SPECIAL GUEST EDITOR SECTION

Coupling Ion Chromatography to Q-Orbitrap for the Fast and Robust Analysis of Anionic Pesticides in Fruits and Vegetables

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Ion chromatography coupled to a quadrupole Orbitrap mass analyzer was used to develop a multiresidue method for the determination of highly polar pesticides and their metabolites (chlorate, perchlorate, fosetyl-aluminum, glyphosate, aminomethylphosphonic acid (AMPA), phosphonic acid, *N*-acetyl AMPA, and *N*-acetyl glyphosate) in fruits and vegetables. After extraction with methanol, samples were diluted 5× with water. No derivatization was applied. Pesticides were separated in an anion-exchange column. Water was used as the ion chromatography mobile phase. A gradient was created by increasing the concentration of KOH in the mobile phase. Ion chromatography provided good and stable retention and separation for all studied compounds. All investigated pesticides had an LOQ of 0.01 mg/kg and a linear range of 0.01–0.50 mg/kg. The ion ratio of the *m*/*z* ions produced was stable and adequate (deviation <30%) in all cases. The obtained mass errors (always in full-scan MS and MS2 mode) were <0.2 mDa. The high resolution (>100 000) provided by the Orbitrap analyzer with the low *m*/*z* ions obtained (e.g., *m*/*z* 80) was effective in obtaining low background matrix signals. The influence of postcolumn infusion of organic solvent on sensitivity was investigated. Acetonitrile was found to be more effective than methanol, increasing the sensitivity 3× with respect to water. The method was validated for five vegetable-based matrixes. Both the sample processing and the analytical measurement were very fast. Hence, the methodology is ideal for high-throughput work.

Ion chromatography (IC) was introduced in 1975 (1). In this technique, polymeric ion exchangers are used as stationary phases. Cation or anion exchangers are used depending on the charge of the analytes. In the case of anions, a high-pH eluent is used. Deprotonated acids can interact with a positively charged stationary phase. Coulombic interactions are the main forces responsible for the retention of the analytes (2). Since the introduction of IC, column technology has improved. Current columns are characterized by higher ion-exchange capacity, higher column efficiency, reductions in column diameters, and a new chemistry of bonded functional groups (3).

Various detectors are used with IC. Examples of the application of conductivity (4, 5), UV (6), and mass detectors (7, 8), can be found in the literature. Because of the typical high content of nonvolatile salts, an ion chromatograph cannot be connected directly to a mass spectrometer. The presence of nonvolatile salts has a negative influence on sensitivity, and salts can precipitate in the ionization source (2).

Highly polar pesticides are challenging analytes. First, they have very low extractability in typical multiresidue methods such as QuEChERS, ethyl acetate, and mini-Luke (9). Second, they have poor retention in reversed-phase LC, which is used for the analysis of the majority of pesticides (10). The problem with extractability can be overcome by the use of a more-polar solvent such as methanol or methanol plus water (9). One of the approaches to solve the poor retention problem is the derivatization of the analyte, as proposed by Goscinny et al. (11). These researchers determined glyphosate and aminomethylphosphonic acid (AMPA) levels in cereals by using 9-fluorenylmethyl chloroformate as a derivatization agent. After that, glyphosate and AMPA became amenable for analysis with a C18 column. The same derivatization agent was used by another group to analyze glyphosate and AMPA in surface water (12). Another possibility to analyze polar pesticides using reversed-phase LC is the application of ion-paring reagents. Hernández et al. (13) validated a method for the determination of fosetyl-aluminum in lettuce. Thanks to the application of tetrabutylammonium acetate, fosetyl-aluminum was properly retained in a C18 column. An alternative approach to achieve the retention of polar compounds is to change reversed-phase chromatography to another type of chromatography, e.g., normal-phase chromatography or IC. Vass et al. (10) evaluated various normal-phase columns for the analysis of 24 polar pesticides in orange matrix. They achieved good results using a hydrophilic interaction LC (HILIC) column for the majority of the tested pesticides, except for glyphosate and AMPA. Better results for those two compounds were obtained by use of zwitterionic-type columns (i.e., Obelisc N; 10). The suitability of zwitterionic columns for glyphosate analysis was also demonstrated in another work (14). The HILIC column was also found to be suitable for analysis of polar pesticides in olive oil and olives; however, glyphosate and AMPA were not present on the target list (15). Glyphosate in various matrixes (corn, carrot, apple, and spicy cabbage) was successfully analyzed on a HILIC column by Ding et al. (16). Nevertheless, the method was a single-residue method, only for

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glyphosate. It was demonstrated that polar pesticides (e.g., chlorate, perchlorate, and fosetyl-aluminum) can be retained on a Hypercarb column (17). However to analyze polar pesticides, the Hypercarb column typically requires a very time-consuming preparation process and sometimes isotopically labeled standards. To block active sites of the column, it is recommended to run, e.g., 50 injections of 50 µL spinach extract (18).

