PAPER IN FOREFRONT

Liquid chromatography Orbitrap mass spectrometry with simultaneous full scan and tandem MS/MS for highly selective pesticide residue analysis

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Abstract This paper describes the application of LC/Q-Orbitrap MS for the analysis of pesticide residues in fruit and vegetable commodities. LC/Q-Orbitrap MS working in full scan simultaneously with a single MS/MS scan was used to analyse 139 pesticide residues in QuEChERS extracts of tomato, pepper, orange and green tea. Full scan data were obtained at a resolution of 70,000 whereas MS/MS data were obtained at a resolution of 17,500. Quantitation and detection was carried out using full scan data while MS/MS data were used only for identification. MS/MS scans did not have a negative influence on quantitation under the applied conditions. Some peak area reproducibility problems were the consequence of the low sensitivity for some compounds (aldicarb, chlorpyriphos methyl, fenitrothion and fipronil) under the applied conditions. The relation between the operational parameters (viz. automatic gain control (AGC) target, maximum injection time (IT), underfill ratio, isolation window and apex trigger) and the number of automatically identified compounds was investigated. Mass error and minimal intensity of selected fragment ions were also studied. Various working modes were compared, such as full scan with single MS/MS scan and full scan with multiple MS/MS scans. In both cases, the number of automatically reported pesticides was the same. However full scan with single MS/MS scan ensured more points per peak in full scan mode and better peak area reproducibility. The evaluation of the identification and quantitation capabilities of the instrument was performed through the analysis of 100 real samples. The samples were also analysed by LC–QqQ MS/MS and the results of both analytical systems were compared. The comparison revealed that the two instruments were consistent with each other. They found the same pesticides and neither false positive nor false negatives were reported. Nevertheless the Q-Orbitrap MS allowed one to work in high resolution mass spectrometry, increasing the selectivity and, in full scan mode, permitting the retrospective analysis of the data feature that cannot be achieved with QqQ.

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Introduction

The Orbitrap mass analyser was described for the first time in 2000 by Alexander Makarov [1] who demonstrated that single stage Orbitrap can compete with triple quadrupole mass spectrometers as a tool for pesticide analysis [1]. Examples of the application of Orbitrap to various analytes and matrices can be found in the literature. Single stage Orbitrap (Exactive) was applied to the detection of pesticides [2–4], veterinary drugs [5], mycotoxins [6] and plant toxins [7].

The Orbitrap mass analyser is also installed in hybrid instruments. LTQ Orbitrap (hybrid with a linear ion trap) was

the first mass spectrometer equipped with Orbitrap. The instrument was successfully applied for pesticide analysis [8].

The hybrid of Orbitrap and quadrupole is called LC/Q-Orbitrap MS and it was introduced into the market in 2011. For this instrument, the user can select one of four resolution values: 17,500, 35,000, 70,000 or 140,000. These values refer to m/z 200. In the Orbitrap mass analyser, the resolution is inversely proportional to the square root of m/z. Thus for higher m/z, the observed resolution is lower.

The presence of a quadrupole mass filter enables the selection of the parent ion which is subsequently fragmented, and the obtained fragments are finally analysed in the Orbitrap. Depending on the selected mode of acquisition, the data obtained in MS/MS analysis can be used for compound identification as well as for quantitation.

Amongst several workflows which are available in LC/Q-Orbitrap MS, a very convenient one is the data-dependent mode. In this mode the user defines a list of target parent ions and retention time window for each one. During the analysis, data acquisition is carried out in full scan mode. However, if any of the parent ions from the target list are detected at the correct retention time window, the instrument switches automatically to MS/MS mode, carries out one scan in this mode and just after the system continues in full scan mode. Data obtained in full scan mode are used for detection and quantitation whereas data from the MS/MS mode are used for identification purposes [9].

LC/Q-Orbitrap MS working in the data-dependent mode was applied for various types of small molecules. Mycotoxins were analysed in dietary supplements [10] and dairy products [9]. Some authors stated that in the case of complicated matrices LC/Q-Orbitrap MS provides more unequivocal identification than triple quadrupole [10]. Fedorova et al. used LC/Q-Orbitrap MS for the analysis of illicit drugs in wastewater; they also compared results obtained by LC/Q-Orbitrap MS working in the data-dependent mode with those obtained by triple quadrupole and concluded that LC/Q-Orbitrap MS is equal to or better than QqQ [11]. Data-dependent analysis was also found to be a convenient tool for the detection of illegal adulterants in herbal medicines. Shi et al. stated that resolution of 70,000 was enough to eliminate all isobaric ions and the peaks thus obtained in full scan were free of interferences [12]. Kumar et al. tested various LC/Q-Orbitrap MS workflows in the analysis of synthetic hormones in animal urine. They compared various combinations of full scan and MS/MS workflows (targeted MS/MS, data-dependent MS/MS, all ions fragmentation). Nevertheless, the list of target compounds was short and contained only ten [13]. LC/Q-Orbitrap MS was also applied for pesticide residue analysis [14, 15].

