Identification and quantitation of pesticides in vegetables by liquid chromatography time-of-flight mass spectrometry

Imma Ferrer, Juan Francisco García-Reyes, Amadeo Fernandez-Alba

This overview covers pesticide-residue determination in food samples by liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS). We present the application of LC-TOF-MS in terms of accuracy, sensitivity and robustness for the quantitative analysis of pesticides in fruit and vegetable samples. The analytical performance of the methodology is validated for various types of vegetables matrices.

Accurate mass measurements (with accuracy better than 3 ppm error) for both the (de)protonated molecule and characteristic fragment ions together with the high-resolution chlorine isotopic signature present in a large number of pesticides and the retention time represent reliable identification criteria for these species in such complex samples. We demonstrated linearity of response over two orders of magnitude ($r > 0.99$) with no significant matrix effects (less than 30%). Low limits of detection at the low mg/kg level, depending on the commodity and pesticide studied, were obtained, all within European Union regulations for food-monitoring programs. The high degree of confirmation for target pesticides by accurate mass measurements demonstrated the applicability of TOF-MS techniques in routine analysis.

This overview is a valuable indicator of the potential of LC-TOF-MS to provide high-order structural information for the unequivocal identification of both targeted and non-targeted pesticide residues present in fruit and vegetables.

Keywords: Food; Liquid chromatography; Mass spectrometry; Pesticides; Time-of-flight; TOF; Vegetables

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1. Introduction

Pesticides are widely used in agricultural practices. The main applications can be classified in production and post-harvest treatment of agricultural commodities for transport purposes. In this sense, production agriculture comprises the main category of use of pesticides subject to control requirements and, therefore, legal action levels [e.g., maximum residue limits (MRLs) or tolerances] have been fixed to assess food safety. For example, the European Union (EU) has set new Directives for pesticides at low levels in vegetables in order to meet these health concerns. New laws, such as the European Directive 91/414/EEC or the Food Quality Protection Act (FQPA) in the USA, have increased the standards for human health, and worker and environmental protection. The quality standards include the re-assessment of the MRLs, which are typically lower than the previous ones, so EU Directives are setting different MRLs for each pesticide within each food group. Typically, the MRLs are in the range 0.01–3 mg/kg depending on the combination commodity and pesticide [1]. For fruits and vegetables intended for production of baby food, an MRL of 0.01 mg/kg is applicable for all pesticides [2], and, finally, banned compounds typically have MRLs at 0.01 mg/kg considered to be lowest limits of detection (LODs) achievable. Finally, review programs have withdrawn authorizations for many crop-protection products currently on the market. This means that a reduction of about 50% in the number of authorized compounds can be expected in Europe and USA in the near future.

Furthermore, these regulations have special provisions for infants and children, including additional safety factors. Two Baby Food Directives in Europe set zero LODs for 11 substances being phased out and LODs close to zero for another five.

All aspects commented upon above have an important impact on LC–MS method development, because it is clear

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that all these factors are interdependent with the required performance of the analytical method. In addition, the EU institutions stress that monitoring residues in food significantly affects the development of new and high-performance multi-residue methods for pesticides. Low MRLs have promoted the development of more powerful, sensitive analytical methods to meet the requirements in complex samples, such as food. In this sense, liquid-chromatography tandem mass spectrometry (LC–MS^2) with triple quadrupole (TQ) in selected reaction monitoring (SRM) mode has become the most widely used technique for the quantitation of (polar) pesticides in food so far, as reported extensively in the literature [3–13]. On the other hand, high-resolution MS techniques, such as time-of-flight MS (TOF-MS) have been applied mainly for structure elucidation or confirmation purposes for environmental analyses [14–18].

However, the limitations of applying TOF-MS to pesticide-residue analysis in comparison with other MS techniques have occurred because of its low efficiency in obtaining quantitative information. The need for low LODs (e.g., 0.01 mg/kg), adequate linear ranges and robustness have prevented TOF analyzers being efficient in pesticide-residue control with respect to other analyzers, such as TQ mass spectrometers. Nevertheless, new improvements in this technique with respect to the number of scans per second and new digital sampling techniques [18] have overcome the initial drawbacks.

LC-TOF-MS has been applied for confirmatory analyses mainly because of well-known limitations, such as the narrow dynamic linear range obtained with this type of instrumentation. Another disadvantage has been the lack of accuracy of some instruments to achieve the 2–5 ppm error level, usually needed when analyzing complex matrices for a correct identification of the target analytes [18,19]. However, the use of TOF-MS techniques has become necessary in the last few years for the unequivocal identification of contaminants in veterinary drugs in meat [20] and to achieve the EU requirements regarding the number of identification points for a positive finding [21]. In addition, TOF-MS has the capability of non-target identification, because the full spectrum is recorded at all times, which is not possible with standard monitoring practices that use single-ion monitoring or multiple reaction monitoring (MRM) techniques.

LC–MS techniques, especially LC–MS^2 under selected reaction monitoring (SRM) mode, have shown their superiority in target-pesticide determinations due to the excellent sensitivity, selectivity and efficiency achieved. However, when it is suspected that unknown pesticide residues are present in the sample, these MS^2 techniques usually do not offer identification and structural elucidation of non-targeted compounds. In this sense, full-scan TOF analyses improve the structural information achieved at expense of sensitivity.

Using LC–MS^2 techniques in this way, pesticides not previously incorporated as standards for the multi-residue methods (MRMs) applied are out of the analytical range, so they are not detected. This can cause significant oversights in detection due to the high number of possible compounds that could be present and the difficulties (e.g., cost and time required) of laboratories managing large numbers of compounds as targets (e.g., 300). In recent years, TOF mass analyzers have gained considerable acceptance by offering high selectivity under full-scan conditions, resolutions of around 10,000, and especially high mass accuracies as a result of very stable calibration [22]. All these characteristics allow the presence of analytes to be confirmed in single MS mode and also with the same performance as tandem mass spectrometers.