In many cases, these analytical difficulties have resulted in routine laboratories not having the capability to determine these compounds, as noted by the European Food Safety Authority in the 2014 European Union (EU) Report on Pesticide Residues in Food, in which only 22 EU countries included glyphosate in their official monitoring programs (19).

IC has previously been proposed as a technique for the analysis of polar pesticides. Perchlorate was analyzed in lettuce, cantaloupe, bottled water, and milk (20). In another work, the determination of glyphosate in cereals by coupling IC to triple-quadrupole MS was described (21). A flow injection technique was also tested for the detection of polar pesticides in apple, lettuce, and wheat flour; however, quantitative determination was possible only using isotopically labeled standards (9).

This work describes the application and evaluation of IC coupled to high-resolution MS using a quadrupole (Q)-Orbitrap MS instrument for the multiresidue detection of polar pesticides in fruits and vegetables. The validated method is fast and straightforward in order to be implemented in routine laboratories. It provides an appropriate LOQ relative to EU maximum residue limits in all cases, and the linearity, retention time, precision, mass accuracy, and repeatability cover the most stringent criteria of QC performance for routine food control.

Experimental

Reagents and Materials

High-purity pesticide standards were obtained from LGC Standards (Wesel, Germany) and Sigma-Aldrich (Steinheim, Germany) and were stored at −30°C. Individual pesticide stock solutions (1000–2000 mg/L) were prepared in water and methanol and were stored in plastic vials in the dark at −20°C. A mixed-standards solution was prepared from the stock standards. Pesticides used in the study were chlorate, perchlorate, fosetyl-aluminum, glyphosate, AMPA, phosphonic acid, *N*-acetyl AMPA, and *N*-acetyl glyphosate.

Water was obtained from Fisher Scientific (Fair Lawn, NJ), and methanol and acetonitrile were obtained from Fluka Analytical (Steinheim, Germany). Formic acid was purchased from Sigma-Aldrich. Pierce LTQ Velos ESI Negative Ion Calibration Solution was provided by Thermo Fisher Scientific (Waltham, MA).

IC-MS Analysis

For the IC separation, a Dionex Integrion IC system (Thermo Scientific, San Jose, CA) was used. The mobile phase was water. The gradient was created by increasing the concentration of KOH. Separation was carried out on a Dionex IonPac AS19 column. The length, diameter, and particle size were 250 mm, 2 mm, and 4 µm, respectively. To protect the column, a guard column was used (Dionex IonPac AG19). The length, diameter, and particle size of the guard column were 50 mm, 2 mm, and 4 µm, respectively. The column was thermostatted at 40°C. The gradient started at 5 mM KOH and increased to 20 mM KOH in 8 min; from 8 to 12 min, KOH increased to 60 mM and was held at 60 mM up to 22 min. At 22.1 min, KOH decreased to 5 mM and was maintained for 4 min for re-equilibration. The injection volume was 50 µL. The autosampler was thermostatted at 15°C. The mobile phase flow rate was 0.35 mL/min. A 2 mm anion electrolytically regenerated suppressor was used. The suppressor current was set to 52 mA. The flow rate through the suppressor was 0.4 mL/min. Water for the suppressor was provided by an external pump, independent of the Dionex Integrion IC system. The role of the suppressor was to neutralize KOH and to convert salts into acids.

The postcolumn organic solvent (acetonitrile) flow rate was 0.4 mL/min. Acetonitrile was provided by another external pump.

A Q Exactive™ Focus mass spectrometer (Thermo Scientific, Bremen, Germany) was equipped with a heated electrospray ionization source (HESI-II; Thermo Scientific). The spectrometer was operated in negative polarity. The HESI-II parameters were as follows: sheath gas flow rate: 32; auxiliary gas flow rate: 10; sweep gas flow rate: 0; spray voltage: 3.50 kV; capillary temperature: 380°C; S-lens radio frequency level: 55.0; heater temperature: 350°C.

MS analysis was carried out simultaneously in MS and MS2 mode. In MS, two mass ranges were registered, *m/z* 79–212 to acquire data for all the analytes, and *m/z* 109.5–110.5 for AMPA only (to compensate for its low sensitivity). The resolution was set to 70 000 (for *m/z* 200), the automatic gain control (AGC) target was set to 1e6, and the maximum injection time (max IT) was set to auto. For MS2, a resolution of 17 500 was selected. The AGC target and maximum IT were set to 1e6 and auto, respectively. The precursor ion was filtered with an isolation window of 1 Da (precursor mass ±0.5 Da). The collision energy was optimized for each of the analytes. The list of analyzed compounds, masses of ions, and collision energies are shown in Table 1.