The aim of this work was to evaluate LC/Q-Orbitrap as a tool for the detection, identification and quantitation of pesticides in food matrices and optimisation of the operational settings.

Materials and methods

Reagents and materials

High purity pesticide standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and Riedel–de Haën (Selze, Germany) and were stored at −30 °C. Individual pesticide stock solutions (1000–2000 mg/L) were prepared in acetonitrile and ethyl acetate and were stored in amber screw-capped glass vials in the dark at −20 °C. Individual standard solutions, used for the optimization, along with standard mixed solutions, used for the calibration, were prepared from the stock standards.

Water was obtained from Fisher Scientific (Fair Lawn, NJ, USA) and methanol from Fluka Analytical (Steinheim, Germany). Ammonium formate and formic acid were purchased from Sigma–Aldrich (Steinheim, Germany).

Pierce LTQ Velos ESI Positive Ion Calibration Solution was provided by Thermo Fisher Scientific (Waltham, MA, USA).

LC–MS analysis

For the LC separation, a UHPLC Dionex Ultimate 3000 (Thermo Scientific, San Jose, USA) was used. Mobile phase A was 98 % water and 2 % methanol whereas mobile phase B was 98 % methanol and 2 % water; both mobile phases contained 5 mM of ammonium formate and 0,1 % formic acid. Separation was carried out on a Thermo Scientific Accucore aQ C18 column. The length, diameter and particle size were 150 mm, 2.1 mm and 2.6 μm, respectively. The column was thermostatted at 25 °C. Three minutes before injection, the column was equilibrated with 100 % of mobile phase A. From 0 to 4 min, the amount of mobile phase B increased to 20 %, from 4 to 5.5 min to 40 %, and from 5.5 to 10 min to 100 %. Then 100 % of B was maintained until 13 min. Following this, the mobile phase was changed to 100 % A and maintained over 5 min for re-equlibration. The injection volume was 10 μL. The autosampler was thermostatted at 10 °C.

An LC/Q-Orbitrap MS (Thermo Scientific, Bremen, Germany) mass spectrometer was equipped with a Heated Electrospray Ionization Source (HESI II). The HESI parameters in positive polarity were as follows: sheath gas flow rate, 40; auxiliary gas flow rate, 5; sweep gas flow rate, 1; spray voltage, 3.00 kV; capillary temperature, 280 °C; S–lens RF level, 55.0; heater temperature, 350 °C. In full scan mode the LC/Q-Orbitrap MS settings were as follows: resolution, 70,000; AGC target, 3E06; maximum injection time (IT), 100 ms; scan range, 140–750 m/z. In MS/MS mode the LC/Q-Orbitrap MS worked with a resolution of 17,500; AGC target, 2E04; maximum IT, 50 ms; loop count, 10; MSX

count, 1; underfill ratio, 1 %; isolation window, 1.3; first fixed mass, 70 *m/z*; apex trigger, 2–4 s.

The external mass calibration and the quadrupole calibration were carried out daily. For the calibration, a mixture containing *n*-butylamine, caffeine, Ultramark 1621 and MRFA was used.

The type of adduct, molecular mass and retention time for all the pesticides analysed are presented in Table S1 in the Electronic Supplementary Material (ESM).

TraceFinder 3.2 (Thermo Scientific, San Jose, USA) was used for qualitative and quantitative analysis. Automatic detection and quantification was followed up by a manual verification.

Sample preparation

Tomato, pepper, orange and green tea were bought in a local shop in Almeria. These selected matrices can be considered representative of the widest difficulty range expected in a laboratory focused on fruit and vegetable analysis. Matrices were extracted according to the methods described elsewhere (tomato, pepper, orange [16]; green tea [17]).

Extracts were spiked with 139 pesticides, all included in the European Union Monitoring Program. Blank extract (in the case of tomato, pepper and orange, 100 μL; and in the case of green tea, 500 μL) was evaporated under a gentle stream of nitrogen and reconstituted with 100 μL of acetonitrile containing a mixture at 10, 50, 100 or 500 μg/kg of the analysed pesticides. Subsequently, 400 μL of ultrapure water was added to dilute the sample and reduce the percentage of acetonitrile. Because of precipitation, green tea samples were filtered with 0.45-μm PTFE filters. The final samples contained 0.2 g of matrix per 1 mL of extract and pesticide concentrations equal to 2, 10, 20 and 100 μg/kg.

Real samples were extracted in the same way as for tomato, pepper and orange. To prepare real samples for injection, 100 μL of QuEChERS extract was added to 400 μL of ultrapure water.

All the concentration values which appear in this paper refer to the concentration before dilution.