This article presents an overview of the main achievements of LC-TOF-MS in multi-residue analysis of food. It also discusses the advantages and the difficulties encountered when using this technique for qualitative and quantitative analyses of fruit and vegetables after fast, inexpensive extraction. It also describes present strategies and technical developments for solving the problems of identification and quantification.

2. Identification by LC-TOF-MS

Recently, LC-TOF-MS has been successfully applied to the identification and the determination of several classes of pesticides in vegetable samples [22–25]. The identification and confirmation criteria are provided by accurate mass measurements for each molecular ion and subsequent fragments, allowing unambiguous identification when measurements involve isobaric compounds in the sample matrix [23]. The following sections describe each of the capabilities of LC-TOF-MS for the determination of pesticides in detail.

2.1. Accurate mass measurements

The list of tentative empirical formulae can be dramatically reduced by refining the minimum and the maximum number of atoms for each molecule and the error level. This is the case for analytes with one or more chlorine atoms, a number that can be easily deduced from the isotopic profile of the accurate mass spectra. As an example, the accurate mass spectrum of acetamiprid obtained in a matrix-matched standard pepper sample is shown in Fig. 1. As can be seen in Fig. 1, both the pattern and the abundance of the chlorine isotope in the molecular ion can be easily used as a valuable tool for identification [14]. In addition, a large number of proposed formulae are often ''chemically incoherent'' because they contain atoms that are not present in most organic compounds. This also helps in unequivocal

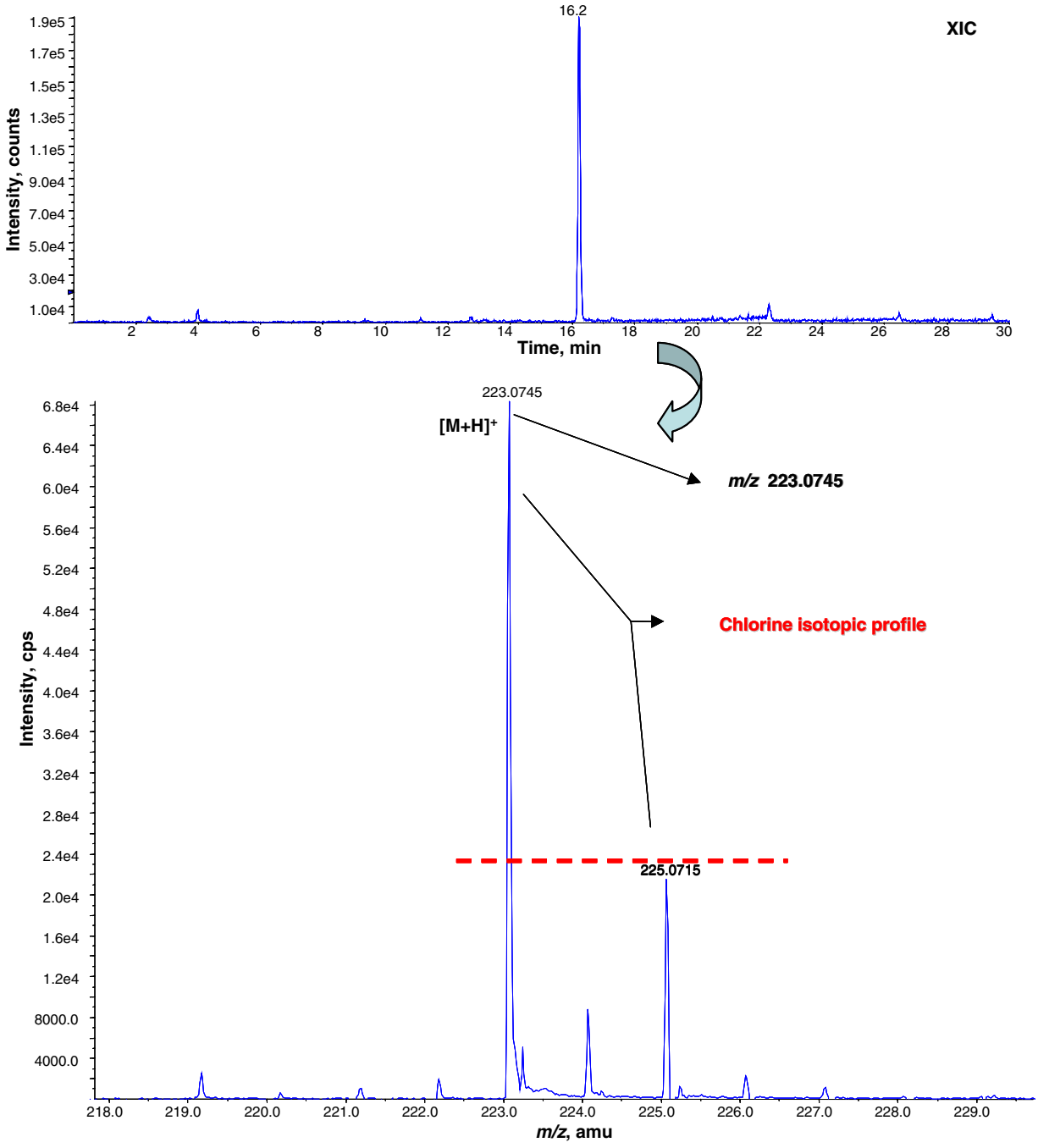

Figure 1. LC-TOF-MS extracted ion chromatogram (XIC) for acetamiprid at *m/z* 223 and corresponding mass spectrum showing the chlorine-isotope signature.

identification of the targeted species and assignment of correct elemental composition.