The external mass calibration was carried out daily. For the calibration, a mixture containing Ultramark 1621, sodium dodecyl sulfate, and sodium taurocholate (Pierce LTQ Velos ESI Negative Ion Calibration Solution) was used. The lowest mass present in the mixture was *m/z* 265.14790. Chloride anion (*m/z* 82.95414) was added to the calibration mixture to improve mass accuracy for low masses.

TraceFinder 4.1 (Thermo Scientific) was used for qualitative and quantitative analysis. Automatic detection and quantification was followed up by manual verification.

Spiking Procedure

For recovery studies, samples obtained from the local market were spiked with the standard solution in water at the appropriate level. Prior analysis of the samples was performed to ensure that it did not contain any of the studied compounds, and those samples were selected as a blank for spiking, calibration curves, and recovery purposes. A 70 g portion of minced matrix was weighed and transferred to a beaker, the sample was fortified homogenously with a 700 µL aliquot of the appropriate mix, and the mixture was blended for 30 min. The samples were allowed to stand at room temperature before analysis. The final spiking concentration levels in the sample used for recovery studies were 0.01 and 0.05 mg/kg.

Table 1. List of analyzed compounds, masses of ions found and collision energies. In bold and italic quantifier and in bold qualifier

	MS		MS2				
Compound	Formula	*m/z*	CE, eV	*m/z*			
AMPA	CH_5NO_3P	110.0012[a]	25	62.9637[b]	78.9588	—[c]	—
Chlorate	ClO_3^-	82.954[a]	55	66.9589[b]	50.9637	—	—
Glyphosate	$C_3H_7NO_5P^-$	168.0067	25	62.9637[a]	78.9588[b]	80.9745	110.0011
Glyphosate ^{13}C	$^{13}CC_2H_7NO_5P$	169.0101[a]	—	—	—	—	—
Perchlorate	ClO_4^-	98.9491[a]	25	82.9540[b]	66.9588	—	—
Fosetyl	$C_2H_3O_3P^-$	109.006	25	62.9637[a]	78.9589[b]	80.9745	—
Phosphonic acid	$H_2PO_3^-$	80.9747	25	62.9637[a]	78.9588[b]	—	—
N-acetyl AMPA	$C_3H_8NO_4P$	152.0118	25	62.9637[a]	78.9588[b]	80.9745	110.0011
N-acetyl glyphosate	$C_5H_{10}NO_6P$	210.0173	45	62.9637[a]	78.9588[b]	80.9745	—

[a] Quantifier ion.

[b] Qualifier ion.

[c] — = No data.

Sample Preparation

All matrixes were extracted according to the following protocol. A 10 g portion of homogenized sample was weighed into a 50 mL PTFE centrifuge tube. Next, 10 mL methanol and an appropriate volume of water (0.5 mL for tomato; 1 mL for carrot, melon, and onion; and 1.5 mL for orange) was added (18). In the case of recovery studies, 50 µL 10 mg/L ^{13}C glyphosate was added. The samples were shaken in an automatic axial extractor (AGYTAX®; Cirta Lab S.L., Madrid, Spain) for 4 min. The extract was then centrifuged (3500 rpm) for 5 min. The extracts were transferred to a plastic vial. Samples were diluted 5× with water before the injection.

To evaluate peak area repeatability and linearity, blank extracts were spiked. To 100 µL of blank extract, a 100 µL aliquot of standard solution containing an appropriate concentration of the target pesticides was added. Subsequently, 300 µL ultra-pure water was added to dilute the sample.

Results and Discussion

MS Analysis

The MS analysis was carried out simultaneously in both MS and MS2 mode. Enough chromatographic data points (>12) for quantitation were recorded with this acquisition. The mass range for MS was between *m/z* 79 and *m/z* 212. It was enough to cover all targeted compounds, from phosphonic acid (*m/z* 80.9747) to *N*-acetyl glyphosate (*m/z* 210.0173). This narrow range had a positive influence on the sensitivity of the method, because the number of matrix ions entering the C-trap was smaller so that more ions of the analytes were present in the transient analyzed in the Orbitrap. However, the sensitivity was still not high enough for AMPA. Thus, in the same run, an additional selected-ion monitoring (SIM) analysis for AMPA was included. Figure 1 presents extracted-ion chromatograms of 0.01 mg/kg AMPA registered in the same run. Figure 1A shows the ions extracted