Results and discussion

Full scan mode

Full scan analysis was carried out at a resolution of 70,000 because of the reasons described previously [18]. This resolution ensured 3 scans per second which was enough to achieve a satisfactory number of points per chromatographic peak and avoid most of the possible interferences due to isobaric compounds from the matrix. Resolution of 70,000 and mass tolerance of 5 ppm were enough to obtain good peak shapes for

quantitation [18]. This resolution was also considered as optimal by other authors [12]. The protonated molecule or the ammonium adduct peak acquired in full scan mode was used for detection and quantitation. In simple matrices (tomato and pepper) at a level of 10 μg/kg, only three compounds (chlorpyriphos methyl, fenitrothion and fipronil) showed an area relative standard deviation (RSD) higher than 20 %. However, this poor reproducibility was a consequence of low sensitivity and cannot be assigned to the influence of MS/MS scans. Problems with those pesticides also appeared when samples were analysed only in full scan mode, as was shown in our previous work [18]. In general, very good reproducibility and peak shape were obtained even in the portions of the chromatogram where many pesticides eluted with similar retention times. In orange and green tea, the number of pesticides yielding RSDs above 20 % (at a level of 10 μg/kg) was higher (8 and 15, respectively), and some compounds were not detected at a level of 10 μg/kg in any of the difficult matrices as a consequence of very high ion suppression. In some cases even at the 100 μg/kg level some problems with reproducibility were observed for those two matrices. Orange and green tea provide much more matrix ions than tomato and pepper and worse results were a consequence of ionisation suppression in the ESI source. Because of strong ion suppression cymoxanil in green tea was detected only at a level of 500 μg/kg. But, the number of cases with this type of problem can be considered as low and therefore the introduction of changes in the extraction procedure is not critical.

Linearity was investigated in the 2–100 ng/mL range (which corresponded to 10–500 μg/kg in the sample before dilution). In almost all of the cases, the value of r^2 was greater than 0.995. Lower values were obtained in orange (fenitrothion, prothioconazole) and green tea (fenithrothion, chlorpyriphos-methyl) extracts. Figures 1a–d show the percentage of detected compounds, results of reproducibility and linearity (detailed data are presented in ESM Table S2).

MS/MS scans did not negatively affect the possibility of quantitation in full scan mode. All the encountered problems were a consequence of the low sensitivity for some pesticides.

Data-dependent parameters

The influence of AGC target, maximum IT, underfill ratio, isolation window, and apex trigger on data-dependent analysis was checked. For all tests pepper extract spiked with 139 pesticides at 10 μg/kg was used, and each combination of settings was tested by three consecutive injections of the sample.

AGC target and maximum IT control the work of the C-trap (radiofrequency trap which is placed directly before the Orbitrap mass analyser). AGC target represents the number of ions which will be accumulated in the C-trap and subsequently injected into the Orbitrap. Maximum IT defines the
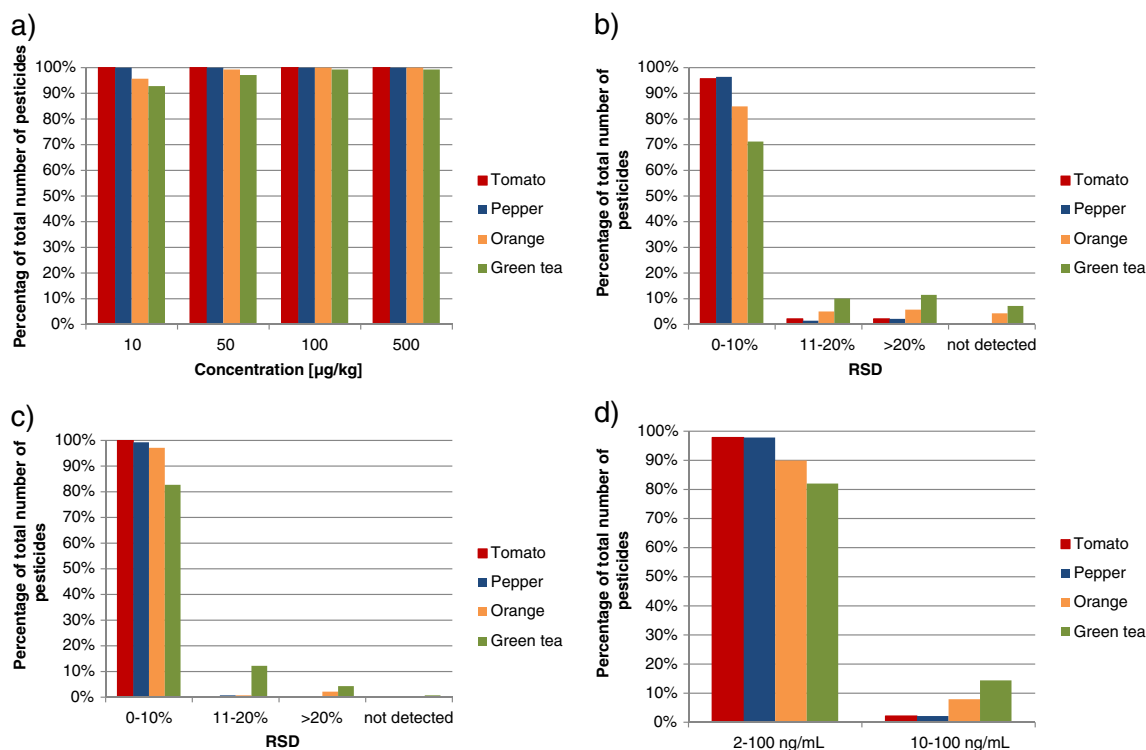
Fig. 1 Detection and quantitation with Q-Orbitrap: **a** percentage of detected pesticides, **b** reproducibility expressed as relative standard deviation of peak area at 10 µg/kg, **c** reproducibility expressed as relative standard deviation of peak area at 100 µg/kg, **d** linearity range