The accuracies obtained in the mass measurements of the protonated molecules of the selected pesticides on matrix-matched standards are shown in Table 1 [25] (using a pepper extract fortified with 0.05 mg/kg of each pesticide as an example). The errors obtained were less than 2 ppm in most cases. The widely accepted accuracy threshold for confirmation of elemental compositions was established as 5 ppm, so mass-measurement accuracy, along with characteristic retention time, usually provides highly reliable identification of the target species. In addition, mass measurement accuracy is also easily achieved for all the characteristic fragment ions, thus, providing two sets of information for unequivocal identification.

Table 1. Liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS): accurate mass measurements in a pepper extract fortified with a pesticide mixture (concentration level: 0.05 mg/kg)

Compound	Formula	Selected ion	$m/z_{experimental}$	$m/z_{calculated}$	Error	
					mDa	ppm
Cyromazine	$C_6H_{10}N_6$	$[M + H]^+$	167.1031	167.10397	−0.9	−5.2
Carbendazim	$C_9H_9N_3O_2$	$[M + H]^+$	192.0767	192.07675	−0.05	−0.3
Thiabendazole	$C_{10}H_7N_3S$	$[M + H]^+$	202.0435	202.04334	0.15	0.76
Methomyl	$C_5H_{10}N_2O_2S$	$[M + Na]^+$	185.0351	185.03552	−0.4	−2.3
Imidaclopriil	$C_9H_{10}N_5O_2Cl$	$[M + H]^+$	256.0596	256.05957	0.02	0.08
Acetamipricl	$C_{10}H_nN_4Cl$	$[M + H]^+$	223.0741	223.07450	−0.4	−1.8
Thiacloprid	$C_{10}H_9N_4ClS$	$[M + H]^+$	253.0308	253.03092	−0.12	−0.5
Spinosyn A	$C_{41}H_{65}NO_{10}$	$[M + H]^+$	732.4672	732.46812	−0.9	−1.2
Spinosyn D	$C_{42}H_{67}NO_{10}$	$[M + H]^+$	746.4822	746.48377	−1.6	−2.1
Dimethomorph	$C_{21}H_{22}NO_4C1$	$[M + H]^+$	388.1306	388.13101	−0.4	−1.1
Azoxystrobiti	$C_{22}H_{17}N_3O_5$	$[M + H]^+$	404.1243	404.12409	0.2	0.5
Triflumizol	$C_{15}H_{15}N_3OF_3Cl$	$[M + H]^+$	346.0932	346.09285	0.35	1.0
Hexaflumuron	$C_{16}H_8N_2O_3F_6Cl_2$	$[M + H]^+$	460.9882	460.98889	−0.7	−1.5
Teflubenzuron	$C_{14}H_6N_2O_2F_4Cl_2$	$[M + H]^+$	380.9817	380.98152	0.2	0.5
Lufenuron	$C_{17}H_8N_2O_3F_8Cl_2$	$[M + H]^+$	510.9863	510.98570	0.6	1.2
Flufenoxuron	$C_{21}H_{11}N_2O_3F_6Cl$	$[M + H]^+$	489.0425	489.04351	−1.0	−2.1

In the example shown in Table 1 [25], the good accuracy of the results obtained can be attributed to the way instruments process ''all the data, all the time'' and calculate the accurate mass. The new generation of TOF instruments usually uses a dual-nebulizer ion source to perform accurate mass calibration automatically, introducing the reference masses at a very low flow rate (continuously or otherwise) along with the output flow stream of the high-performance liquid chromatography (HPLC) system. This feature is combined with advanced software, which constantly auto-calibrates and records the results of the internal reference masses along with the raw data. This approach enhances accuracy compared to many previous TOF instruments, in which mass calibration was external [14].

The effect of different concentration levels and matrix complexity on the accuracy of mass measurements was also evaluated in all matrices tested at different concentration levels across the working range (0.01–0.5 mg/kg). No significant differences were observed in the accuracy obtained in the various matrix-matched standards compared to those prepared with pure solvents, the error being kept far below 5 ppm, with average values of about 1 ppm for all the pesticides [25].

2.2. Fragmentation by LC-TOF-MS

The fragmentor role in LC–MS is crucial for efficient transmission of the ions to obtain the best balance between sensitivity and fragmentation. The generation of alternative confirmatory ions happens at the expense of molecular ion intensity. Usually, the fragmentor voltage cannot be fixed for each pesticide independently because of the proximity of other target analytes, so it plays an important role in both sensitivity and fragmentation. This parameter is important, because it provides valuable structural information (characteristic fragmentation for each pesticide), making attainable the accurate mass of each characteristic fragment ion together with its elemental composition [16,17], which can be used with confidence to meet the criteria for identifying the molecular ion.

Usually, fragmentor voltages of 250 V or higher lead to extensive fragmentation, even of the reference masses. Voltages of about 120 V provide minimal fragmentation in most pesticides. The following were explored as optimal fragmentation voltages [25]: 190 V [which provides a mild in-source collision-induced dissociation (CID) fragmentation]; and, 230 V (for extensive fragmentation). Table 2 compares the typical fragment ions obtained and summarizes their relative abundances. Some compounds presented characteristic fragmentations at a higher fragmentor voltage, such as neonicotinoid pesticides (imidacloprid, acetamiprid, and thiacloprid). Similarly, cyromazine, carbendazim, azoxystrobin and dimethomorph also needed a high voltage to fragment. However, methomyl gave abundant fragmentation even at the low fragmentor voltage of 190 V. Finally, thiabendazole, hexaflumuron, teflubenzuron, lufenuron, flufenoxuron and spinosad did not fragment clearly even at a high fragmentor voltage. As a compromise value between sensitivity for quantitation (using the protonated molecule) and ''rich'' information mass spectra, 190 V was chosen for further experiments. In some exceptional cases (i.e., triflumizol), a lower voltage yields better sensitivity as well as enhanced fragmentation.

