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Identification/quantification of multiple pesticide residues in food plants by ultra-high-performance liquid chromatography-time-of-flight mass spectrometry

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ABSTRACT

In this study, the potential of ultra-high-performance liquid chromatography coupled with the time-of-flight mass spectrometry (UHPLC–TOF MS) to enable rapid and comprehensive analysis of 212 pesticide residues in QuEChERS extracts obtained from four plant matrices has been investigated. Method optimization is discussed in detail. In addition to molecular adducts, also fragment ions were provided for all target pesticides, thus obtaining at least three identification points required by European Decision 2002/657/EC was achieved. To get maximum information on analytes present in the extracts, each sample was examined within two injections, the first in a positive and the next one in a negative ionization mode. Under UHPLC conditions, both analyses were completed within 24 min. For more than 96% of pesticides involved in this study, the limit of quantification was $\leq$10 μg/kg. As a part of the work, strategy enabling screening of non-target pesticides and their metabolites is demonstrated on analysis of real-life samples.

1. Introduction

Over 800 pesticides representing various chemical groups have been registered for control of undesirable pests and weeds in food crops. To address health concerns on exposure to these chemicals, maximum residue limits (MRLs) have been set in EU for a wide range of pesticide/food commodity combinations.

Pesticide residues were traditionally monitored mainly by GC-based multiresidue methods [1–3]. However, many modern (semi)polar and/or ionic pesticides cannot be determined directly in this way due to their poor thermal stability or volatility. Thus, liquid chromatography coupled with tandem mass spectrometry (LC–MS–MS) has become dominating technique widely used in multiple residues analysis. As documented in many recent studies [4–7], high sensitivity and selectivity of pesticide residues detection can be achieved by tandem mass analyzers operated in a selective reaction monitoring mode (SRM) providing operation parameters were carefully optimized for each target analyte. This approach, however, does not enable to identify non-targeted compounds. In other words, detection of analytes for which reference substance is not available is a rather complicated (or even impossible) task. Another problem encountered when using a number of MS–MS is moving of an analyte out of an originally fixed time window, what requires its re-adjustment.

Recently, a novel approach represented by LC–TOF MS has been introduced into analysis of pesticide residues in food. The potential of this technique for both, target and non-target analyses, has been demonstrated in several studies [8–11]. Ongoing developments in instrument design have resulted not only in extending dynamic range allowing improved quantification, but also in high attainable accuracy of mass measurements (typically 2–5 ppm). This in combination with high spectral resolution (5000–12,000 FWHM, full width at half of maximum) enables identification of unknowns based on elemental composition.

For target pesticide analysis, at least three identification points (IPs) are recommended by EU legislation 2002/657/EC [12] for unequivocal interpretation of mass spectrometric data. When using TOF MS, additional structural information can also be obtained by in-source collision-induced dissociation (CID) fragmentation.

To compensate rather limited selectivity typical when using TOF as compared to triple quadrupole instruments, attention has to be paid to the optimization of the LC part of LC–MS setup. In this context, the use of sub-2 μm UHPLC columns provides additional chromatographic resolution and sensitivity gain is also an option.

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The aim of this study is to demonstrate the capability of UHPLC–TOF-MS for (i) fast and accurate analysis of pesticide residues in high moisture, low fat fruit and vegetable matrices and (ii) evaluation of possibilities of non-target analysis.

2. Experimental

2.1. Chemicals and reagents

Certified pesticide standards were purchased from Dr. Ehrenstorfer GmbH (Germany) and/or Riedel de Haen (Germany). Individual pesticide stock solutions (concentrations in the range 0.3–3 mg/mL) were prepared in either methanol, acetonitrile or acetone:acetonitrile mixture (1:3, v/v) depending on the solubility of particular pesticide. Stock standard mixture was then prepared in acetonitrile (each analyte 10 μg/mL) and stored at −18 °C. The working standard mixtures (0.010–4.5 μg/mL) used for calibration were prepared from stock mixture by further dilution with acetonitrile.

Deionized water for preparation of a mobile phase was produced by Milli-Q apparatus (Millipore, Germany). Ammonium formate ($HCOONH_4$) for mass spectrometry and anhydrous magnesium sulfate ($MgSO_4$) was obtained from Fluka (Germany). Acetonitrile (Sigma–Aldrich, Germany) and methanol (Merck, Germany) were HPLC gradient grade solvents for pesticide residue analysis. Sodium chloride and acetone were obtained from Penta (Czech Republic). The lock-mass internal calibration was provided by leucine-enkephalin (Sigma–Aldrich, Germany).

2.2. Sample preparation

Blank matrices – apples, strawberries, tomatoes and spinach – were obtained from organic farms. Simplified QuEChERS method (acronym for Quick, Easy, Cheap, Effective, Rugged and Safe) was employed for the extraction of fruit and vegetable samples. Briefly, 10 g of thoroughly homogenized sample was weighted into PTFE centrifugation tube (50 mL). 10 mL of acetonitrile were added and the tube was shaken vigorously for 1 min. After addition of $MgSO_4$ (4 g) and NaCl (1 g) sample was immediately shaken again for 1 min to prevent formation of coagulated $MgSO_4$. The extract was centrifuged (Hettich, Germany) at 11,000 RPM for 5 min. An aliquot of supernatant was filtered through a 0.2 μm PTFE filter (Cronus, UK) and transferred into a vial. Spiked matrix extracts (1–450 μg/kg) were used for validation of the method.

2.3. Matrix-matched standards preparation

100 μL of particular working standard mixture were added to 900 μL of blank extracts prepared as described above to obtain matrix-matched standards corresponding to concentration level 1, 2, 5, 10, 20, 50, 100, 200, 300 and 450 μg/kg.

2.4. UHPLC–TOF MS analysis

The UHPLC analyses were performed using Acquity Ultra-Performance LC system (Waters, USA) equipped with an Acquity UPLC HSS T3 separation column (100 mm × 2.1 mm i.d., 1.8 μm particle size, Waters, USA) maintained at 40 °C. The mobile phase consisted of methanol (A) and 0.005 M ammonium formate (B); the optimized UHPLC gradient is shown in Table 1. The Acquity UPLC operating pressure was 8000 psi at initial gradient conditions and maximum pressure did not exceed 13,000 psi. Sample injection volume 2 μL was used in all experiments and the sample temperature was maintained at 10 °C.

UHPLC system was connected to orthogonal accelerated time-of-flight mass spectrometer Waters LCT Premier XE (Waters, USA) operated in both, positive (ESI+) and negative (ESI−) electrospray ionization mode. In ESI+ mode capillary voltage was 3500 V, cone voltage 30 V, source temperature was maintained at 120 °C and desolvation temperature was 350 °C. Nitrogen was used as desolvation and cone gas at flow rate 700 L/h and 10 L/h, respectively. In negative ESI capillary voltage was −2000 V and cone voltage −30 V, temperature of source and desolvation and nitrogen flow rates were the same as in positive ESI mode. The instrument was tuned using leucine-enkephalin to provide a resolution higher than 11,000 FWHM (m/z 556.2672 in ESI+ and m/z 554.2615 in ESI−). Raw mass spectra were acquired in the m/z range 50–1000 and summed each 0.11 s to produce final spectrum. As far as the function "Dynamic Range Enhancement" (DRE) was switched on, the acquisition of each final spectrum took 0.22 s. The mass calibration in both ionization modes was performed by sodium formate solution (0.5%). The mass accuracy was maintained within the whole acquisition period by using a lock spray with the leucine-enkephalin as the reference compound. Three acquisition functions with different in-source collision-induced dissociation (CID) fragmentation were employed. Monitoring of molecular ions was conducted with "aperture 1" 5 V for monitoring of fragments the voltage was increased up 30 V and 50 V, respectively. MassLynx 4.1 equipped with application manager QuanLynx was used for data acquisition and processing.

Table 1
Optimized UPLC gradient.

Time (min)	Flow (mL/min)	A (%)	B (%)
Initial	0.30	10	90
1.00	0.30	50	50
6.50	0.30	100	0
7.75	0.30	100	0
9.50	0.60	100	0
11.50	0.45	10	90

3. Results and discussion

3.1. The choice of extraction technique and UHPLC separation strategy

As documented in earlier published studies [13–15], the choice of sample preparation strategy (extraction, clean-up) fundamentally influences the performance characteristics achievable by respective MRM. Currently, one of the most popular approaches in analysis of multiple pesticide residues in fruits and vegetable is QuEChERS. Procedure originally developed by Anastassiades et al. [16] was based on acetonitrile extraction/partition followed by dispersive solid phase purification step employing primary–secondary amine (PSA) sorbent. Since its introduction, several limitations of QuEChERS procedure, such as loss of acidic pesticides due to their adsorption on sorbent and/or degradation of base-sensitive analytes, have been documented [17]. To avoid these difficulties and in order to cover the widest possible analytes range, PSA clean-up was omitted in our experiments.

Optimization of sample introduction into reversed phase LC system is a critical step not only to reduce problems associated with broadening of polar pesticides, but also to achieve good performance characteristics for all other analytes. In our preliminary study, we tested solvent exchange of QuEChERS extract to initial LC mobile phase. Due to higher polarity of mobile phase (10% of methanol in water), many less polar analytes were removed from solution and the drop of recoveries as high as 50–100% occurred. This phenomenon was undoubtedly due to the sorption of pesticides with more hydrophobic i.e. poorly soluble matrix co-extracts.