maximum time of C-trap filling. Injection into the Orbitrap is carried out when the assumed IT or AGC target is reached. By changing the maximum IT, AGC target and underfill ratio, the user can adjust the intensity threshold value. The latter parameter informs one about the minimum intensity of the parent ion in the full scan mode which is necessary to initiate a data-dependent scan. Four values of AGC target were tested: 2E04, 1E05 and 2E05 gave the same result i.e. 95 % of detected compounds were identified; whereas an AGC target of 5E05 reduced the number of identified compounds to 88 %. Changes of maximum IT in the range of 50–200 ms did not influence the number of confirmed compounds. In general an intensity threshold above 4E04 reduced the number of identified compounds. In the case of values higher than 4E04, the signal intensity of some pesticides for which sensitive was low (e.g. aldicarb, chlorpyriphos-methyl, isocarbofos, tolclofos-methyl, propargite) was not sufficient to allow a data-dependent scan. However an extremely low value of intensity threshold also can have a negative influence. For example, MS/MS scan can be triggered by low abundant isobaric matrix ions. In those cases LC/Q-Orbitrap MS carries out the MS/MS scan too early, not in the apex of the peak.

Isolation window refers to the quadrupole filter. Six isolation windows from m/z 0.4 (narrowest possible) to m/z 5 were tested. Theoretically a narrow isolation window limits the number of ions entering the Orbitrap and thereby makes the spectra simpler and decreases the possibility of interferences during mass measurement. On the other hand, pesticide ions containing target isotopes such as chlorine or bromine are not fragmented; thus in the MS/MS spectra only fragments containing lighter isotopes are present. The best results were obtained with isolation windows of m/z 1.3 and 2. With those two values 95 % of compounds detected in full scan were identified by the fragments obtained in the data-dependent scan. Increases of the isolation window caused reductions of the number of identified pesticides (only 87 % of confirmed pesticides with an isolation window of m/z 5). Figure 2 presents a comparison of MS/MS spectra of flonicamid obtained with different isolation windows. Differences between these spectra could be the result of an interaction between ions in the collision cell or in the C-trap. Results obtained with an isolation window of m/z 0.4 were unexpected. Only 92 % of the compounds were identified, which means 3 % less than when working with an isolation window of m/z 1.3.

Apex trigger is a parameter that helps to locate a peak apex, with the aim of obtaining more intense peaks in the MS/MS spectrum. In analyses without use of apex trigger, the percentage of identified compounds decreased from 95 % to 92 %. However, in most cases, the MS/MS scan was carried out at the beginning of the chromatographic peak.