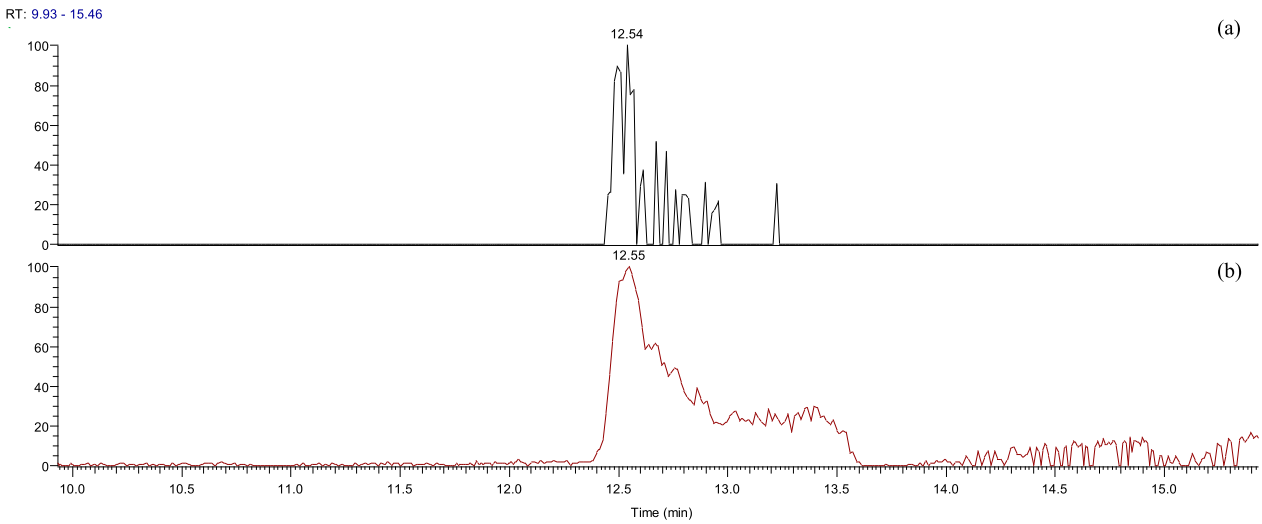

Figure 1. Improvement of the sensitivity in SIM mode compared to full-scan MS for 0.01 mg/kg AMPA in onion. 110.0012 ± 5 ppm extracted from (A) full-scan MS (*m/z* 79–212) and (B) SIM MS (*m/z* 109.5–110.5).

from the data registered in the range *m/z* 79–212, whereas the SIM data are shown in Figure 1B. All MS data were acquired with a resolution of 70 000 to maximize the selectivity of the analysis. This value was specified for *m/z* 200. In an Orbitrap mass analyzer, the resolution is inversely proportional to the square root of *m/z*. All targeted ion (except *N*-acetyl glyphosate) *m/z* values are <200. Thus, the resolution was higher than 70 000. In the case of the smallest ion (phosphonic acid, *m/z* 80.9747), the experimental mass resolution was around 110 000.

For the acquisition of MS2 data, the parallel reaction monitoring (PRM) mode was selected. In this mode, precursor ions from the inclusion list were isolated with a narrow quadrupole window (precursor mass ±0.5 Da). Next, they were fragmented in the collision cell, and then the obtained fragment ions were analyzed in the Orbitrap. In addition to the masses of the precursor ions, the inclusion list contained retention time windows in which each precursor ion was expected. In the retention time windows specified in the inclusion list, MS and MS2 scans were carried out alternately. With the final MS acquisition parameters the scan speed of the instrument was not a limitation for two reasons. First, no more than two compounds were coeluting. Thus, the cycle time was not longer than 750 ms. Second, because the peaks in IC are broader than in UHPLC, the typical base peak width observed was around 0.35 min (except for AMPA, which suffered from tailing in the applied chromatographic conditions). Hence, there was no problem with obtaining a sufficient number of points per chromatographic peak. Thanks to the application of MS and PRM MS2, it was possible to extract a chromatographic peak from both modes (MS and MS2) for all analyzed pesticides. Collision energies were optimized to obtain abundant signals of MS2 fragment ions. The resolution applied for the analysis of MS2 fragment ions was 17 500 at *m/z* 200. Masses of analyzed fragments were in the range of *m/z* 62.9637–82.9540. Thus, the practical resolution was between 31 150 and 27 125. Obtained MS2 spectra were relatively noncomplex, so this resolution value was satisfactory and it helped to shorten cycle time. In Orbitrap, resolution is proportional to the analysis time. In other words, when a higher resolution is desired, the scan takes more time.