In the next experiment, sample extract was diluted with water (1:9, v/v) prior to injection. No visible precipitation of matrix com-

ponents was observed and the peak shapes of polar compounds were fairly improved. Higher volume (20 μL) of injection compensated the increase of detection limits that might occur when fewer matrix is introduced. However, the drop of intensity of many analytes was observed due to their limited stability in aqueous solution (this was most pronounced for base-sensitive compounds such organophosphates, other esters such as haloxyfop-methyl and haloxyfop-etotyl, some pyrethroids, tolylfluanid, dichlofluanid, etc.). While the breakdown of many of these pesticides was reduced or even inhibited (most of organophosphates) by acidification (0.1% acetic acid), degradation of some phenylureas, clofentezine, cycloxydim, diazinon, piperonil butoxide, propaquizafop, quinoxyfen, tepraloxydim and terbufos analytes was accelerated by reducing pH value of diluted sample extract. The drop of their concentration during 24 h long storage in cooled autosampler tray (4 °C) was in range 20–100%.

Considering the above mentioned analytes stability problems, direct injection of small volume (2 μL, equivalent to 2 mg of original matrix) of crude QuEChERS extract was identified as the most promising compromise. Under these conditions (content of organic solvent in injected sample at least 85% [16]), band broadening was observed for less retained polar pesticides represented by methamidophos, acephate and omethoate. No distortion of elution bands of other analytes due to introduction of strong solvent occurred.

As a part of optimization of LC–TOF MS method, attention was also paid to the UHPLC separation. Significantly better retention of polar analytes as compared to our previous study [5] employing BEH C18 column, was obtained on later introduced Waters Acquity HSS T3 column.

Further improvement of peaks shapes with a low retention was achieved by mobile phase gradient tuning. Since relatively low amount of polar matrix co-extracts eluting close to a void volume of reversed phase LC column were contained in QuEChERS extract, faster gradient was applied in the first phase of separation (0–1 min). It should be noted that most of pesticides elute when methanol content in mobile phase is at least 50% and compression (narrower bands) of less retained compounds was obtained in this way (Fig. 1).

3.2. Identification of pesticides by UHPLC–ESI-TOF MS

The mass resolution of employed TOF MS detector is declared as high as >10,000 FWHM (what allows setting of a very narrow mass window thus improving selectivity), it might be not sufficient enough for discrimination of isobaric interferences. Setting the optimal width of a mass window is a rather difficult task—while a wide mass window worsens selectivity, the narrow mass window can remove some analytes from chromatogram due to unavoidable uncertainties of exact mass measurement, especially for low intensity ions [18]. Taking into account mass resolution/mass accuracy of TOF MS analyzer used in our experiments, following two mass windows were set: (i) up to *m*/*z* 150, mass window at 100 ppm, and (ii) above *m*/*z* 150, 50 ppm mass window was used. Due to the limited selectivity of TOF instruments and rather poor structural information of mass spectra originated under ESI conditions, comprehensive assessment of generated data is needed to avoid false positive results.

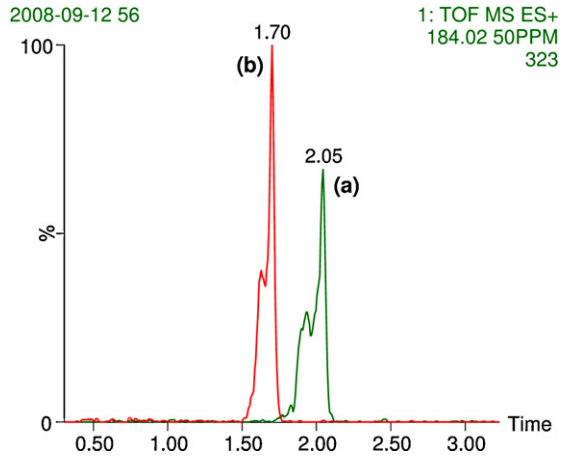

Fig. 1. Comparison of elution of polar pesticides (acephate, *m*/*z* 184.0197) with linear (a) and optimized (b) gradient. The peak eluted with optimized gradient is 1.6× higher. Apple matrix-matched standard, 10 μg/kg.

According to the document No. SANCO/2007/3131 [19], additional confirmation of residues at, or above the reporting limit, is required for unbiased identification. A system of IPs [13] is commonly used for this purpose and to get additional IPs, an in-source CID fragmentation has to be employed [8–11]. In principle, two alternative approaches are conceivable in TOF MS measurement: (i) mass spectra are acquired by application of a few (2–3) preset fragmentation voltages (the spectral information is obtained either simultaneously in a single run, or within repeated runs conducted for each voltage) [9,10,20–22], or (ii) mass spectra are generated under conditions of medium fragmentation, thus both molecular and fragment ions are recorded simultaneously [8]. The latter approach was not employed in this study, since for some analytes, relatively labile, highly specific molecular ion adducts ($[M+H]^+$, $[M+NH_4]^+$) may disappear from mass spectrum. Another adverse consequence of loss of molecular ion is a potential failure of non-target analysis which is based on interpretation of compound molecular mass. In Supporting Materials, there is a comprehensive overview of exact masses of molecular ions and respective fragments obtained in our study by in-source CID fragmentation for 212 pesticides. To compose and validate the entries in the ion database, the data published in earlier studies concerned with application of TOF MS in multiresidue pesticide analysis [8,9,21] as well as information on the masses of pesticide fragments obtained in studies employing tandem MS [4–7,23] were considered.

As regards the fragmentation conditions set over LC run, these were optimized to achieve as many intensive product ions as possible. The aperture 1 voltage (in-source CID fragmentation energy) was tested in the range from 5 V to 70 V using 5 V steps. The requirement for obtaining at least three IPs for most analytes was met at combination of voltages for mild (30 V) and extensive (50 V) fragmentation. The third aperture voltage included in the final, optimized method, was 5 V for monitoring of molecular adduct ions. The same setting of collision energies was used in ESI− experiments. Negative ionization mode has been used only in a few publications [24,25] concerned with analysis of multiple pesticide residues in fruit and vegetable (the scope of most existing multiresidue methods involves only those pesticides providing ions in positive mode). Although in this way, the majority of registered pesticides can be determined, there are some widely used active ingredients (for instance phenoxyalkanoic acids), which ionize exclusively in ESI−, thus escaping detection by "ESI+" multiresidue methods. In addition to extending method scope, monitoring of both positive and negative ions in particular sample offers obtaining additional IPs for compounds ionizing in both modes, such as ureas, neionicotinoids and triazoles. The triazoles provide formate adducts only without any fragment formation, because quasi-molecular ion comes to break-up to formate ion and neutral analyte. Under experimental conditions used in this study, negative ions of approx. 30 analytes were intensive enough to enable reliable detection at target concentration level 10 μg/kg that corresponded to EU MRL for baby food [26].

A few isobaric ions originated either from matrix/mobile phase impurities or other pesticides were observed within validation of determinative step. In addition to the biased identification of some analytes with identical molecular masses, false negative results were encountered in some cases due to incorrect centroiding of mass spectra caused by abundant co-eluting mass interferences [27]. For instance, we failed to detect acetamiprid (*m*/*z* 223.0750) when it was overlapped by intensive ion of diethyl phthalate (*m*/*z* 223.0970), an impurity contained in one of water batches used for preparation of mobile phase.

Ferrer and Thurman [8] noted four pairs of pesticides with the same molecular formula, where high mass resolution could not help to resolve such analytes. Then, the unbiased identification can be obtained only on the basis of fragment ions. In our experiments, closely eluting prometryn/terbutryn, ethiofencarb/methiocarb and totally co-eluted desmetryn/simetryn were three most critical pairs. Limits of quantification in case of the last pair were rather increased since selective fragment ions applicable for this purpose were less intensive. Suggested fragmentation pathway of desmetryn and simetryn with fragments used for quantification are illustrated in Fig. 2.

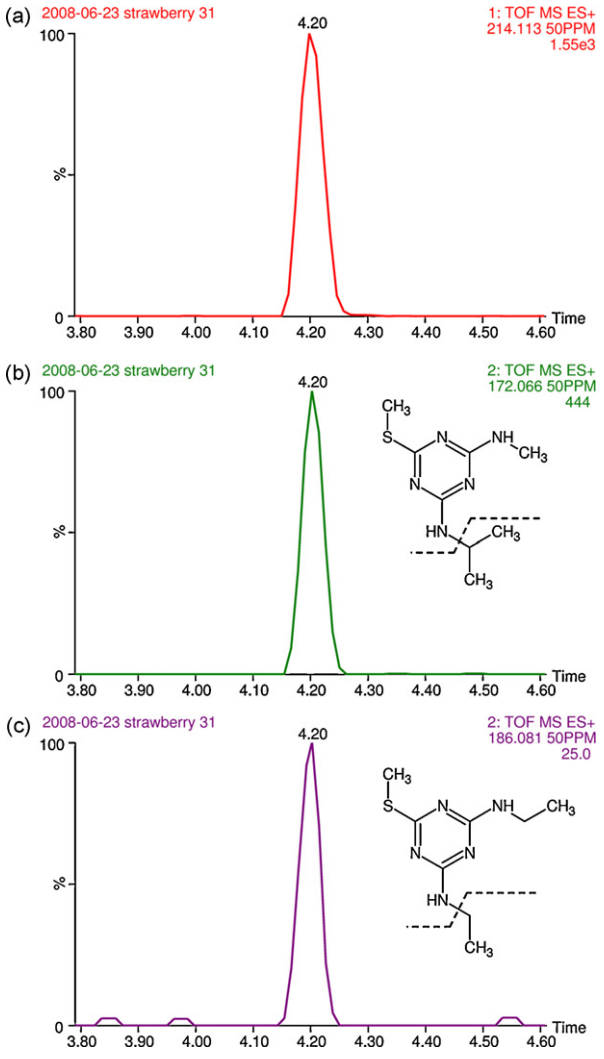

Fig. 2. Identification of isobaric, co-eluted pesticides desmetryn and simetryn in strawberry matrix-matched standard at 10 μg/kg. Extracted chromatogram of (a) desmetryn and simetryn molecular ion (*m*/*z* 214.1126), (b) selective fragment of desmetryn $[C_5H_{10}N_5S]^+$ (*m*/*z* 172.0657) and (c) selective fragment of simetryn $[C_6H_{12}N_5S]^+$ (*m*/*z* 186.0813).