Using the capabilities for optimizing in-source fragmentation, LC-TOF-MS becomes an attractive tool for the unequivocal identification of pesticides. The proposed

Table 2. Typical fragmentation obtained by in-source collision-induced dissociation (CID) in electrospray time-of-flight mass spectrometry (ESI-TOF-MS): study of fragmentor voltage [25]

	m/z	Relative abundance	
		190 V	230 V
Cyromazine	167[b]	100	100
	125	<5	22
	85	<5	24
Carbendazim	192[b]	100	17
	160	43	100
Thiabendazole	202[b]	100	100
	175	<5	9
Imidacloprid	278[a]	–	38
	256[b]	100	48
	210	24	20
	209	20	56
	175	24	100
Acetamiprid	245[a]	–	25
	223[b]	100	77
	126	15	100
	99	–	12
Thiacloprid	275[a]	–	24
	253[b]	100	83
	126	16	100
	99	–	11
Hexaflumuron	483[a]	100	100
	461[b]	94	80
Teflubenzuron	403[a]	100	100
	381[b]	64	30
Azoxystrobin	404[b]	100	19
	372	31	100
	344	–	11
Dimethomorph	388[b]	100	100
	301	<5	10
Triflumizol	346[b]	24	5
	278	100	100
Methomyl	185[a]	13	7
	163[b]	6	–
	106	32	14
	88	100	86
	73	20	100
Lufenuron	533[a]	100	100
	511[b]	70	70
Flufenoxuron	511[a]	100	100
	489[b]	92	46
Spinosad A	732	100	100
	544	<5	<5
Spinosad D	746	100	100
	558	<5	<5

[a]Sodium adduct.
[b]Protonated molecule (used for quantitation).

approach fulfils all the European Commission (EC) criteria for the spectrometric identification and confirmation of organic residues and contaminants, even those based on the use of identification points (IPs). EC Decision 2002/657/EC established the need to obtain three IPs to confirm organic residues of drugs in food (four if they are banned substances) [21]. In this respect, for LC-single quadrupole MS analysis at low resolution, three or four ions (depending of the commodity or pesticide) are necessary for confirmation. In MS^2 analysis, the selection of one precursor ion and the recording of two product ions at low resolution would result in four points for a safe positive finding, which is enough for any combination of pesticide and commodity. Using the accurate mass of the protonated molecule along with that of an additional characteristic fragment ion, the proposed technique meets these regulations. It is clear that the application of these criteria for pesticide-residue analysis should convert many positive findings usually reported into questionable or negative findings.

2.3. Selectivity with accurate mass

The selectivity of LC-TOF-MS relies on the resolving power of the instrument on the *m/z* axis. The higher the resolution provided by the instrument, the better the selectivity for unequivocal identification. Taking into account that the resolving power of a TOF instrument is in the range of 5000–10,000 [18], it can discriminate between ''isobaric'' interferences within 0.05 Da mass difference (e.g., using an ion at 350 *m/z*). An isobaric interference in LC-TOF-MS analyses would therefore arise only if an interfering species with the same time retention of the target analyte had the same exact mass (differences less than 0.05 Da). This selectivity is significantly higher than that provided by any other LC–MS instrument. In addition and as an alternative, using an optimized fragmentor voltage, the accurate mass of any other characteristic fragment ion could be employed for quantitation in order to avoid this potential isobaric interference.

However, compared with TQ instruments, the accurate mass measurements of TOF instruments can provide valuable evidence for an unavoidable isobaric interference, which might occur in complex samples. This kind of interference yields well-known overestimation errors (e.g., in LC-Q-MS and LC-(TQ)-MS^2 instruments). For example, overestimation errors due to the contribution from the signal of the ^{13}C isotope of an interferent specie cannot be avoided (or even detected) with other LC–MS (-MS) instruments in selected ion monitoring (SIM) or SRM modes. Taking advantage of the *m/z* resolving power of the instrument, accurate mass measurements with LC-TOF-MS can at least reveal the existence of these inevitably isobaric interferences. In this sense, to circumvent these interferences, other characteristic fragment ions from accurate mass spectra of the targeted species could be employed *a posteriori*, since LC-TOF-MS records the full-scan spectra at all times.

Matrix interferences are less important when exact mass measurements are performed because the number of coincident ions between matrix and pesticides can be considered negligible for mass-accuracy levels higher than 5 mDa. This feature reinforces the usefulness of bench-top TOF mass spectrometers applied to analyses of pesticides in food. Fig. 2 shows an example of the selectivity achieved by TOF-MS. When a wide amu window is selected in the extracted ion chromatogram (XIC) for $m/z = 253$ (thiacloprid), other interferences might be present in the sample matrix, as observed in the peak at 8.6 min. When the same window is narrowed down, the interferences disappear leading to a more selective identification for the target compound and also to an enhanced signal-to-noise ratio.

2.4. Identification of unknown species by LC-TOF-MS

In general, the most common way to proceed in multi-residue analysis is to develop methods for selected lists of compounds taking into consideration all the aspects commented upon above. But the situation is much more complicated when the goal is identification of non-target pesticides and no selective sample extraction or clean-up procedure is applied, as usually happens. This goal has become more important in recent years with a clear trend to using new pesticides, many times unexpected or not ''controlled'' by routine laboratories because of the different speeds of introduction and approval of new substances for agricultural practices by the respective authorities.

For detecting and identifying unknown pesticides, a single quadrupole mass spectrometer is clearly insufficient due to its low sensitivity in full-scan mode and the lack of information when SIM is applied. Triple quadrupole mass spectrometers provide different options for operation: product-ion, parent-ion and neutral loss modes. However, MS^2 fragmentation may be limited and insufficient to elucidate a structure fully.