Also the influence of MSX count was studied. Selection of MSX from 1 to 10 did not produce any difference.
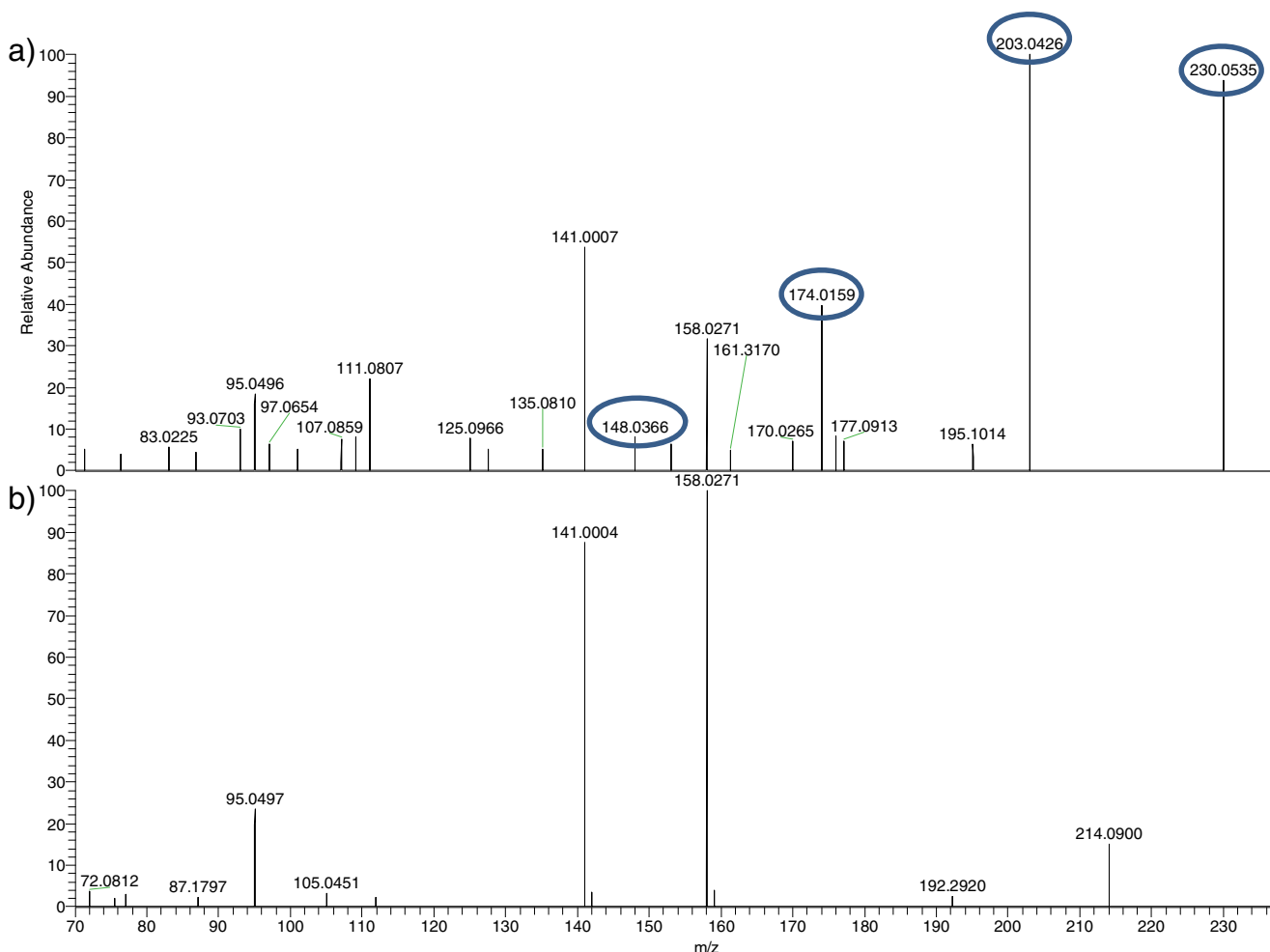
Fig. 2 Comparison of MS/MS spectra of flonicamid obtained with different isolation windows. **a** m/z 1.3 and **b** m/z 5. Marked ions are fragments of flonicamid

Additional MS/MS scans

Data-dependent mode provides one scan per chromatographic peak. However in some situations more scans were observed. Some compounds eluting at the beginning of the chromatogram were wide and therefore up to 3 MS/MS scans per peak were performed. More than one scan was also observed in the case of high concentration levels. This was a consequence of the low intensity threshold which was optimised to obtain good results at the level of 10 µg/kg. Additional scans at 100 or 500 µg/kg did not have a negative impact on quantitation and identification.

Identification parameters

For detection and identification of pesticides, the retention time and at least two ions, one in full scan and one in MS/MS, measured with high resolution were used. Mass accuracy of 5 and 10 ppm was selected for full scan and MS/MS, respectively. Detected MS/MS fragments had to fulfil criteria of absolute

and relative abundance as well as mass error (error lower than 5 ppm and ratio variability of 30 %). Criteria that are too strict criteria could produced false negatives results whereas those that are too broad would have produced false positives. A database was built up for identification which contained the list of expected fragments. Typically for each pesticide four or five experimental MS/MS fragments were known.

Intensity thresholds from 100 to 10000 counts were studied. A higher intensity threshold gives better protection from false positives; however, pesticides whose fragments have low abundance can be considered by software as false negatives. The number of identified compounds did not change between intensity threshold 100 and 1000. However a further increase to 5000 caused a small reduction in the number of identified compounds. Nevertheless this reduction was only observed at the 10 µg/kg level.

In the case of compounds for which sensitivity was low (e.g. fipronil, aldicarb), even an intensity threshold of 100 counts was too high to detect fragment ions at a level of 10 µg/kg. Apart from the problems with sensitivity, aldicarb also exhibited problems with fragmentation (normalized collision energy was optimised

in the 10–95 range). At levels of 10 and 50 μg/kg, fragments were not encountered in any of the investigated matrices.

Characteristic fragments were searched with mass tolerances of 5, 10 and 20 ppm. In full scan mode it is advised to use a mass tolerance of 5 ppm [19]. However in MS/MS mode such narrow limits are not necessary because the quadrupole filter rejects most of the unwanted parent ions.