3.3. Validation of UHPLC–TOF MS analysis

As mentioned earlier, the uncertainty of QuEChERS sample handling step has not been subject of this study, the quality of data obtained by our laboratory through this approach was demonstrated in several EU proficiency tests. The focus of our experiments was to investigate the potential of UHPLC–TOF MS in pesticide residue analysis. In paragraphs below, validation data are discussed in detail.

3.3.1. Limits of quantification

Limits of detection (LODs)/limits of quantification (LOQs) are, undoubtedly, key parameters in residue analysis. Optimal mode of analytes detection was searched in this phase of experiments. The LCT Premier XE offers two modes of measurement, (i) "V mode" using one reflection of ions package that enables higher intensity of signal on account of lower mass resolution and (ii) "W mode" with three reflections resulting in higher mass resolution, while ion intensity is rather reduced. Based on the assessment of obtained data, "W mode" was found more suitable for analysis of complex matrices; because of better selectivity. Although low sample equivalent was injected (2 mg), only 8 analytes from total 212 were not detected below 10 μg/kg, the EU MRL for baby food [26] (see Table 2).

Worth to notice, LODs and LOQs of analytes were not determined in this study since their extrapolation based on results obtained for the lowest calibration point and pre-defined target S/N ratio was practically impossible. When working with narrow mass window (particularly 50 ppm), the chemical noise was either very low or undetectable what may lead to unrealistically high S/N value, so lowest calibration levels (LCLs) were estimated.

Relatively worse LCLs (as high as 20 μg/kg) were obtained in case of some pyrethroids. In addition to inherently weak ES ionization, a bulk of less polar matrix eluting in the late part of chromatogram caused severe ion suppression. This problem was most pronounced in the spinach.

3.3.2. Working range

The linearity of LCT Premier XE was tested in the concentration range 1–450 μg/kg, which corresponded to contamination levels found in real-life samples. TDC used for digitalization of pulses from detector offers higher resolution for low masses, but on the other hand, this setup suffers from narrow dynamic range. Although mathematical algorithm "dead time correction" is commonly employed to improve dynamic range of TDC, it cannot work with higher number of arriving ions. Therefore, modern instruments incorporating TDC use a special option to improve dynamic range of TOF MS acquisition system called "Dynamic Range Enhancement" (DRE). This software function replaces saturated data in a mass spectrum with unsaturated ones, which are acquired with defocused ion beam, when fewer ions are introduced into the flight tube. As far as this function is turned on, each spectrum is acquired twice: under normal condition and with defocused ion beam. Significant improvement of a detection capability of abundant sample components obtained in this way is demonstrated in Fig. 3. Although the increase of dynamic range with DRE function is usually by factor 25–30, extension of a linear range of calibration curves is typically fairly lower, not more than 2–4 times. Generally, the most pronounced impact of DRE function was observed for analytes with *m*/*z* above 250. Linearity range for those few compounds with masses up to *m*/*z* 200 (e.g. methamidophos, *m*/*z* 142.0091; methomyl, *m*/*z* 163.0541; metolcarb, *m*/*z* 166.0868; carbendazim, *m*/*z* 192.0773; etc.) was better without DRE function. Comparison of calibration curves of high and low molecular weight compounds (methomyl and azoxystrobin, respectively) as measured under alternative conditions are shown

Table 2
Validation results in tested matrices ($n = 6$), fortified at 10 μg/kg, except LOQ > 10 μg/kg (fortification at 100 μg/kg).

Compound	Ionization	Apple		Strawberry		Spinach		Tomato	
		LCL–MAX$^+$ (μg/kg)	RSD (%)	LCL–MAX$^+$ (μg/kg)	RSD (%)	LCL–MAX$^+$ (μg/kg)	RSD (%)	LCL–MAX$^+$ (μg/kg)	RSD (%)
Acephate	ESI+	1–200	7.14	2–450	4.67	5–300	5.44	1–450	2.51
Acetamiprid	ESI+	1–300	5.20	2–450	6.11	2–300	8.53	2–300	4.88
Acrinathrin	ESI+	2–450	10.02	2–300	8.84	2–450	6.14	2–300	5.97
Alachlor	ESI+	2–200	3.31	2–450	4.77	5–300	3.65	2–450	4.77
Aldicarb	ESI+	2–200	5.24	1–300	5.97	1–200	5.43	5–200	7.00
Aldicarb-sulfone	ESI+	1–200	3.91	2–200	3.54	1–300	3.43	1–200	1.90
Aldicarb-sulfoxid	ESI+	2–200	4.34	5–300	3.71	2–200	5.31	2–450	8.75
Ametryn	ESI+	1–450	4.57	2–450	3.19	2–300	4.76	1–450	7.74
Atrazin	ESI+	1–200	4.51	1–300	4.96	1–300	5.93	1–450	5.82
Azinphos-ethyl	ESI+	2–450	3.53	2–300	2.61	5–300	3.27	2–300	4.65
Azinphos-methyl	ESI+	1–450	5.49	1–300	4.69	1–300	4.30	1–450	4.01
Azoxystrobin	ESI+	1–450	3.16	1–300	5.10	1–300	3.68	1–450	4.10
Benalaxyl	ESI+	1–200	3.20	1–300	7.27	1–300	6.18	1–450	6.84
Bifenthrin	ESI+	20–200	6.34	5–450	7.14	20–450	10.10	2–450	2.69
Bitertanol	ESI+	1–450	6.51	1–450	4.43	1–300	4.29	2–450	7.77
Bupirimate	ESI+	1–200	4.29	1–300	5.51	1–300	6.53	1–300	4.13
Buprofezin	ESI+	1–300	6.98	1–450	6.57	1–200	4.62	1–450	5.72
Cadusafos	ESI+	5–450	5.17	1–450	5.67	1–300	5.19	1–450	3.83
Carbaryl	ESI+	2–200	5.07	1–300	5.39	2–300	3.11	1–450	3.91
Carbendazim	ESI+	1–200	4.69	1–100	5.87	2–100	5.74	1–450	4.40
Carbofuran	ESI+	2–300	4.23	1–300	3.56	1–200	4.14	1–200	6.66
Carbofuran-3-OH	ESI+	5–300	2.92	2–300	5.02	5–200	6.13	1–450	5.41
Carbophenothion	ESI+	5–300	5.96	2–300	5.53	2–200	5.05	2–450	6.65
Chlorfenvinphos	ESI+	1–450	6.69	1–300	3.94	1–300	4.24	1–450	3.42
Chloroxuron	ESI+	1–300	6.47	1–300	3.41	1–200	3.52	1–30	1.95
Chlorpyrifos	ESI+	1–450	6.24	1–450	4.52	5–300	8.64	2–200	4.55
Chlorpyrifos-methyl	ESI+	5–300	3.31	5–300	5.08	5–450	8.99	10–450	13.34
Clofentezine	ESI+	1–300	5.15	1–300	4.49	5–200	3.65	5–300	3.96
Clomazone	ESI+	1–300	2.25	1–450	4.49	1–300	3.62	1–450	3.96
Clothianidin	ESI+	2–300	5.54	2–300	6.09	5–450	5.60	1–300	6.76
Cyanazine	ESI+	1–200	4.86	1–200	2.55	2–200	5.08	1–300	3.13
Cyazofamid	ESI+ESI+	1–300	3.91	1–300	5.10	1–200	5.48	1–450	5.08
Cymoxanil	ESI+	10–200	4.71	5–300	5.38	5–300	4.62	10–300	4.51
Cypermethrin	ESI+	10–450	6.36	10–450	6.98	10–450	6.81	10–450	7.30
Cyproconazole	ESI+	2–450	3.59	2–300	3.89	5–300	3.38	2–450	5.80
Cyprodinil	ESI+	1–300	3.56	1–450	5.70	1–200	5.98	1–450	2.85
Deltamethrin	ESI+	10–300	11.62	10–450	8.00	5–450	7.84	5–450	6.21
Demeton-S-methyl	ESI+	1–300	2.80	2–450	5.11	2–300	4.16	2–200	6.13
Demeton-S-methylsulphon	ESI+	1–300	5.13	1–200	2.99	1–300	1.93	1–450	5.89
Desmetryn	ESI+	1–300	2.84	2–300	2.66	2–300	2.14	1–300	4.49
Diazinon	ESI+	1–300	8.22	1–450	6.09	1–300	4.26	1–450	3.02
Dichlofluanid	ESI+	1–300	3.39	2–300	4.20	5–200	7.01	2–300	6.26
Dichlorvos	ESI+	5–300	4.08	5–450	10.71	5–300	4.52	5–200	11.45
Diclofop-methyl	ESI+	2–300	8.19	2–200	2.68	2–450	6.93	5–300	4.96
Dicrotophos	ESI+	1–300	4.28	2–300	7.61	1–200	4.66	1–300	5.65
Diethofencarb	ESI+	1–450	4.52	1–450	2.83	1–300	4.35	1–450	5.62
Difenoconazole	ESI+	2–300	3.43	1–450	5.23	1–200	5.97	1–450	5.26
Diflubenzuron	ESI+	2–300	4.74	1–450	3.33	1–200	5.56	1–450	3.04
Diflufenican	ESI+	1–300	4.78	2–450	5.14	1–200	4.06	2–200	4.48
Dimethoate	ESI+	1–300	2.76	1–200	6.90	1–300	6.62	1–300	5.52
Dimethomorph	ESI+	1–450	1.57	1–450	2.77	1–300	6.51	1–450	3.95
Dimoxystrobin	ESI+	1–300	2.57	1–450	4.41	1–200	6.68	1–450	5.45
Disulfoton-sulfon	ESI+	1–450	4.12	1–300	5.06	1–300	5.40	1–300	6.85
Disulfoton-sulfoxide	ESI+	1–300	4.33	1–450	2.95	1–300	6.86	1–300	4.32
Diuron	ESI+	1–300	2.90	1–300	6.66	1–300	6.50	1–300	5.74
DMSA	ESI+	5–300	4.01	1–200	6.67	2–200	3.70	2–200	3.69
DMST	ESI+	1–300	5.39	1–200	5.05	1–100	4.15	1–300	5.15
Dodine	ESI+	1–300	5.82	1–300	4.19	1–450	3.66	1–300	6.06
Epoxiconazole	ESI+	1–450	5.14	1–300	5.11	1–300	5.22	1–450	6.91
Ethiofencarb	ESI+	1–300	5.38	1–300	5.59	1–300	3.83	1–200	4.63
Ethion	ESI+	1–450	4.30	1–450	6.21	1–200	4.36	1–300	5.44
Ethofumesate	ESI+	1–300	3.34	1–300	4.43	1–300	3.40	1–450	4.73
Ethoprophos	ESI+	1–300	3.70	1–300	6.75	1–200	4.35	1–450	5.85
Ethoxyquin	ESI+	10–450	11.05	2–300	6.66	5–300	10.54	2–300	7.02
Etofenprox	ESI+	2–300	4.21	1–300	5.08	2–300	5.74	2–450	6.39
Etrimfos	ESI+	1–300	3.04	1–100	4.40	1–300	5.55	1–450	3.55
Fenamiphos	ESI+	1–300	4.66	1–300	5.85	1–200	5.64	1–450	6.62
Fenamiphos-sulfon	ESI+	1–450	5.01	1–200	5.69	1–300	3.85	1–450	6.34
Fenamiphos-sulfoxide	ESI+	1–450	3.39	1–450	4.87	1–300	3.70	1–450	3.67
Fenarimol	ESI+	10–300	7.69	10–450	6.43	20–100	9.77	5–300	8.35
Fenazaquin	ESI+	1–300	4.95	1–450	3.60	1–300	5.51	1–300	3.12
Fenbuconazole	ESI+	5–450	2.55	2–450	5.16	1–450	3.77	2–450	6.86
Fenhexamid	ESI+	1–300	2.87	1–300	4.97	1–300	5.96	2–450	5.91