A more promising strategy is to screen similar compounds with selected functional groups. A wide variety of functional groups exhibits characteristic fragmentation properties, which can detect compounds with a specific moiety using different MS^2 approaches. In this sense, neutral loss scans can indicate pesticides losing a

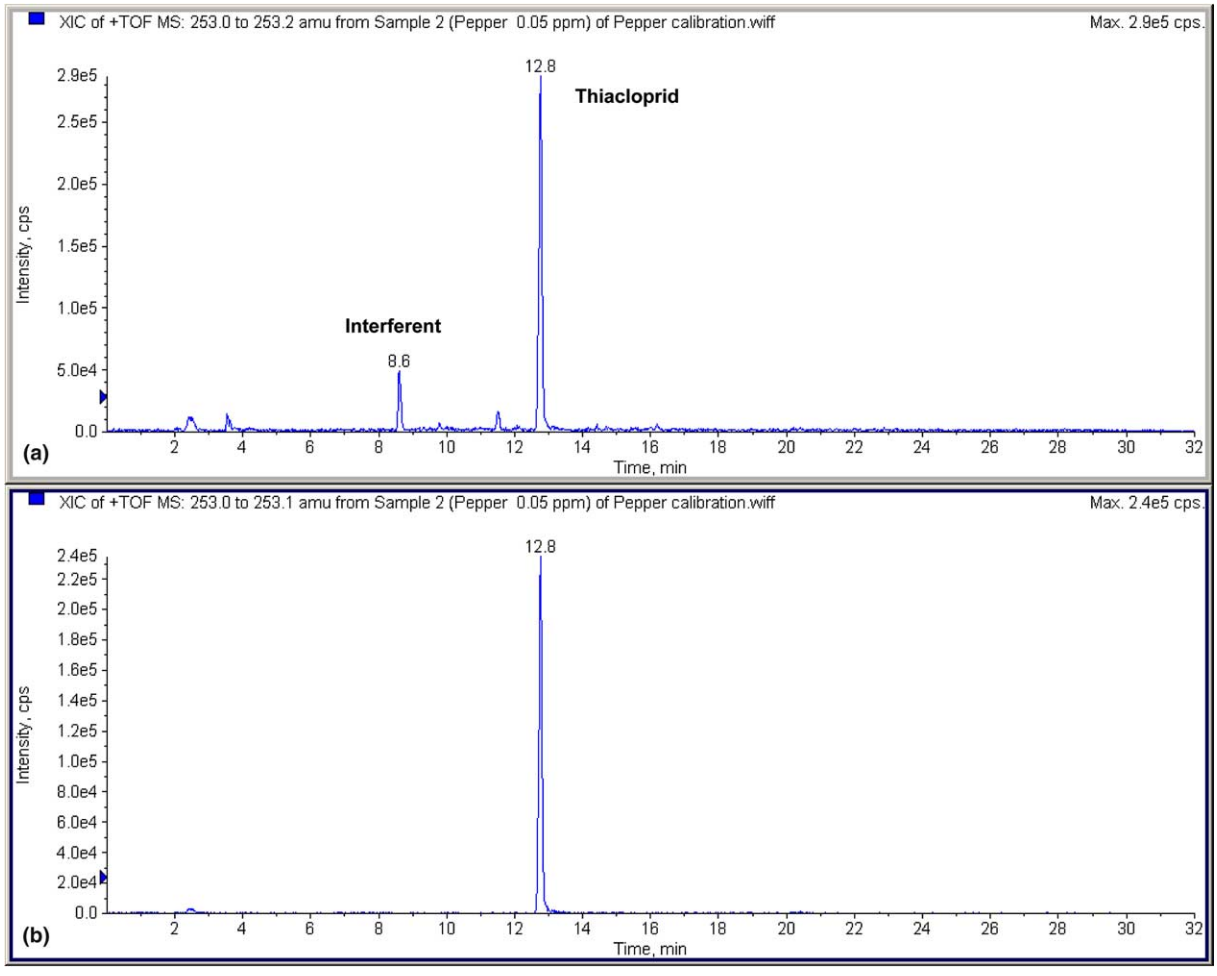

Figure 2. LC-TOF-MS extracted ion chromatogram (XIC) for thiacloprid at m/z 253 using two different extraction windows: (a) 0.2 Da; (b) 0.1 Da.

specific non-ionic fragment. The selection of a constant neutral loss (CNL) along with the chromatogram combined from parent-ion scans provides molecular mass identification and subsequent confirmation. The potential members of a particular compound class in the samples can be detected in this way. This concept has been successfully applied to water and effluent samples for identifying compounds with characteristic fragmentation [26–28]. For example, CNL of methylisocyanate from *N*-methylcarbamates (constant *m*/*z* difference 57) can screen and identify this class of compounds [29].

Depending on the experimental conditions, some variations can be expected; for example some *N*-methylcarbamates are very prone to form adducts $[M + NH_4]^+$ and the neutral loss value can change. However, this rule has important exceptions with *N*-methylcarbamates typically used in food samples that do not yield the characteristic moiety, such as aldicarb, ethiofencarb and fenoxycarb. As a consequence, due to the relevant structural differences that are typically present, even in pesticides of the same family, this procedure has important limitations. Moreover, the sensitivity decreases markedly when the TQ is operated in neutral loss mode.

Another mode of operation is the precursor ion scan that gives typical or diagnostic fragment ions. For example, some of the neonicotinoid insecticides, which are relevant in food analysis, present a diagnostic ion at *m*/*z* 126 [30,31].