For all the compounds, one MS ion and at least two MS2 fragment ions were found. This provided some flexibility in the selection of ions for detection, identification, and quantitation. In the case of AMPA, chlorate, and perchlorate, their deprotonated molecules acquired in MS were used for detection and quantitation. For identification, the fragment ions acquired in MS2 were used. In the case of chlorate and perchlorate, this approach helped to avoid the use of isotope ions for identification. This strategy is commonly used in the analysis of chlorate and perchlorate in triple-quadrupole MS. It is difficult to obtain two sensitive transitions from the same precursor. Therefore, for quantitation, transitions with ^{35}Cl (83 to >67 for chlorate and 99 to >83 for perchlorate) are used, whereas for identification, the transitions with ^{37}Cl; (85 to >69 for chlorate and 101 to >85 for perchlorate) are used (18). In the case of phosphonic acid, fosetyl-aluminum, glyphosate, *N*-acetyl AMPA, and *N*-acetyl glyphosate, two MS2 fragment ions were used for detection, identification, and quantitation. The reason for this was the higher sensitivity in MS2 than in MS. Figure 2 shows three extracted-ion chromatograms for glyphosate, including the precursor ion extracted from full-scan MS (Figure 2A) and two fragment ions extracted from PRM MS2 (Figure 2B and C). It is obvious that in this case, the MS2 mode provided better sensitivity. For both modes (MS and MS2), the AGC target (number of charges injected into Orbitrap in one transient) was set to 1e6. In MS mode, this value was reached in a relatively short time (<2 ms). Most of the detected ions were matrix ions. Thus, glyphosate ions were only a small fraction of the total population of the transient. In MS2 mode, the precursor ion of glyphosate was filtered in the quadrupole (168 ± 0.5 Da)

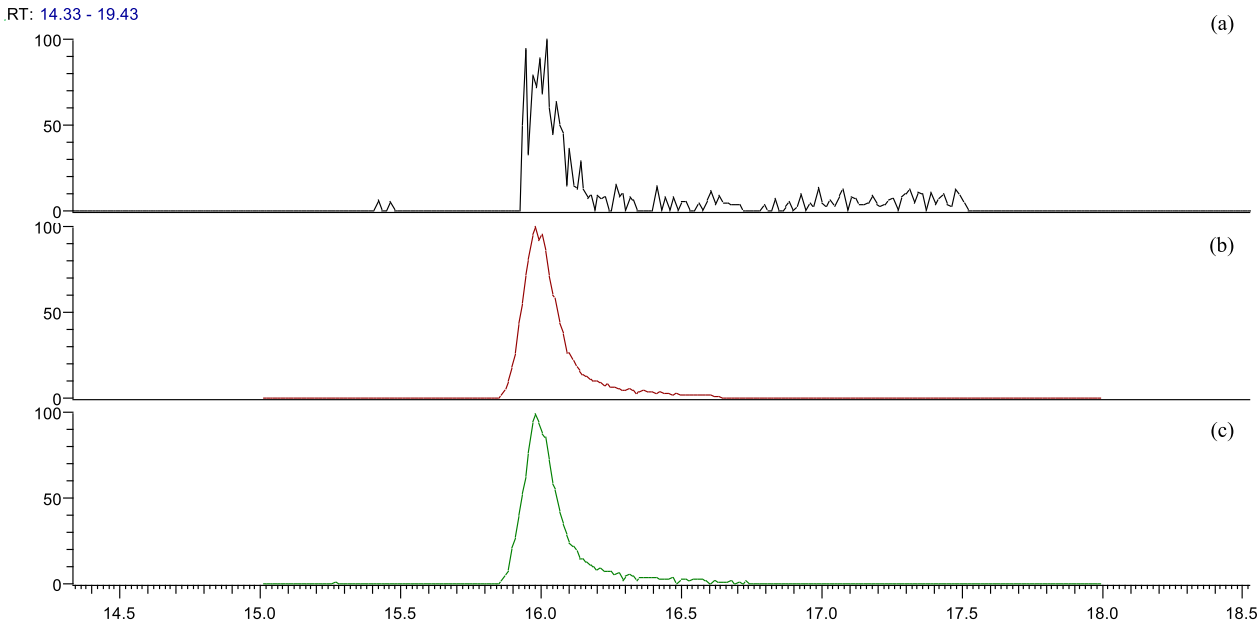

Figure 2. Improvement of the sensitivity in PRM MS2 compared to full-scan MS for 0.01 mg/kg glyphosate in carrot. (A) 168.0067 ± 5 ppm extracted from full-scan MS (*m/z* 79–212). (B) 62.9637 ± 5 ppm extracted from PRM MS2. (C) 78.9588 ± 5 ppm extracted from PRM MS2.