Table 2 (*Continued*)

Compound	Ionization	Apple		Strawberry		Spinach		Tomato	
		LCL–MAX$^+$ (μg/kg)	RSD (%)	LCL–MAX$^+$ (μg/kg)	RSD (%)	LCL–MAX$^+$ (μg/kg)	RSD (%)	LCL–MAX$^+$ (μg/kg)	RSD (%)
Fenoxycarb	ESI+	1–450	5.81	1–300	2.60	1–300	5.74	1–450	3.58
Fenpropathrin	ESI+	5–450	6.79	5–450	5.31	5–300	6.74	5–450	4.87
Fenpropidin	ESI+	1–300	4.72	1–450	2.43	1–200	5.44	1–450	3.92
Fenpropimorph	ESI+	2–200	3.07	1–200	3.18	5–100	3.56	1–450	2.04
Fenpyroximate	ESI+	1–300	6.37	1–450	2.52	1–200	2.28	1–300	4.75
Fenthion	ESI+	1–300	6.79	2–200	4.24	1–200	9.82	2–200	5.34
Fipronil	ESI+	5–300	9.54	5–300	4.98	5–300	11.22	5–450	5.01
Fluazifop-P-butyl	ESI+	1–300	3.08	1–450	5.43	1–300	4.47	1–450	5.80
Flufenoxuron	ESI+	1–300	4.78	1–300	3.78	1–300	5.09	1–450	4.69
Fluoxastrobin	ESI+	1–200	3.65	1–300	1.98	1–200	3.49	1–300	4.80
Fluquinconazole	ESI+	5–300	2.17	5–200	4.19	5–200	7.10	2–450	4.63
Flusilazole	ESI+	1–300	1.90	1–300	3.91	1–200	4.01	1–450	3.72
Fonofos	ESI+	5–300	5.91	5–200	3.93	2–300	4.56	5–300	5.45
Haloxyfop-etotyl	ESI+	1–300	5.76	1–300	6.80	1–300	6.02	1–450	3.62
Haloxyfop-P	ESI+	5–450	5.24	5–450	5.31	2–300	8.20	5–450	6.01
Haloxyfop-P-methyl	ESI+	1–300	5.92	1–300	6.50	1–300	5.26	1–450	5.01
Heptenophos	ESI+	1–300	5.50	1–200	6.45	1–450	5.05	1–450	5.75
Hexaconazole	ESI+	1–450	3.60	2–450	5.04	1–300	5.46	2–450	4.37
Hexythiazox	ESI+	1–300	3.41	2–300	4.97	2–450	3.82	2–200	6.11
Imazalil	ESI+	1–300	4.33	1–450	2.88	1–200	4.77	1–450	3.38
Imidacloprid	ESI+	2–300	5.06	1–200	5.49	1–300	4.16	1–450	4.24
Indoxacarb	ESI+	1–450	5.13	1–450	5.58	1–200	4.18	1–450	8.24
Iodosulfuron-methyl	ESI+	2–300	4.57	2–300	4.04	1–200	4.53	1–300	5.64
Iprovalicarb	ESI+	2–300	5.16	2–300	4.16	5–300	4.55	2–450	5.54
Isofenphos	ESI+	2–300	4.86	2–300	5.81	2–100	3.42	1–300	3.91
Isoproturon	ESI+	5–300	5.76	5–300	5.10	5–200	3.70	5–450	5.79
Kresoxim-methyl	ESI+	1–450	4.79	1–450	3.83	1–300	4.27	1–450	5.09
Lenacil	ESI+	5–300	6.13	2–450	4.04	2–200	4.74	2–450	4.36
Linuron	ESI+	1–300	3.28	1–450	4.37	1–200	5.72	1–450	4.27
Malaoxon	ESI+	1–300	6.15	1–450	4.41	2–200	7.36	1–300	3.14
Malathion	ESI+	2–300	5.61	5–450	5.33	5–300	8.71	1–300	6.98
Mecarbam	ESI+	1–300	4.15	1–200	5.39	1–200	4.89	1–300	5.76
Mefenpyr-diethyl	ESI+	1–300	4.07	1–200	5.33	1–300	5.64	1–300	7.14
Mepanipyrim	ESI+	2–200	6.69	2–300	4.40	2–200	4.69	2–200	5.10
Metalaxyl-M	ESI+	1–300	4.89	1–300	2.45	1–300	4.18	1–300	4.61
Metamitron	ESI+	2–200	6.68	2–300	7.34	5–100	9.81	2–200	2.28
Metconazole	ESI+	1–450	3.09	2–450	3.06	2–300	3.21	1–450	6.53
Methacrifos	ESI+	5–300	8.26	5–300	8.40	2–300	8.04	5–450	8.69
Methamidophos	ESI+	2–450	4.91	5–450	5.25	2–300	6.17	1–450	5.47
Methidathion	ESI+	1–450	6.49	1–200	4.38	1–300	3.12	1–450	3.23
Methiocarb	ESI+	1–300	3.40	1–300	5.48	1–300	6.92	1–450	4.47
Methiocarb-sulfon	ESI+	2–300	6.44	1–300	6.18	1–300	3.60	1–450	2.98
Methiocarb-sulfoxide	ESI+	1–300	5.59	2–450	2.33	1–200	4.83	1–450	2.36
Methomyl	ESI+	2–50	5.86	5–200	9.44	1–100	7.07	2–100	5.27
Methoxyfenozide	ESI+	1–300	4.31	1–300	4.54	1–200	3.07	1–300	5.17
Metobromuron	ESI+	1–200	2.00	1–200	5.34	1–300	4.66	1–300	5.18
Metolachlor	ESI+	1–300	5.50	1–300	1.74	1–300	1.57	1–450	2.55
Metolcarb	ESI+	1–200	3.57	2–100	5.55	5–100	6.65	2–300	6.16
Metoxuron	ESI+	1–50	3.44	1–50	7.42	1–100	4.76	1–100	4.73
Metribuzin	ESI+	1–200	2.48	1–300	4.44	1–300	3.71	1–300	3.51
Mevinphos	ESI+	1–300	4.94	1–300	5.04	5–100	8.92	1–450	3.84
Monocrotophos	ESI+	1–300	6.92	2–220	5.30	5–450	6.76	2–450	3.46
Monolinuron	ESI+	1–300	2.85	1–300	4.69	1–300	3.89	1–300	2.83
Monuron	ESI+	1–50	5.98	1–50	3.14	1–50	2.97	1–100	4.55
Myclobutanil	ESI+	2–450	3.66	5–450	4.36	2–300	2.11	1–300	1.85
Naled	ESI+	5–200	6.60	5–200	7.78	5–200	8.86	5–200	8.90
Neuron	ESI+	1–300	3.40	1–300	5.41	1–200	2.98	1–300	5.86
Norflurazone	ESI+	1–450	4.30	1–450	5.27	1–300	4.69	1–450	5.08
Omethoate	ESI+	1–300	3.47	1–300	3.88	1–100	5.76	1–450	3.81
Oxadixyl	ESI+	5–300	5.57	1–450	2.92	5–200	4.29	2–450	5.88
Oxamyl	ESI+	2–300	6.33	5–300	5.24	2–200	5.98	2–450	4.09
Oxydemeton-methyl	ESI+	1–300	5.48	1–100	6.43	1–300	3.99	1–450	8.18
Paclobutrazol	ESI+	2–300	5.25	1–300	4.45	20–200	4.90	1–300	4.82
Penconazole	ESI+	1–200	4.05	1–200	2.78	1–300	10.98	1–200	5.59
Pencycuron	ESI+	1–200	3.99	1–200	3.20	2–300	3.98	1–300	3.71
Pendimethalin	ESI+	5–300	5.39	2–300	2.80	2–300	4.73	2–200	5.34
Permethrin	ESI+	10–450	4.78	2–450	4.47	20–450	7.99	5–450	7.05
Phenmedipham	ESI+	1–450	5.62	1–450	1.48	1–450	5.09	1–450	4.01
Phenothrin	ESI+	5–450	5.66	5–450	5.92	20–450	4.87	5–450	2.44
Phenthoate	ESI+	1–300	3.53	1–300	5.37	1–200	3.20	1–450	4.35
Phorate	ESI+	2–300	5.30	5–450	6.15	5–200	6.60	2–450	5.02
Phorate-sulfone	ESI+	1–300	2.58	1–450	3.45	1–200	4.05	1–300	6.05
Phorate-sulfoxide	ESI+	1–300	5.05	1–300	4.78	1–200	5.62	1–300	5.33
Phosalone	ESI+	1–300	3.81	1–200	5.83	1–200	5.65	1–200	4.72