In our experiments some MS/MS fragments had mass errors higher than 5 ppm. Error expressed in ppm is a relative error so ions with low values of m/z were especially problematic. The percentage of the pesticides with an error of 5–10 ppm was between 15 and 19 % and was independent of pesticide concentration and matrix.

Increase of mass tolerance in MS/MS from 5 to 10 ppm helped to identify 3 % more compounds in tomato and pepper, 5 % more in orange and 6 % more in green tea. Further increase to 20 ppm was less effective, and more pesticides were identified in only two cases.

False positive detections were used (see "Real samples") to evaluate the maximum mass error that would be acceptable. Mass tolerance was increased until a false detection was confirmed. For example, in spectra of onions a fragment ion with an error of 20.612 ppm that could be assigned to prochloraz was found but prochloraz was not present in the sample. Thus mass tolerance in MS/MS of around 20 ppm may be a source of false positive results, especially with difficult matrices. For that reason the operational parameters stated above were selected.

It seems that mass tolerance in MS/MS of 10 ppm is a better choice than 5 ppm. Increasing from 5 to 10 ppm did not produce any false positives and helped to avoid false negatives. Table 1 presents the percentage of identified compounds in each of the studied matrices.

At a level of 10 μg/kg for 65 % of pesticides in tea and 85 % in tomato at least two MS/MS fragment ions were obtained. In those cases the relative abundance of the fragments was stable comparing injections in different matrices (variation below 30 %), providing more tools for a proper identification of the compound.

Settings of 70,000 in full scan and 17,500 in MS/MS mode were selected by many other authors [9, 11, 12, 14, 15] for analysis of small molecules by LC/Q-Orbitrap MS.

To check the influence of resolution in MS/MS analysis, results of triplicate injection of 10 μg/kg in orange extract acquired with 70,000/17,500 and with 70,000/35,000 were compared. At both resolutions the number of compounds confirmed by the fragments was the same. In three injections with an MS/MS scan resolution of 17,500, 1178 fragment ions from the database (with mass error below 10 ppm and intensity higher than 1000 counts) were detected, whereas 1183 were detected in the case of a resolution of 35,000. The average mass error was equal to 2.38 and 2.06 ppm for resolution of 17,500 and 35,000, respectively. This comparison shows that in MS/MS analysis, an increase of resolution did not improve results.

Table 1 Percentage of identified pesticides

	Tomato	Pepper	Orange	Green tea
10 μg/kg	97 %	95 %	89 %	86 %
50 μg/kg	97 %	96 %	95 %	94 %
100 μg/kg	99 %	98 %	98 %	96 %
500 μg/kg	99 %	98 %	99 %	98 %

Full scan/targeted MS/MS

LC/Q-Orbitrap MS offers a workflow which is similar to the data-dependent mode described above. It is combination of full scan and targeted MS/MS. Targeted MS/MS is an analogous mode to the product ion scan mode used in triple quadrupole mass spectrometers. When targeted MS/MS is combined with full scan mode in the portions of the chromatogram which are not covered by any retention time windows of targeted MS/MS, LC/Q-Orbitrap MS works in full scan mode; in the other portions full scans and MS/MS scans are carried out alternately and data acquired in full scan mode do not have any influence on MS/MS mode.

A comparison between full scan/data-dependent MS/MS and full scan/targeted MS/MS was performed using an orange extract spiked with pesticides at the 10 μg/kg level. Experiments were carried out with multiplexing because many pesticides had very similar retention time. In both workflows the same number of pesticides was identified by the MS/MS fragments. However, the data-dependent mode has a very important advantage over the targeted MS/MS. Acquisition in the full scan/data-dependent MS/MS mode permits one to gather a larger number of points to build up the chromatographic peak in full scan than when acquiring with full scan/targeted MS/MS mode. In data-dependent mode, the MS/MS scan for identification purposes is carried out only when parent ion form in the target list is detected. In targeted MS/MS scans are carried out always and across the whole retention time window. Therefore, in the portion of the chromatogram covered by the retention time windows, a half of the points from the full scan chromatogram are lost.

Incidental software problems

During these studies some difficulties with the data-dependent mode workflow were faced, such as location of MS/MS scan, lack of MS/MS scan and automatic identification of pesticides by the used software.