Table 2. Sensitivity gain after postcolumn addition of organic solvent[a]

Compound	MeOH 0.2 mL/min, %	Acetonitrile 0.2 mL/min, %	Acetonitrile 0.4 mL/min, %
AMPA	169	269	254
Chlorate	121	381	434
Glyphosate	145	269	235
Perchlorate	132	365	454
Fosetyl	242	347	339
Phosphonic acid	139	280	283
N-acetyl AMPA	147	254	250
N-acetyl glyphosate	138	268	255

[a] Peak area without organic solvent addition = 100%.

and then fragmented in the collision cell. Fragment ions were sent to the C-trap; however, during this time, the C-trap was filled only with fragmentation products of 168 ± 0.5 Da. Therefore, the majority of detected ions were fragment ions of glyphosate. The same explanation is also correct for four other pesticides (phosphonic acid, fosetyl-aluminum, *N*-acetyl AMPA, and *N*-acetyl glyphosate).

Postcolumn Infusion

In IC, water is used as the mobile phase. In the case of anions, the final effluent (after passing through the column and suppressor) is an aqueous solution of various acids. However, in electrospray, the desolvation process from an aqueous solution is not as efficient as is desolvation from an aqueous/organic solution. This is mainly a consequence of differences in volatility (22, 23). To improve the desolvation process, an organic solvent was added to the effluent by postcolumn infusion. Methanol and acetonitrile were tested. The addition of 0.2 mL/min methanol had a considerable influence on the signal intensity of fosetyl. The peak area with methanol was 242% of the peak area without methanol. Improvements for the rest of the compounds were smaller (121–169% of water-obtained peak area). The second tested solvent (acetonitrile) was more effective. A flow of 0.2 mL/min resulted in signal gain between 269% (glyphosate and AMPA) and 381% (chlorate). When the flow of acetonitrile was increased to 0.4 mL/min, the signal gain was even higher. The most affected analytes were chlorate (434% of initial peak area) and perchlorate (454% of initial peak area). Higher flows were not tested for several reasons: the obtained improvement of sensitivity was satisfying; too high a flow negatively influences ionization efficiency and dilutes the sample; and lastly, a higher flow of organic solvent increases the cost of analysis. Detailed information about the sensitivity gain after the addition of organic solvent is shown in Table 2.

Column Selection

Two columns (AS11-HC and AS19) were evaluated for the selected compounds. Both provided similar results, except for perchlorate. The AS11-HC column showed a very high affinity toward perchlorate anion. Consequently, the retention time of perchlorate was beyond 25 min of run. This problem was not observed with the AS19 column and, therefore, it was chosen for the method validation.

Mass Accuracy and Ion Ratio

According to DG SANTE guidelines (24), the detected ion should display a relative mass error of <5 ppm or <1 mDa for *m/z* values <200. In the case of the pesticides considered in this work, all their ions were below *m/z* 200 (except the protonated molecule of *N*-acetyl glyphosate). The mass accuracy obtained in this study was excellent. Regardless of the matrix and concentration tested, all ions exhibited relative mass errors of <0.2 mDa, and 85% of them were <0.1 mDa.

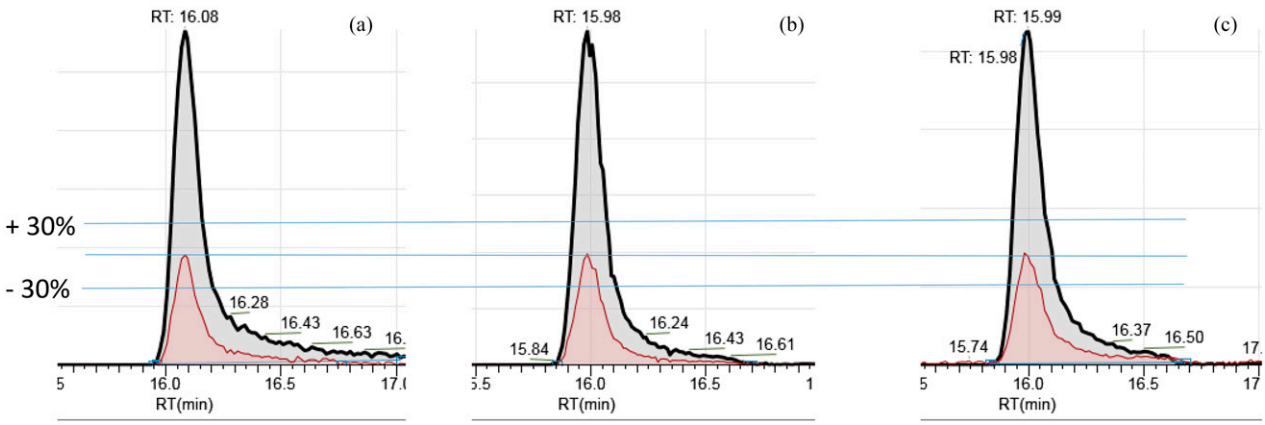

Figure 3. Stability of ion ratio for 0.01 mg/kg in (a) solvent, (b) carrot, and (c) onion. Acceptable ion ratio deviation is ±30%.