Table 2 (*Continued*)

Compound	Ionization	Apple		Strawberry		Spinach		Tomato	
		LCL–MAX$^+$ (μg/kg)	RSD (%)	LCL–MAX$^+$ (μg/kg)	RSD (%)	LCL–MAX$^+$ (μg/kg)	RSD (%)	LCL–MAX$^+$ (μg/kg)	RSD (%)
Phosmet	ESI+	1–450	2.87	1–450	4.47	1–300	6.25	1–450	3.75
Phosphamidon	ESI+	2–300	4.50	1–200	5.30	2–450	2.12	1–300	5.53
Picoxystrobin	ESI+	1–200	3.73	1–300	5.27	1–200	3.96	1–200	2.87
Piperonyl butoxide	ESI+	1–200	3.70	1–200	2.04	2–200	6.58	1–300	7.19
Pirimicarb	ESI+	1–300	3.79	2–450	5.67	2–200	3.67	1–300	4.24
Pirimiphos-ethyl	ESI+	1–300	3.62	1–300	3.67	1–200	9.04	1–300	4.33
Pirimiphos-methyl	ESI+	1–300	2.25	1–300	4.39	1–200	3.60	1–450	5.88
Prochloraz	ESI+	1–300	5.01	1–300	4.75	1–300	5.17	1–300	4.62
Profenofos	ESI+	1–300	5.84	1–450	3.23	1–200	2.84	1–300	3.62
Prometon	ESI+	1–300	3.69	1–300	6.05	1–300	5.73	1–450	5.22
Prometryn	ESI+	1–300	5.46	1–450	5.57	1–200	5.24	1–450	3.53
Propachlor	ESI+	1–100	4.80	1–100	3.46	1–200	6.73	1–200	4.58
Propamocarb	ESI+	1–300	3.26	1–300	3.89	1–300	6.48	1–450	3.37
Propaquizafop	ESI+	1–450	6.70	1–300	4.24	1–450	4.02	1–450	4.70
Propargite	ESI+	1–300	2.87	1–450	5.70	1–300	6.84	1–450	3.69
Propham	ESI+	10–450	7.83	10–450	8.38	5–450	6.83	10–300	9.82
Propiconazole	ESI+	1–300	6.39	1–450	2.62	1–300	4.93	1–450	4.82
Propoxur	ESI+	1–300	6.78	1–200	3.77	1–100	6.46	1–50	4.12
Propyzamide	ESI+	1–300	5.92	1–300	1.60	1–300	2.34	1–300	4.04
Prosulfocarb	ESI+	1–450	3.66	2–450	4.01	1–300	4.25	2–450	3.26
Pymetrozine	ESI+	5–200	5.27	1–200	6.21	2–100	2.87	1–100	7.58
Pyraclostrobin	ESI+	1–300	4.99	1–300	5.73	1–200	2.48	1–450	6.84
Pyrazophos	ESI+	1–300	4.23	1–300	3.82	2–300	4.10	1–300	6.90
Pyridaben	ESI+	2–300	6.95	1–300	4.17	1–300	3.37	1–300	2.65
Pyrimethanil	ESI+	1–300	4.91	1–450	3.93	1–30	3.16	1–450	4.58
Pyriproxyfen	ESI+	1–300	4.30	1–300	3.11	1–300	6.95	1–450	4.72
Quinalphos	ESI+	1–300	4.63	1–300	4.02	1–300	4.65	1–450	6.21
Quinmerac	ESI+	1–200	4.21	1–100	1.62	1–100	6.44	1–100	5.82
Quinoxyfen	ESI+	1–450	5.31	1–450	4.80	2–300	4.92	1–450	3.79
Resmethrin	ESI+	2–300	3.50	2–200	1.68	5–450	9.21	5–450	4.57
Rimsulfuron	ESI+	2–300	5.61	5–300	7.17	2–200	2.79	2–300	3.85
Simazine	ESI+	1–100	5.54	1–100	4.06	1–100	2.94	1–100	5.56
Simetryn	ESI+	5–300	6.30	5–300	5.05	5–300	3.27	5–300	8.30
Spinosyn A	ESI+	2–300	3.86	5–200	3.72	5–300	6.05	5–300	3.65
Spinosyn D	ESI+	5–200	3.78	5–200	4.67	10–300	5.98	10–200	7.05
Spiroxamine	ESI+	1–450	2.70	1–300	6.84	1–300	4.34	1–450	4.43
Sulfotep	ESI+	1–300	5.79	1–300	5.91	1–300	4.60	1–450	6.01
tau-Fluvalinate	ESI+	5–300	5.50	5–450	7.87	10–450	11.98	2–450	7.68
Tebuconazole	ESI+	1–200	1.28	1–200	4.60	1–300	2.09	1–200	5.44
Tebufenozide	ESI+	2–200	6.18	1–200	5.04	2–200	6.60	2–200	6.55
Teflubenzuron	ESI+	10–300	16.28	10–300	8.42	10–450	15.57	10–450	7.97
Terbufos	ESI+	5–300	6.00	5–450	7.38	5–200	13.63	5–450	9.96
Terbuthylazine	ESI+	1–300	7.18	1–300	5.94	1–300	5.85	1–300	6.76
Terbutryn	ESI+	1–450	4.73	1–300	5.51	1–300	4.06	1–300	5.06
Tetraconazole	ESI+	1–300	3.95	1–200	4.03	1–200	6.80	1–300	4.59
Thiabendazole	ESI+	1–300	5.91	1–200	4.80	1–300	4.28	1–450	6.28
Thiacloprid	ESI+	1–300	6.97	1–450	1.75	1–300	4.46	1–300	3.48
Thiamethoxam	ESI+	5–300	7.14	2–100	7.55	2–300	2.30	1–200	3.18
Thiodicarb	ESI+	1–300	6.13	1–300	2.02	2–200	8.15	1–450	5.03
Thiophanate-methyl	ESI+	1–450	3.40	1–450	3.68	1–300	1.43	1–300	4.22
Tolylfluanid	ESI+	1–200	2.60	1–300	3.98	1–100	6.32	1–300	7.22
Triadimefon	ESI+	1–450	4.33	1–450	3.17	1–300	1.85	1–450	3.14
Triadimenol	ESI+	2–300	6.27	5–300	2.12	20–200	3.22	2–450	5.70
Triazophos	ESI+	1–300	5.56	1–300	4.94	1–300	4.68	1–300	4.97
Trichlorfon	ESI+	5–300	5.65	20–450	7.30	5–300	5.15	5–300	8.64
Trifloxystrobin	ESI+	1–300	4.97	1–300	5.96	1–300	4.51	1–450	4.45
Triflumuron	ESI+	2–300	5.00	1–200	3.08	5–300	6.43	2–450	5.76
Triforine	ESI+	20–450	4.04	20–450	5.41	20–300	2.73	20–450	4.49
Vamidothion	ESI+	1–200	4.10	1–200	4.63	1–300	2.43	1–300	3.70
2.4-D	ESI-	10–450	7.10	5–200	5.31	10–200	5.63	5–200	6.92
Acetamiprid	ESI-	20–200	5.28	5–200	5.16	10–200	13.53	10–200	6.28
Acrinathrin	ESI-	5–300	6.68	5–300	6.50	2–300	7.74	2–200	5.24
Bentazone	ESI-	1–450	6.31	1–300	6.83	1–300	2.44	1–300	3.57
Chloroxuron	ESI-	20–200	4.04	10–200	4.10	10–300	12.23	5–200	7.11
Clothianidin	ESI-	1–50	5.53	1–200	3.31	1–200	3.48	1–100	4.20
Cyanazine	ESI-	5–200	9.09	20–300	4.35	10–200	7.67	10–450	9.33
Cymoxanil	ESI-	5–200	10.19	2–300	6.07	5–100	3.84	2–200	6.19
Cyproconazole	ESI-	2–200	5.00	2–300	4.35	2–200	3.12	2–200	5.15
Diflubenzuron	ESI-	2–100	2.93	2–300	7.05	1–200	6.70	2–200	7.46
Diflufenican	ESI-	5–200	7.64	2–200	3.65	2–300	3.85	2–200	4.78
Diuron	ESI-	2–100	3.13	1–100	4.05	2–200	5.17	1–200	4.35
DMSA	ESI-	10–200	5.85	5–200	5.97	5–200	5.22	5–300	7.94
DMST	ESI-	5–300	3.84	5–200	7.42	10–300	8.84	5–200	6.51
Fenarimol	ESI-	5–200	6.50	20–300	4.10	10–300	8.23	10–300	4.29