Problems associated with the MS/MS scan being too early (before or at the beginning of the peak) were discussed in the "Data-dependent parameters" section. However, in some cases the MS/MS scan was carried out after the peak had eluted. In the case of isocarbofos which had good peak shape, the lack of MS/MS scan
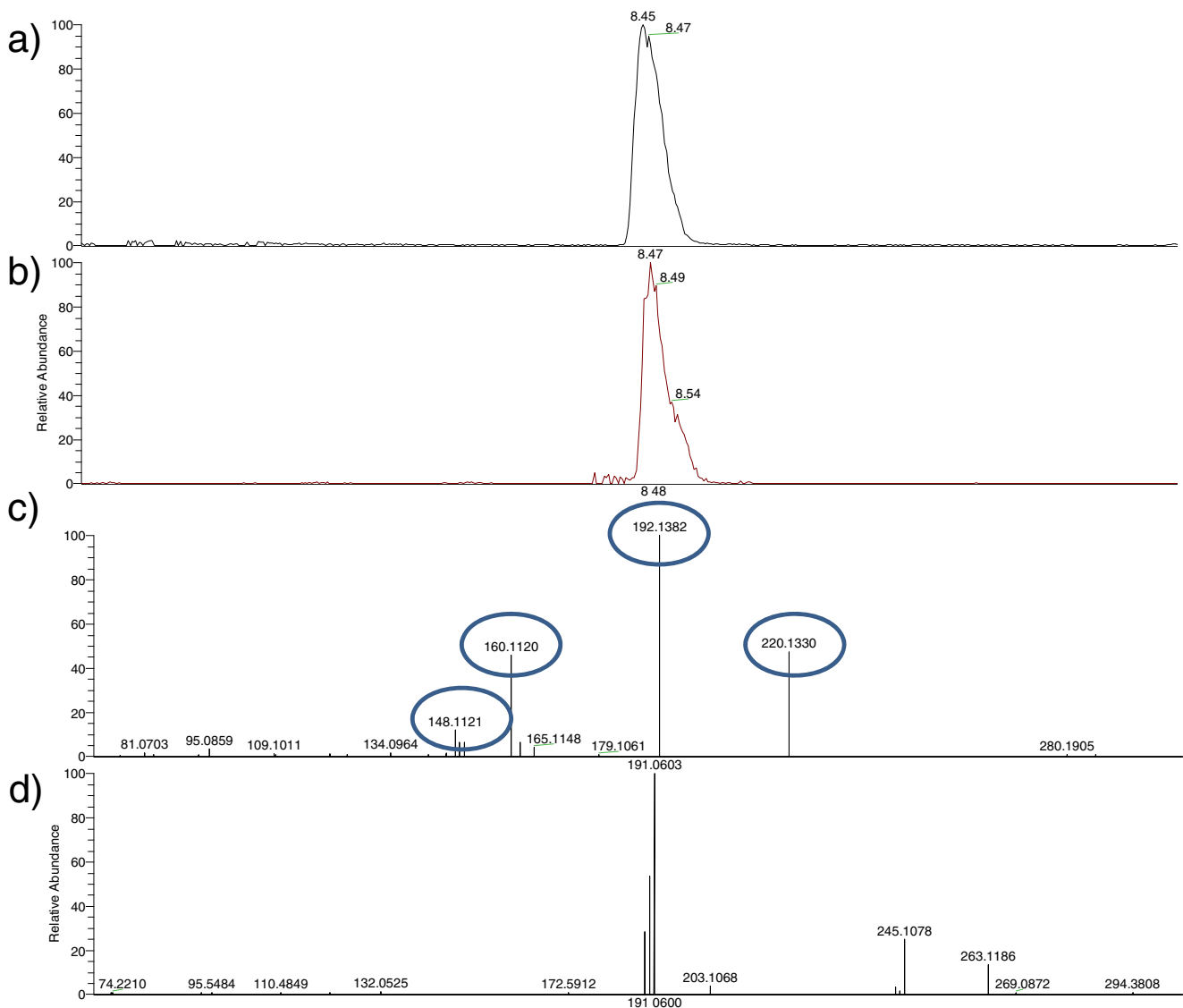
Fig. 3 Metalaxyl-M (XIC *m/z* 280.1543±5 ppm) **a** standard and **b** real sample. MS/MS spectra of **c** standard and **d** real sample. Marked ions are fragments of metalaxyl-M

was observed. The method is not very sensitive toward osocarbofos but the problems were independent from the concentration, i.e. they were noted at low concentration levels as well as at higher ones, either in all investigated matrices or in pure solvent.

A randomly occurring incident due to the TraceFinder software was also noticed. In some infrequent cases the MS/MS scan was carried out twice per peak. Usually, the first scan was at the very beginning of the peak and the intensities of the fragment ions were not enough to identify the compound. Luckily the second scan was performed in a more suitable part of the peak but TraceFinder considered only the scan with shorter retention time, in such cases forcing the manual verification of all detected but not identified peaks. This has to be improved in the next software update.