Table 3. Recoveries obtained with and without formic acid

	With formic acid		Without formic acid	
Compound	Recovery, %	RSD, %	Recovery, %	RSD, %
AMPA	76	7	84	2
Chlorate	61	10	97	2
Glyphosate	64	9	92	5
Perchlorate	48	5	110	3
Fosetyl	58	8	104	2
Phosphonic acid	72	8	106	2
N-acetyl AMPA	67	7	89	3
N-acetyl glyphosate	63	9	94	3

The other criterion related to MS confirmation is the ion ratio of the two ions selected for identification. It should be within ±30% (relative) of the average of calibration standards from the same sequence. In this work, the ion ratio was calculated between qualitative peak area and quantitative peak area. In all cases, the obtained values of ion ratio were within ±30%. Figure 3 presents an example of ion ratio stability.

Retention Time Stability

Acceptable retention time deviation is <0.1 min (24). This condition was fulfilled using the data within a single investigated matrix. However, some bigger differences were observed between the retention times of different matrixes. Typically, the longest retention times were observed in solvent and shorter retention times were observed in matrixes, with the shortest retention times seen in orange. The highest observed difference between retention time in solvent and in orange extract was in the case of perchlorate (0.58 min). 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. Taking into account the data from the conductivity detector, the extracts used in this study contained many more compounds than the solvent blank. In the case of blank tomato, the total area of all peaks detected by the conductivity detector was 19× higher. In the case of blank orange and blank melon, the area was 50× larger. In 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. In melon and orange, a huge peak at the end of the chromatogram was observed. This means that orange and melon contained some compounds with very strong affinity to the stationary phase. The shapes of matrix peaks are proof of the high matrix load. Some of them did not have Gaussian shapes. Non-Gaussian shape suggests overloaded column (25). Thus, the matrix compounds were retained more easily and more strongly than the investigated analytes, with an average change in retention time of 0.2 min observed. Therefore, it is recommended to calibrate every batch with matrix-matched standards of a commodity similar to the samples tested.

Recoveries, Repeatability, and Linearity

The recoveries were checked at two spiking levels: 0.01 and 0.05 mg/kg. Samples were extracted with a modified QuPPe method (18). With this extraction method, no phase separation