Table 2 (*Continued*)

Compound	Ionization	Apple		Strawberry		Spinach		Tomato	
		LCL–MAX⁺ (μg/kg)	RSD (%)	LCL–MAX⁺ (μg/kg)	RSD (%)	LCL–MAX⁺ (μg/kg)	RSD (%)	LCL–MAX⁺ (μg/kg)	RSD (%)
Fenbuconazole	ESI-	2–200	7.63	2–200	3.68	1–200	6.19	2–200	5.38
Fenhexamid	ESI-	5–300	6.37	2–200	6.52	2–200	6.64	5–200	8.53
Fipronil	ESI-	1–200	5.14	1–200	5.15	1–200	7.32	1–200	8.58
Fluazinam	ESI-	1–300	3.07	1–200	4.70	1–300	5.94	1–300	4.69
Fludioxonil	ESI-	1–450	7.26	1–300	7.68	1–300	6.74	1–450	6.99
Flufenoxuron	ESI-	2–450	5.04	2–200	2.79	1–200	2.24	1–450	3.31
Fluquinconazole	ESI-	5–200	5.85	5–300	6.91	5–200	7.74	5–200	5.23
Haloxyfop-P	ESI-	2–200	5.45	2–200	7.58	2–200	8.93	2–450	4.59
Hexaconazole	ESI-	2–200	5.97	1–300	7.97	5–300	6.67	2–300	3.96
Imidacloprid	ESI-	10–300	8.90	5–200	9.76	5–200	6.39	5–200	7.23
Iodosulfuron-methyl	ESI-	2–300	3.84	2–200	3.86	1–200	4.91	1–200	3.80
Iprovalicarb	ESI-	5–300	5.42	5–200	3.01	2–200	6.05	5–200	4.83
Lenacil	ESI-	2–200	3.93	5–200	6.86	5–200	3.73	5–200	3.42
MCPA	ESI-	5–300	5.60	1–100	6.16	2–100	3.83	2–100	5.19
Mecoprop	ESI-	5–450	4.44	2–100	3.55	5–100	3.14	2–100	6.26
Metconazole	ESI-	2–200	4.15	2–300	3.52	5–200	5.70	2–200	3.97
Neburon	ESI-	1–200	3.57	1–200	3.95	1–200	7.07	1–200	4.21
Norflurazone	ESI-	20–450	8.17	20–200	2.54	10–200	6.87	10–200	8.22
Paclobutrazol	ESI-	1–300	3.56	1–450	3.57	2–450	7.44	1–200	4.05
Pencycuron	ESI-	10–300	8.68	5–200	7.34	5–200	8.93	5–300	8.13
Propyzamide	ESI-	10–300	8.56	5–200	7.78	2–200	9.35	5–300	9.51
Rimsulfuron	ESI-	1–100	6.89	5–200	8.33	2–200	4.84	1–200	7.39
tau-Fluvalinate	ESI-	20–300	5.99	20–200	7.16	50–300	7.56	20–300	7.84
Tebuconazole	ESI-	5–300	4.23	2–200	4.32	2–300	4.71	2–200	5.73
Tebufenozide	ESI-	2–100	2.69	1–200	4.73	1–200	6.40	2–200	6.48
Teflubenzuron	ESI-	1–200	5.51	1–200	3.35	1–300	2.94	1–200	4.75
Tetraconazole	ESI-	2–200	3.25	1–200	4.27	1–200	5.34	1–200	3.34
Thiacloprid	ESI-	5–300	4.39	2–200	4.19	5–200	6.74	2–200	2.86
Thiophanate-methyl	ESI-	10–300	7.53	10–300	4.22	2–200	8.90	10–200	8.41
Triadimefon	ESI-	20–200	5.44	20–300	12.95	20–450	14.15	20–200	12.88
Triadimenol	ESI-	5–300	5.52	5–200	6.66	5–200	6.96	5–300	5.06
Triflumuron	ESI-	1–200	6.80	1–300	9.39	10–300	9.98	1–200	8.00

⁺Maximum of linearity with DRE function.

in Fig. 4. The overview of linearity ranges for all analytes is summarized in Table 2.

3.4. Possibilities of non-target screening

The possibility of non-target screening is often mentioned as the unique feature of TOF mass analyzers. Rather surprisingly, there are only few studies concerned with strategies enabling to find and identify unexpected (non-target) pesticide residues or their metabolites occurring in examined sample due to unauthorized use [28–33]. An interesting, simple approach based on concept of diagnostic fragment ions was developed by Ferrer and Thurman [8]. The key assumption employed by authors was that compounds representing the same structure class provide identical 'diagnostic fragments'. In Table 3, there is an overview of such ions corresponding to various pesticide groups. In addition to characteristic fragments reported earlier [8], we have added 12 new, thus expanding searchable structures, of both parent compounds and their transformation products. As shown, based on combination of two ions, several sub-groups might be distinguished within particular pesticide class. For example, fragmentation of organophosphorus pesticides depends on the characteristic arrangement on phosphorus atom (–P=O versus –P=S). While compounds containing –P=S structure yield fragments *m*/*z* 157.0083 and *m*/*z* 142.9926, those with –P=O produce fragments *m*/*z* 141.0311 and *m*/*z* 127.0155. Similarly, different characteristic fragments were obtained from triazole pesticides: either dichlorophenyl (*m*/*z* 158.9768) or chlorophenyl (*m*/*z* 125.0158) ion can be yielded, depending on the fungicide structure. In Fig. 5, a few examples of diagnostic ions detected in apple extract spiked at 10 μg/kg with all 212 pesticides involved in this study are shown. Their presence in chromatogram indicates possible occurrence of a com-

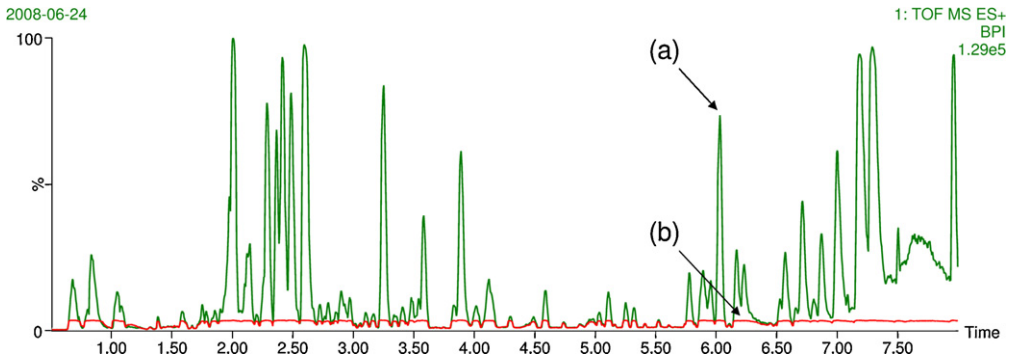

Fig. 3. Comparison of base peak ion (BPI) chromatograms of blank strawberry extract acquired with (a) and without (b) DRE function. The magnification factor (improvement of dynamic range of TDC) is 28.