Ion-trap mass spectrometers present higher sensitivity in the scanning mode and can perform MS^n experiments, so they are very well suited to detecting unknown compounds, provided time and personal skills are available. Until now, there have been no reports in the literature in this promising direction in the analysis of pesticides in food, probably because of the need for high-throughput sampling in methods for analyzing pesticides in foods that makes this procedure not very suitable for routine laboratories.

In this sense, bench-top TOF-MS has important advantages, which make this instrument attractive when screening for unknown compounds. The higher resolution and mass accuracy in full-scan mode can be readily provided by bench-top TOF-MS. Sometimes it can be enough to provide a molecular formula and to confirm or deny a suggested structure [24,32]. The new generation of TOF-MS is very well suited to the purposes of screening food for pesticides [23,24].

The application of TOF-MS for identification of unknowns has been reported [23]. Based on the accurate mass, the elemental composition of the unknown peak of interest is calculated using the elemental composition tool. By using this data and the information of the appropriate number of chlorines in the molecule, determined from the isotopic pattern, the search is performed in a pesticide database, obtaining the unequivocal identification of the unknown with a mass deviation of less than 5 ppm. It is clear that TOF-MS can be very suitable for the main analytical requirements of analyzing pesticides in food. For these reasons, an important increase in the application of these systems to pesticides in food can be expected in the next few years. Even more useful in terms of identification is the hybrid quadrupole-TOF combination (Q-TOF) as it allows MS^2 to be used. Similarly, Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometers can be used, although we consider these instruments outside the scope of routine applications for analyzing pesticides in food.

3. Quantitation by LC-TOF-MS

3.1. Analytical performance

Sensitivity in LC–MS is related to many operational parameters (e.g., flow rate and mobile phase), so the sensitivity achieved by LC-TOF-MS methods has to be considered as a whole. However, on the basis of current LC–MS multi-residue methods, we can evaluate the suitability of LC-TOF-MS.

In recent years, the analytical linearity response of TOF instruments has not enabled its application for quantitative purposes. These instruments usually suffered from narrow dynamic ranges, requiring mathematical algorithms, such as the ''time to digital correction'', in order to attain a longer linear dynamic range, and this therefore severely limited the usability of TOF-MS [18]. This disadvantage was overcome in new instruments, which offered a linear dynamic range of about two orders of magnitude, which was enough to make possible successful quantitative applications in routine analyses of pesticide residues.

As an example, Fig. 3 shows the linearity obtained by TOF-MS, by comparing the slopes obtained from matrix-matched standards solutions with those obtained with pure solvents for two pesticides (thiabendazole and azoxystrobin) [25]. This study was accomplished at six different concentration levels in the range 0.01–0.5 mg/kg.

Table 3 provides another example, which included the results obtained for four selected fungicides (thiabendazole, azoxystrobin, carbendazim and spinosad) in five different vegetable matrices (pepper, broccoli, orange, lemon and melon) [25]. As can be seen, linearity of analytical response across the range studied is excellent, with correlation coefficients higher that 0.98 in most cases. This analytical performance in terms of linearity can compare very well with typical instruments used for quantitation purposes (e.g., single quadrupole or TQ).

By using the peak areas of XICs for the protonated molecule, the typical LODs observed were in the low range of μg/kg (Table 4). The narrower the *m*/*z* window chosen for the XIC, the higher the selectivity obtained, so

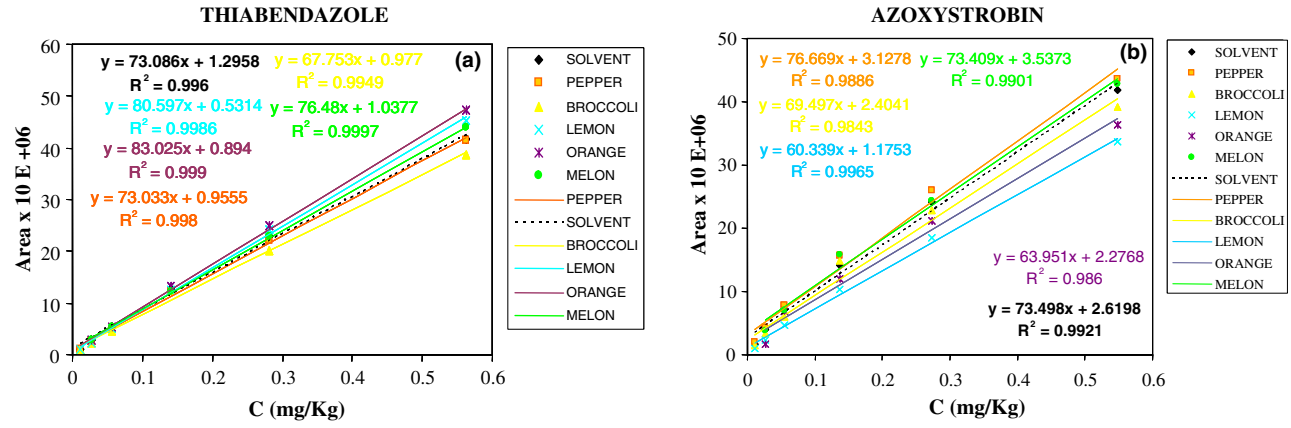

Figure 3. Comparison of the calibration curve slopes obtained for (a) thiabendazole and (b) azoxystrobin, using matrix-matched standards from different fruit and vegetable extracts and solvent-based standards.