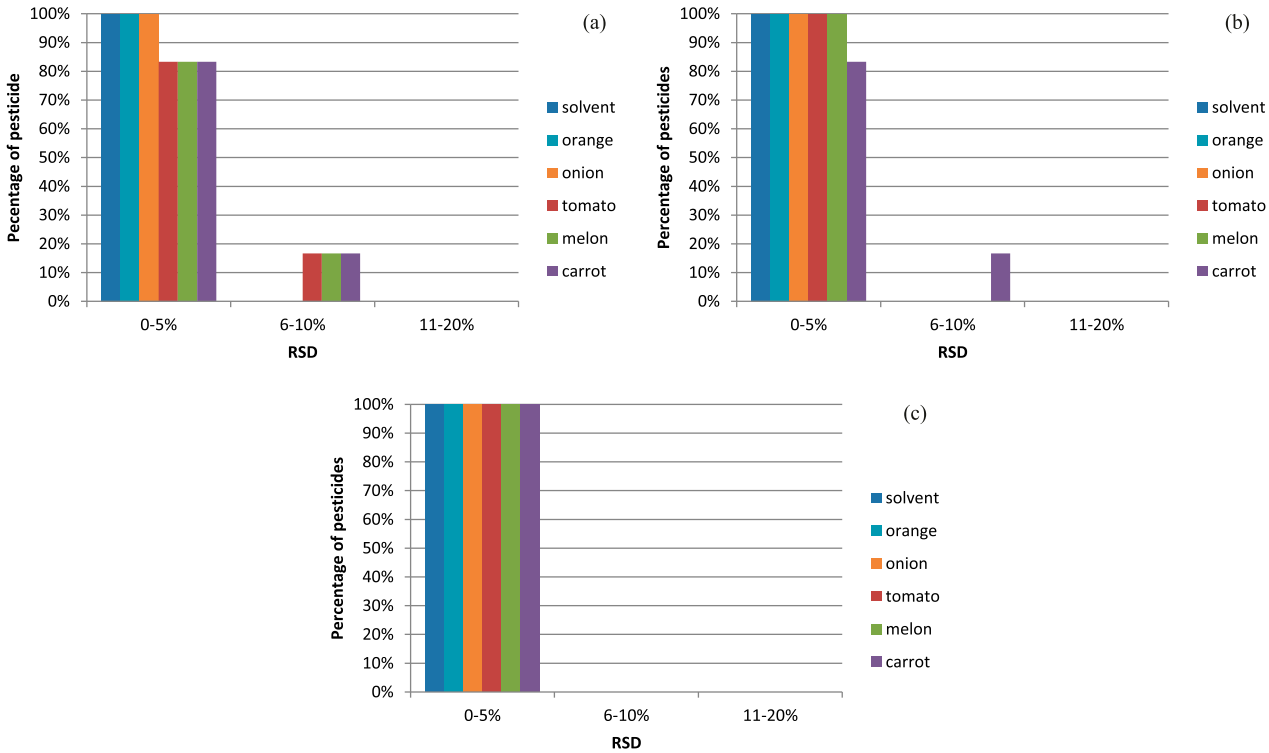

Figure 4. Peak area repeatability in pure solvent and in various matrixes. (A) 0.01 mg/kg, (B) 0.05 mg/kg, and (C) 0.10 mg/kg. Acceptable peak area repeatability is <20%.

Table 4. Positive results in real samples

Sample	Compound	IC concn, mg/kg	Ion ratio in standard	Ion ratio in sample	Hypercarb concn, mg/kg
Baby food	Phosphonic acid	0.205	1.36	1.47	0.180
Aubergine	Chlorate	0.585	0.03	0.03	0.510
Zucchini					
Sample 1	Chlorate	0.045	0.03	0.03	0.044
Sample 2	Perchlorate	0.075	0.55	0.59	0.081
Cabbage					
Sample 1	Chlorate	0.109	0.03	0.03	0.120
Sample 2	Perchlorate	0.229	0.55	0.59	0.270
Orange	Fosetyl	0.239	0.62	0.62	0.230
Watermelon	Phosphonic acid	0.220	1.36	1.49	0.250

occurs. Methanol, added water, and water from the matrix were mixed. Glyphosate labeled with ^{13}C was used to control the final volume of the extract. Obtained recoveries were in the acceptable range (70–120%). However, the key to obtaining these good recoveries was the removal of formic acid from the procedure. When the samples were extracted with acidified methanol, the obtained recoveries were low, in many cases <70%. The removal of formic acid was also found to positively influence RSD values. Table 3 shows recoveries obtained with and without formic acid.

Peak area repeatability was checked at three concentration levels: 0.01, 0.05, and 0.10 mg/kg. Each concentration was injected five times. In solvent neat standard and in five investigated matrixes, all compounds showed excellent repeatability. In all cases, the RSD was <10%. The results of peak area repeatability are shown in Figure 4. Linearity was investigated in the range of 0.01–0.50 mg/kg. The response was considered linear if individual residuals deviated <20%. Weighted linear regression ($1/x$) was used. All the pesticides were linear in all the investigated matrixes in the entire investigated range.

Application of the Proposed Method to Incurred Samples

Twenty real samples were analyzed to check the performance of the system. A total of eight positive results were found in seven samples. All 20 samples were also analyzed by HPLC with the Hypercarb column. The results obtained with both systems are presented in Table 4. In both systems, the same compounds were detected. In all cases, the difference in quantified concentrations was <20%.

Conclusions

IC greatly facilitated the application of multiresidue analysis of polar pesticides. No derivatization was necessary. Retention times were stable within a matrix (deviation <0.1 min). Some higher deviation was observed when retention times in solvent and in matrix were compared.

To obtain good recoveries (70–120%) with the QuPPe method, it is advisable to avoid the addition of formic acid. Extraction without formic acid also improved the reproducibility.

Increasing the sensitivity of the analysis by improving desolvation in electrospray was achieved by the postcolumn addition of organic solvent. For this purpose, acetonitrile was better than methanol.

Simultaneous analysis by MS and MS2 provided a sufficient number of ions for detection, identification, and quantitation. Ion ratios between qualitative and quantitative peaks were stable (deviation <30%). Orbitrap provided excellent mass accuracy (mass errors <0.2 mDa) and very high resolution values (e.g., 100 000) because the *m/z* ions were <100.

Peak area repeatability was <10% for all investigated compounds in all investigated matrixes. In addition, all compounds were linear in the range of 0.01–0.50 mg/kg.

Acknowledgments

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