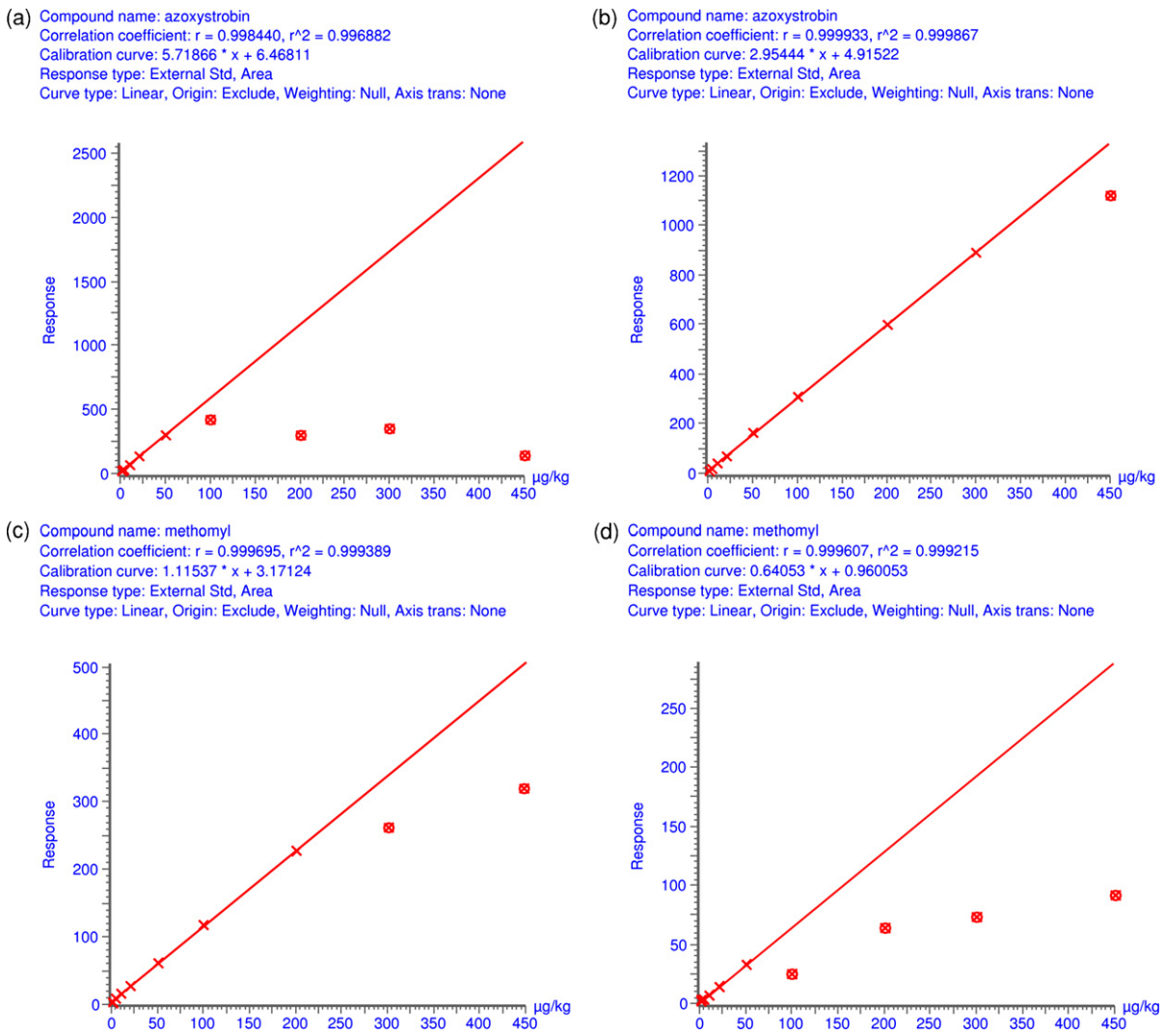

Fig. 4. The influence of DRE on the linearity of compound with different mass: methomyl (*m*/*z* 163.0541) and azoxystrobin (*m*/*z* 404.1246). Calibration of methomyl without DRE function (a) offers better linearity (up to 0.2 μg/kg) than with DRE (b), but linearity of azoxystrobin with DRE function is significantly improved (d) as compared to calibration without DRE (c).

pound related to respective group of target analytes. Obtaining of molecular ion adduct is often possible following background subtraction at particular retention time. As additional evidence, peak with identical shape should be found in record obtained without fragmentation.

Fig. 6 illustrates an example of utilization of diagnostic ions in identification of non-targeted metabolites of (commonly targeted) parent pesticide. When reprocessing TOF MS data acquired in analysis of cherries containing tebuconazole residues (87 μg/kg), also several other signals at different retention times were detected at ions characteristic for triazoles group (*m*/*z* 70.0400 and 125.0158). Based on spectra of these 'unknown' peaks, molecular ion was found and potential elemental composition was calculated within the 5 ppm range. To reduce the number of conceivable formulas which are increasing exponentially with a mass of detected ion, the strategy outlined by Suzuki et al. [34] and Kaufman [35] was employed. In this way, all formulas for which the sum of measured fragment and calculated neutral loss is not corresponding to the proposed formula of molecular ion can be eliminated. Considering the literature data on metabolic pathways of triazoles in plants [36], we concluded that detected compounds are hydroxy derivate of tebuconazole and tebuconazole-glucoside (the experimental mass error was 1.5 ppm and 1.2 ppm, respectively). Since hydroxylation/conjugation could take place at different carbons of tebuconazole skeleton, four peaks of isomeric hydroxyl derivates and two peaks of glucosides (same mass spectra) were observed.

Thanks to the retrospective search based on diagnostic ions we also found and identified in one of apple samples desmethyl-pirimicarb, the degradation product of widely used insecticide pirimicarb. The unknown peak was located due to the occurrence of fragment *m*/*z* 72.0444, which is common for both molecules, parent and its metabolite. In addition to pesticide transformation products, we also found in another apple sample (thanks to the detection of diagnostic fragment ions *m*/*z* 158.0412 and *m*/*z* 141.0146) lufenuron, a benzoylurea insecticide, which was not on the list of our target compounds.

As documented in this paper, the system of diagnostic fragment ions is useful for finding degradation products and non-target pesticides. However, the applicability of this concept is rather limited, many exceptions exist. For instance tolylfluanid and its main degradation product, DMST (*N*,*N*-dimethyl-*N*′-tolylsulfonyldiamide), do not have any identical fragments, hence the use of "diagnostic approach" is not feasible. Also non-target screening of strobilurins, widely used fungicides, cannot be based on a single common fragment ion.

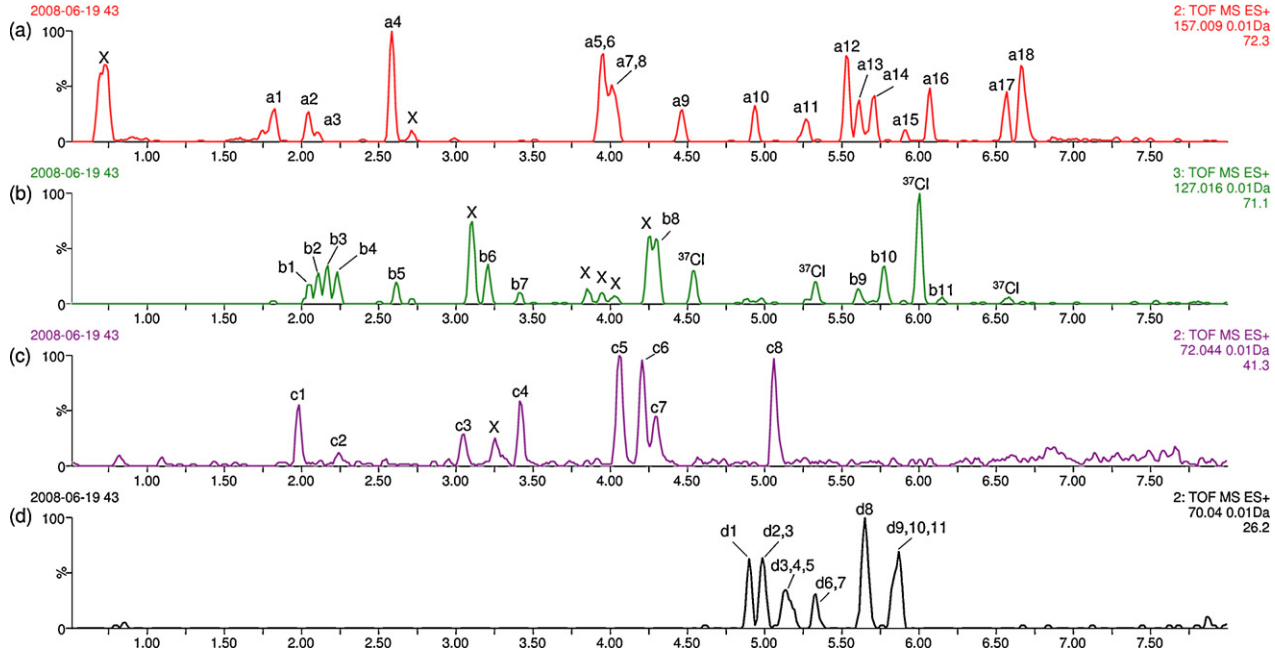

Fig. 5. XIC chromatograms of selected diagnostic fragments at concentration 10 μg/kg in apple matrix-matched standard. The used mass window is 10 mDa. (a) *m/z* 157.0088, [$C_3H_{10}O_3PS$]: a1—omethoate, a2—oxydemeton-methyl, a3—demeton-S-methylsulphon, a4—dimethoate, a5—disulfoton-sulfoxid, a6—phorate-sulfoxid, a7—disulfoton-sulfon, a8—phorate-sulfon, a9—azinphos-methyl, a10—malathion, a11—mecarbam, a12—phenthoate, a13—sulfotep, a14—etrimfos, a15—phosalone, a16—chlorpyrifos-methyl, a17—ethion, a18—chlorpyrifos. (b) *m/z* 127.0160, [$C_2H_8O_4P$]: b1—oxydemeton-methyl, b2—demeton-S-methylsulphon, b3—moncrotophos, b4—dicrotophos, b5—trichlorfon, b6—phosphamidon, b7—dichlorvos, b8—naled, b9—quinalphos, b10—chlorfenvinphos, b11—cadusafos. (c) *m/z* 72.0444, [C_3H_6NO]: c1—oxamyl, c2—dicrotophos, c3–metoxuron, c4—monuron, c5—pirimicarb, c6—isoproturon, c7—diuron, c8—chloroxuron. (d) *m/z* 70.0399, [$C_2H_4N_3$]: d1—paclobutrazol, d2—myclobutanil, d3–cyproconazole, d4—fluquinconazole, d5—tetraconazole, d6—epoxiconazole, d7—fenbuconazole, d8—penconazole, d9—hexaconazole, d10—bitertanol, d11–metconazole. ^{37}Cl—the interference of ^{37}Cl isotope of diagnostic ion [$C_7H_6{}^{37}Cl$] *m/z* 127.0129. Mass difference of 3.1 mDa could not be removed from chromatogram with mass window 10 mDa. X—unidentified isobaric interferences.

