograms of ethephon MMS (1 µg/L) and MES (0.01 mg/kg) in table grapes. The EU residue definition for ethephon is ethephon only and the MRL is set at 1 mg/kg in table grapes.

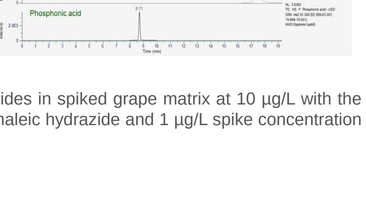


Figure 5. SRM chromatograms of glyphosate MMS (1 µg/L) and MES (0.01 mg/kg) in table grapes. The EU residue definition for glyphosate is glyphosate only and the MRL at 0.5 mg/kg in table grapes.

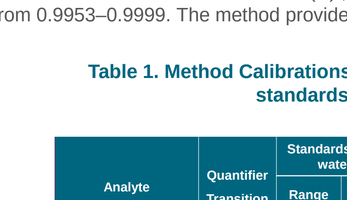


Figure 6. SRM chromatograms of perchlorate MMS (1 µg/L) and MES (0.01 mg/kg) in table grapes. The EU residue definition for perchlorate is perchlorate only and the MRL at 0.1 mg/kg in table grapes.

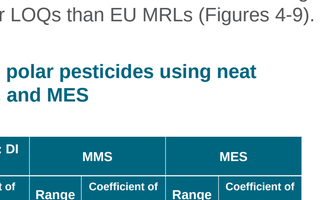


Figure 7 SRM chromatograms of chlorate MMS (1 µg/L) and MES (0.01 mg/kg) in table grapes. The EU residue definition for chlorate is chlorate only and the MRL at 0.01 mg/kg in table grapes.

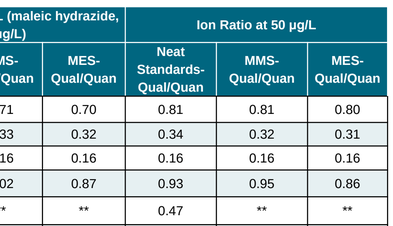


Figure 8 SRM chromatograms of fosetyl MMS (1 µg/L) and MES (0.01 mg/kg) in table grapes. The EU residue definition for fosetyl is the sum of fosetyl, phosphonic acid, and their salts and the MRL at 100 mg/kg in table grapes.

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 at 0.15 mg/kg in wine grapes.

Retention Time Stability, Selectivity, and Recovery

Retention time stability was determined by five replicates of MMS in spiked grape matrix at 10 µg/L. Our results showed good retention time stability within ± 0.1 min. 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 (Table 2). 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 using pure methanol and a Thermo Scientific™ Dionex™ OnGuard™ II RP cartridge as the clean-up step. Glyphosate labeled with 13C15N was used to control the final extract volume. Recoveries against MMS calibration curves were in the acceptable range (70–120%) (Table 3).

Table 2. 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 3. 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	83	2.4	39.9	80	2.5
	8.62 (IS)	86 (IS)		40.1 (IS)	80 (IS)	
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

CONCLUSIONS

We introduced and validated a new IC-MS/MS workflow to directly determine 16 pesticides and related compounds.

- This workflow supported simultaneous multiresidue analysis of polar pesticides in the grape samples using the modified QuPPe method.
- 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 results showed that the sensitivity, linearity, retention time precision, and recovery comply with the SANTE/11813/2017 method performance criteria.

Note: See Thermo Scientific Application Note 72915 for more details[2].

REFERENCES

1. 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 Nov 07, 2018).
2. Thermo Scientific Application Note 72915: Determination of Polar Pesticides in Grapes Using a Compact Ion Chromatography System Coupled with Tandem Mass Spectrometry. Sunnyvale, CA. 2019, https://appslab.thermofisher.com/App/4249/determination-polar-pesticides-grapes.

TRADEMARKS/LICENSING

PO73037-EN0519S