a better signal-to-noise ratio was achieved. The use of narrow mass windows (i.e., accurate mass of the target species ±0.02–0.1 Da) provides a remarkable enhancement of the LODs, as can be observed in Fig. 4. This example showed a typical total ion chromatogram of a 0.01 mg/kg matrix-matched standard from a broccoli sample together with the XICs used for quantification purposes for two of the selected pesticides. In Fig. 4(a), the XIC of azoxystrobin is shown, using three different mass windows [(1) ±0.5; (2) ±0.1; and (3) ±0.02]. By using such narrow mass windows, the signal-to-noise ratio is higher, yielding thus better LODs than those achievable using low resolution instruments [1 Da mass window; (see Fig. 4(a.1) and (b.1)). Similar results are obtained for thiacloprid (Fig. 4(b)). The LODs obtained

Table 3. Linearity obtained by liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS)

	Matrix	Calibration equation	R^2
Thiabendazole	Solvent	$y = 7.3 \times 10^7 x + 1295800$	0.996
	Pepper	$y = 7.3 \times 10^7 x + 955500$	0.998
	Broccoli	$y = 6.8 \times 10^7 x + 977000$	0.995
	Lemon	$y = 8.1 \times 10^7 x + 531400$	0.999
	Orange	$y = 8.3 \times 10^7 x + 894000$	0.999
	Melon	$y = 7.6 \times 10^7 x + 1037700$	0.999
Azoxystrobin	Solvent	$y = 7.3 \times 10^7 x + 2619800$	0.992
	Pepper	$y = 7.7 \times 10^7 x + 3127800$	0.989
	Broccoli	$y = 6.9 \times 10^7 x + 2404100$	0.984
	Lemon	$y = 6.0 \times 10^7 x + 1175300$	0.996
	Orange	$y = 6.4 \times 10^7 x + 2276800$	0.986
	Melon	$y = 7.3 \times 10^7 x + 3537300$	0.990
Carbendazim	Solvent	$y = 4.9 \times 10^7 x + 1221700$	0.993
	Pepper	$y = 4.8 \times 10^7 x + 1109200$	0.995
	Broccoli	$y = 4.9 \times 10^7 x + 637400$	0.993
	Lemon	$y = 5.4 \times 10^7 x + 251200$	0.998
	Orange	$y = 4.9 \times 10^7 x + 2009200$	0.998
	Melon	$y = 5.1 \times 10^7 x + 1783200$	0.997
Spinosad	Solvent	$y = 6.5 \times 10^7 x - 701500$	0.995
	Pepper	$y = 7.3 \times 10^7 x - 414800$	0.999
	Broccoli	$y = 6.5 \times 10^7 x + 467000$	0.997
	Lemon	$y = 7.7 \times 10^7 x + 187000$	0.999
	Orange	$y = 7.3 \times 10^7 x + 47900$	0.999
	Melon	$y = 6.7 \times 10^7 x + 1240700$	0.995

Comparison of the regression equation and regression coefficients (R^2) obtained from the calibration curves of four selected pesticides in five different vegetable matrices [25].

Table 4. Analytical features of liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS) for quantitative pesticide-residue analyses

Pesticide	Intra-day	Inter-day	LOD (µg/kg)
	Amount added (mg/kg)		
	0.05 mg/kg	0.25 mg/kg	
	RSD (%)		
Cyromazine	7.1	5.9	5
Carbendazim	2.7	6.0	5
Thiabendazole	2.2	3.6	10
Methomyl	6.0	10.0	30
Imidacloprid	3.1	6.1	10
Acetamiprid	0.8	7.7	5
Thiacloprid	2.1	10.0	4
Spinosad	2.6	2.6	1
Dimethomorph	2.9	10.4	2
Azoxystrobin	3.1	5.7	0.3
Triflumizol	3.8	9.4	0.9
Hexaflumuron	2.6	11.0	10
Teflubenzuron	5.8	10.0	10
Lufenuron	5.6	8.0	10
Flufenoxuron	5.3	9.2	10

Run-to-run and day-to-day relative standard deviations (RSD (%)) in matrix-matched standards and LODs obtained using a mass window of 0.2 Da in a pepper extract [25].

are near those afforded by TQ instruments. In this sense, the high-sensitivity, full-scan capabilities of TOF instruments and the use of narrow mass windows allow signal-to-noise ratios obtained to be comparable with those obtained with LC-TQ-MS^2 instruments in SIM modes. The LODs observed for the majority of the commodities or pesticides tested meet the requirements regarding the MRLs imposed by the existing European regulations [2].

We studied the reproducibility, repeatability and accuracy of LC-TOF-MS for quantitative purposes on matrix-matched solutions at different concentration levels. As an example, relative standard deviation (RSD) values obtained from run-to-run and day-to-day precision are summarized in Table 4 at two different concentration levels. From the results obtained, LC-TOF-MS was found to be precise (with run-to-run RSD values typically below 5% and day-to-day RSD values between 2% and 10%). These precisions compared very well against those afforded by other LC–MS instruments widely accepted for routine quantitative purposes, such as single quadrupole or TQ instruments in SIM and SRM, respectively.

3.2. Matrix effects

Although some of the interferences are ''invisible'' in the chromatograms, most of the time, co-extracts present during the analysis decrease or enhance the signal.