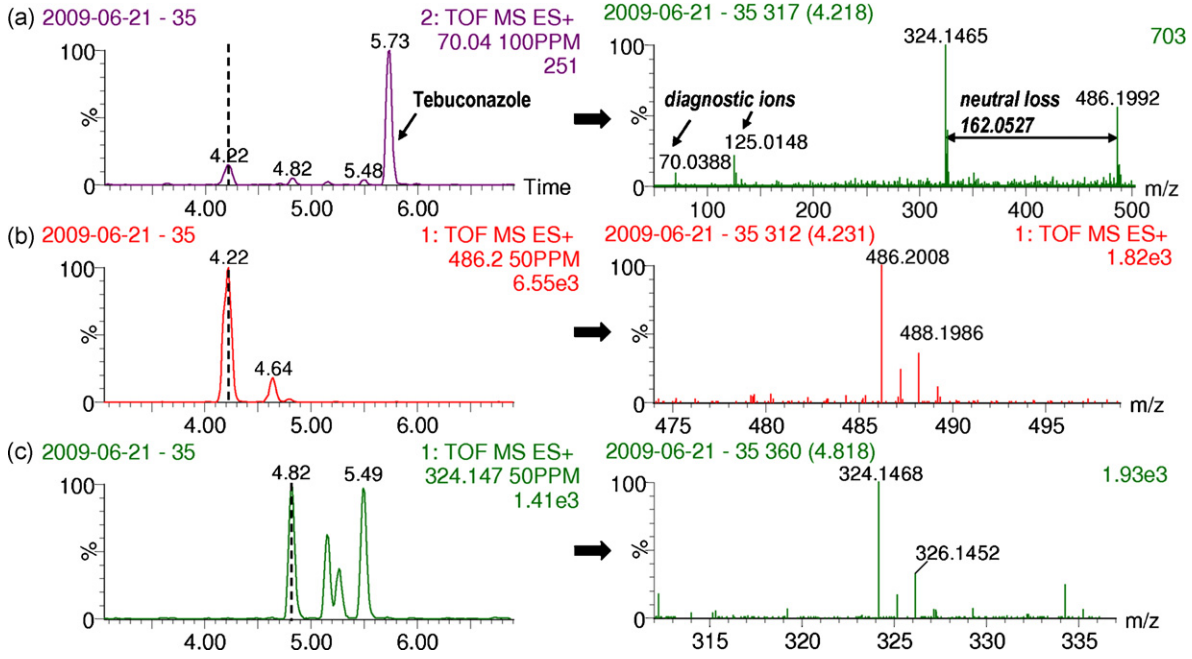

Fig. 6. Example of non-target identification of metabolites of tebuconazole in cherry sample. From the unknown peak of detected diagnostic fragment ion (a) ($C_2H_4N_3$, *m/z* 70.0400) were extracted fragmentation mass spectrum. Except diagnostic fragments is present also fragment corresponding to hydroxyderivate of tebuconazole and molecular ion of glycoside. Neutral loss indicating lost of glucose is calculated. XIC chromatograms of tebuconazole-glucoside (b) ($C_{22}H_{33}ClN_3O_7$, *m/z* 486.2002) and tebuconazole-hydroxide (c) ($C_{16}H_{23}ClN_3O_2$, *m/z* 324.1473) with respective mass spectra without fragmentation.

Table 3
Diagnostic ions of several pesticide classes.

Diagnostic ion (m/z)	Class	Pesticide	Ref.
70.0400 $C_2H_4N_3$	Triazoles	Bitertanol, cyproconazole, epoxyconazole, fenbuconazole, fluquinconazole, hexaconazole, metconazole, myclobutanil, paclobutrazole, penconazole, propiconazole, tebuconazole, tetraconazole, triadimefon, triadimenol	
	Organophosphorus	Dicrotophos	
72.0444 C_3H_6NO	Phenylureas	Chloroxuron, diuron,[a] isoproturon,[a] metoxuron, monuron[a]	[8]
	Carbamates	Oxamyl, pirimicarb	
91.0324 $C_2H_7N_2S$	1,3,5-Triazines	Ametryn, desmetryn, prometryn, simetryn, terbutryn	
96.0562 $C_4H_6N_3$	1,3,5-Triazines	Ametryn, atrazine, cyanazine, simazine, simetryn, terbuthylazine, terbutryn	
	Anilinopyrimidines	Cyprodinil, mepanipyrim	
106.0651 C_7H_8N	Chloracetamides	Propachlor	
	Sulphamides	DMST	
124.9821 $C_2H_6O_2PS$	Organophosphorus	Acephate, azinphos-methyl, chlorpyrifos, chlorpyrifos-methyl,[a] demeton-S-methylsulphon, dimethoate,[a] disulfoton-sulfon, disulfoton-sulfoxid, ethion, etrimphos, malathion,[a] mecarbam, methacrifos, omethoate, oxydemeton-methyl, phenthoate, phorate-sulfon, phorate-sulfoxid, pirimiphos-methyl, sulfotep, triazophos	[8]
125.0153 C_7H_6Cl	Triazoles	Cyproconazole, fenbuconazole, metconazole, myclobutanil, paclobutrazole, tebuconazole	
	Isoxazolidinones	Clomazone	
	Organophosphorus	Heptenophos	
	Phenylureas	Pencycuron	
126.0105 C_6H_5ClN	Neonicotinoids	Acetamiprid,[a] thiacloprid[a]	[8]
	Ureas	Monolinuron, monuron	
127.0155 $C_2H_8O_4P$	Organophosphorus	Cadusafos, chlorfenvinphos, demeton-S-methylsulphon, dichlorvos, dicrotophos, heptenophos, mecarbam, mevinphos, moncrotophos, naled, omethoate, oxydemeton-methyl, phosphamidon, quinalphos, trichlorfon	
141.0146 $C_7H_3F_2O$	Benzoylureas	Diflubenzuron, flufenoxuron, teflubenzuron	
141.0311 $C_3H_{10}O_4P$	Organophosphorus	Demeton-S-methylsulphon, dichlorvos, dicrotophos, heptenophos, mevinphos, monocrotophos, omethoate, oxydemeton-methyl, trichlorfon	
142.9926 $C_2H_8O_3PS$	Organophosphorus	Acephate, azinphos-ethyl, Azinphos-methyl, cadusafos, chlorpyrifos, demeton-S-methyl, demeton-S-methylsulphon, diazinon, dimethoate, disulfoton-sulfon, disulfoton-sulfoxid, ethion, ethoprophos, etrimfos, malaoxon, malathion, mecarbam, methacrifos, oxydemeton-methyl, phenthoate, phosalone, pirimiphos-methyl, pyrazophos, quinalphos, sulfotep, terbufos, triazophos	
157.0083 $C_3H_{10}O_3PS$	Organophosphorus	Azinphos-ethyl, azinphos-methyl, chlorpyrifos, chlorpyrifos-methyl, demeton-S-methylsulphon, diazinon, dimethoate, disulfoton-sulfon, disulfoton-sulfoxid, ethion, ethoprophos, etrimfos, malaoxon, malathion, mecarbam, omethoate, oxydemeton-methyl, phenthoate, phorate-sulfon, phorate-sulfoxid, phosalone, pirimiphos-methyl, quinalphos, sulfotep, triazophos	
158.0412 $C_7H_6F_2NO$	Benzoylureas	Diflubenzuron,[a] flufenoxuron,[a] teflubenzuron[a]	[8]
158.9763 $C_7H_5Cl_2$	Triazoles	Fluquinconazole, hexaconazole, imazalil,[a] penconazole, propiconazole,[a] tetraconazole	[8]
170.9703 $C_3H_8O_2PS_2$	Organophosphorus	Azinphos-methyl, dimethoate, ethion, phorate-sulfon, phorate-sulfoxid	
186.0808 $C_6H_{12}N_5S$	1,3,5-Triazines	Ametryn, simetryn, terbutryn	

[a] Analytes providing particular diagnostic fragment ions reported in earlier study from Ferrer and Thurman [8].

4. Conclusions

The potential of UHPLC–TOF MS technique as a challenging alternative to currently, in pesticide residue analysis well established LC–MS–MS based strategies, has been demonstrated in this study. The outcomes of our research concerned with a validation of this novel approach employed for 212 pesticides representing various chemical classes in four different (in terms of their composition) plant crops, can be summarized as follows:

- Because of the use of a QuEChERS-like extraction procedure (PSA dispersive solid phase extraction omitted) and fast separation step (8 min), the laboratory sample throughput can be increased significantly.
- In line with common regulatory recommendation stated in [12], three identification points are available for unambiguous identification of almost all tested pesticides. In addition to the most intensive molecular ions obtained by ESI+ and fragment ions yielded by in-source CID, identification points for those analytes yielding the ions in ESI− can be obtained within the follow-up run.
- LCLs 10 μg/kg and lower are obtained for 96% of target analytes what means that the method is applicable for control of MRLs established in Commission Directive 2006/125/EC for processed cereal-based foods and baby foods for infants and young children.
- Compared to tandem mass analyzers such as triple quadrupols, the linear dynamic range of LCT Premier XE instrument is rather narrower. However, under real-life conditions, when mainly (not too high) concentrations around MRLs are to be controlled, this limitation does not pose a serious problem, since the accuracy of results for analytes fairly exceeding the regulated level is not a crucial issue.
- In addition to reliable quantification of target analytes, the occurrence of non-target pesticides, compounds yielding diagnostic

ions common for particular group of parent compounds and/or their degradation products/metabolites, can be detected, and, possibly identified on the basis of molecular ions found at respective retention time.

In conclusion, comprehensive and fast information on pesticides extractable by QuEChERS procedure and ionizable by ESI can be obtained by UHPLC–TOF MS, the performance characteristics comply with legislative requirements for residue analyses [19]. As far as both, positive and negative spectra generated at three fragmentation energies are acquired; retrospective searching aimed at recovery of additional information is possible.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2009.11.098.

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