Real samples

To verify the efficiency of the proposed method, 100 real samples of various commodities (fruits, vegetables, herbs and marmalades) were analysed by LC/Q-Orbitrap MS/MS and as a reference by LC QqQ-MS/MS. To identify a pesticide ion detected in full scan mode with accuracy better than 5 ppm, one MS/MS fragment with abundance of at least 1000 counts and error below 10 ppm was necessary. The total number of pesticides detected at a concentration above 10 μg/ kg was 183. A calibration curve was include in every batch of 10 samples. In 18 cases a peak detected in full scan mode was not identified by the data-dependent MS/MS scan. Concentrations of those false detects were from 10 μg/kg up to over 1 mg/kg. Analysis by QqQ confirmed that all 18 cases were false positives as a consequence of isobaric matrix
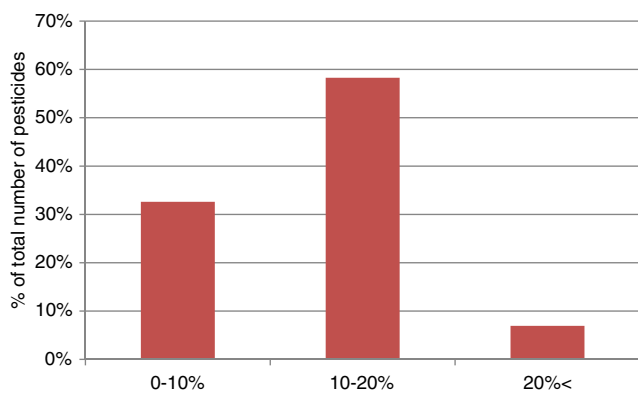
Fig. 4 Difference between concentrations determined by QqQ and Q-Orbitrap

compounds. Figure 3 presents an example of a false positive detection discovered and discarded by the MS/MS scan.

Some coincidences between matrices and false detections in full scan were noticeably. Prochloraz was detected four times in alliums commodities (onion, leek and garlic). False detects of metalaxyl-M appeared in both of the analysed grapefruit samples.

For 93 % of the samples quantified with a calibration curve the difference between concentration determined by QqQ and LC/Q-Orbitrap MS was lower than 20 % (Fig. 4). In fruits and vegetables these differences were observed especially for compounds present in high concentrations (five results with concentrations above 300 μg/kg); in the case of four, the concentration obtained with the LC/Q-Orbitrap MS was 122 %, 123 %, 135 % and 155 % of the concentration obtained with QqQ. In marmalade samples, for seven pesticides the difference quantified was larger than 20 %. Also here, the results obtained by LC/Q-Orbitrap MS were usually higher than those determined by QqQ.

Conclusions

LC/Q-Orbitrap MS working in full scan with single MS/MS scan is a powerful tool for routine analysis of pesticide residues in fruits and vegetables in both identification and quantitation applications. The MS system is very sensitive and thus it was possible to analyse fivefold diluted samples. No saturation effects were observed in the studied concentration range. Single MS/MS scans were carried out without affecting full scan data to a significant degree. To minimise false negatives, it is important to select appropriate settings such as AGC and underfill ratio. To identify pesticides at low concentrations it is important to set the intensity threshold low (1000 counts). The benefit of setting a low intensity threshold in difficult matrices is that it avoids potential problems. It was observed that naturally abundant isotopes of fragment ions are not useful in MS/MS because it is important to select a narrow quadrupole isolation window (m/z). Mass errors observed in MS/MS mode were higher than in full scan; therefore to decrease the number of false negatives it was necessary to increase the mass tolerance for fragments up to 10 ppm. Increasing the resolution in MS/MS from 17,500 to 35,000 did not influence the mass accuracy.

Full scan with multiple MS/MS scans (with multiplexing) was equally effective in identification as full scan with single MS/MS scan. However, in the latter case the number of points per peak (in full scan) was approximately two times higher and better reproducibility of peak area is typically achieved.

Comparison of results between LC/Q-Orbitrap MS and LC/QqQ-MS/MS showed that these two instruments have similar capabilities for quantitation. Moreover, a resolution of 70,000 and one MS/MS fragment have at least the same identification potential as two transition and ion ratio. Nevertheless the Q-Orbitrap allows one to work using high resolution full scan mass spectrometry and to obtain better selectivity and permit the retrospective analysis of the data features that QqQ cannot achieve.
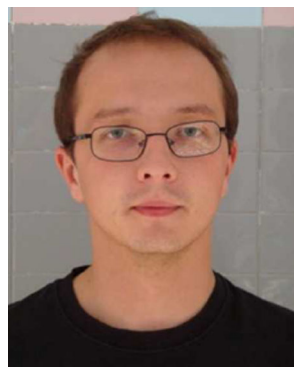
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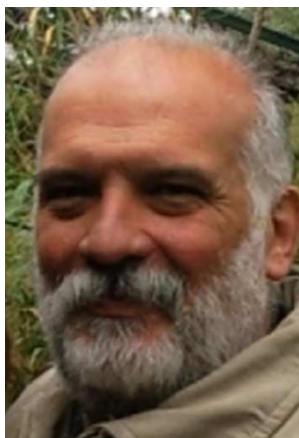
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