The phenomenon of signal suppression is related to the ionization system and therefore not to the analyzer used [8]. The occurrence of matrix effects in LC–MS is well known, playing an important role in the quantitation of the pesticides, especially when electrospray

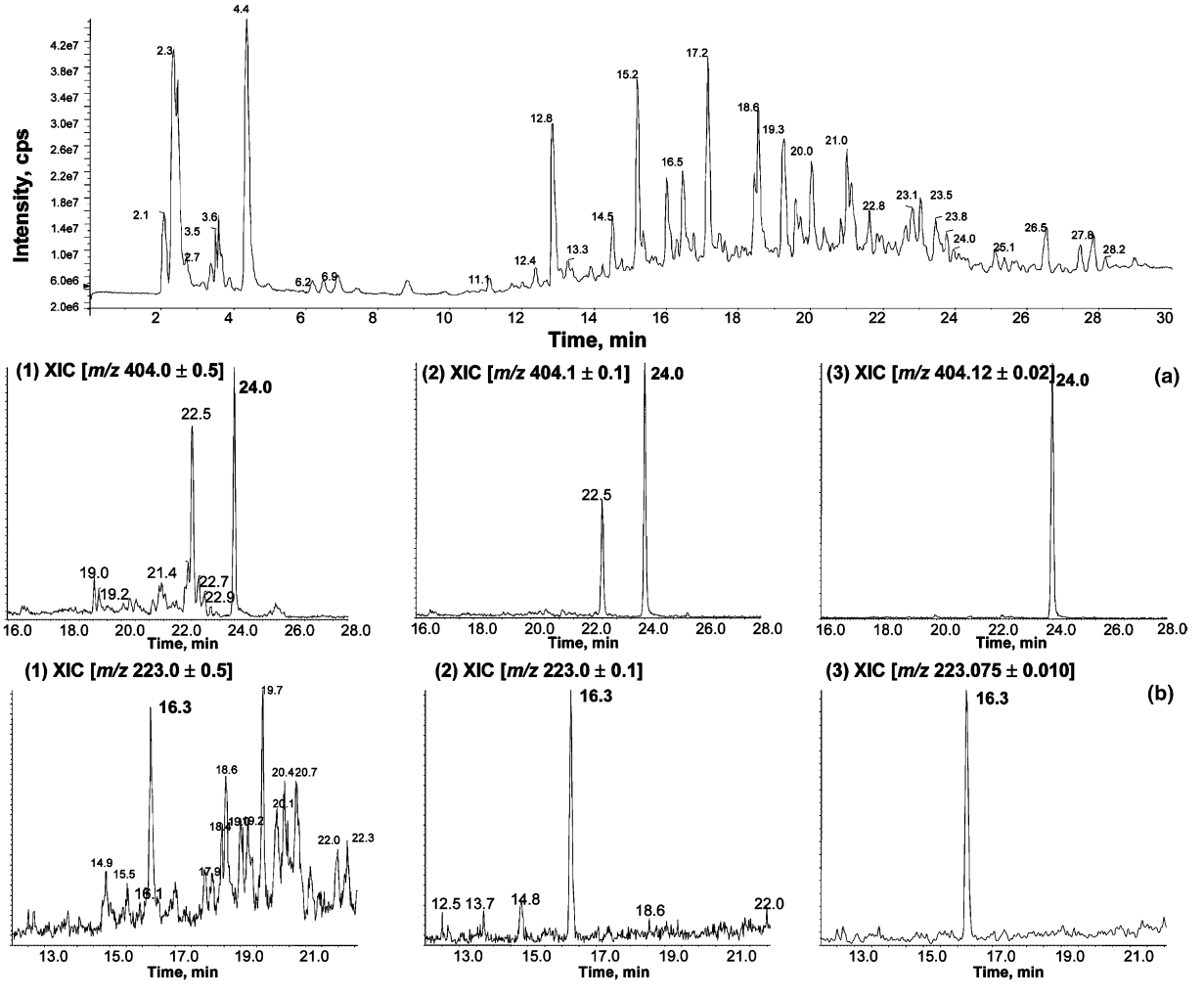

Figure 4. Signal-to-noise ratios using narrow mass windows for quantitative purposes. Above: total ion chromatogram (TIC) of a broccoli extract spiked with 0.01 mg/kg of azoxystrobin and thiacloprid. Below: extracted ion chromatogram (XIC) for (a) azoxystrobin (24.0 min) and (b) thiacloprid (16.3 min), using three different *m/z* windows for each (accurate mass of the protonated molecule (1) ±0.5, (2) ±0.1 and (3) ±0.02 uma for azoxystrobin and ±0.01 for thiacloprid).

ionization (ESI) is used. Matrix effects can both reduce and enhance the response when compared to ''solvent'' standards. The signal suppression or enhancement depends strongly on:

- the interface used (ESI in positive mode usually suffers higher signal suppression/enhancement than negative mode);
- each individual pesticide;
- each matrix tested (i.e., the amount of matrix per mL of extract); and, also,
- the sample-treatment procedure (e.g., extraction solvent and clean-up procedure).

To evaluate the signal suppression/matrix effects, the slopes obtained in calibration with solvent-based standards for each pesticide can be measured and the slope ratios (matrix/solvent) in the various different matrices tested can be calculated. Another way would be by comparing the signals obtained for a specific pesticide present at a certain concentration in different matrices. Fig. 3 shows the linear calibration curves of two selected pesticides in fruit and vegetable matrices along with the standard prepared in pure solvents; the variations in response of both thiabendazole and azoxystrobin are shown for different fruit and vegetable matrices. In this example, the matrix effects are only significant for azoxystrobin, especially in citrus matrices (orange and lemon). However, signal suppression for thiabendazole is practically insignificant.

Fig. 5 shows another example of signal suppression; it plots the differences between the XICs obtained for imidacloprid in different vegetable extracts under the same instrumental conditions. Some significant differences (positive and negative) in the responses can be observed, although the most usual case is a decrease in the response of the analyte due to signal suppression.

The extent of suppression or enhancement of the signal is typically 0–30% but, in some cases, it can be around 100% or higher [31,33] for a specific combination pesticide and commodity when using the most common extraction solvents [7]. As a result, the response of an analyte in pure solvent can differ significantly from that in the matrix sample. For this reason, procedures optimized with standards in pure solvent by adjusting MS parameters during method development could easily lead to wrong conclusions. The best solution to compensate for matrix effects of pesticide residues in food is by using matrix-matched calibration (standards with identical or similar matrix composition to the sample to be analyzed). However, the variability of the matrix between commodities and series makes it

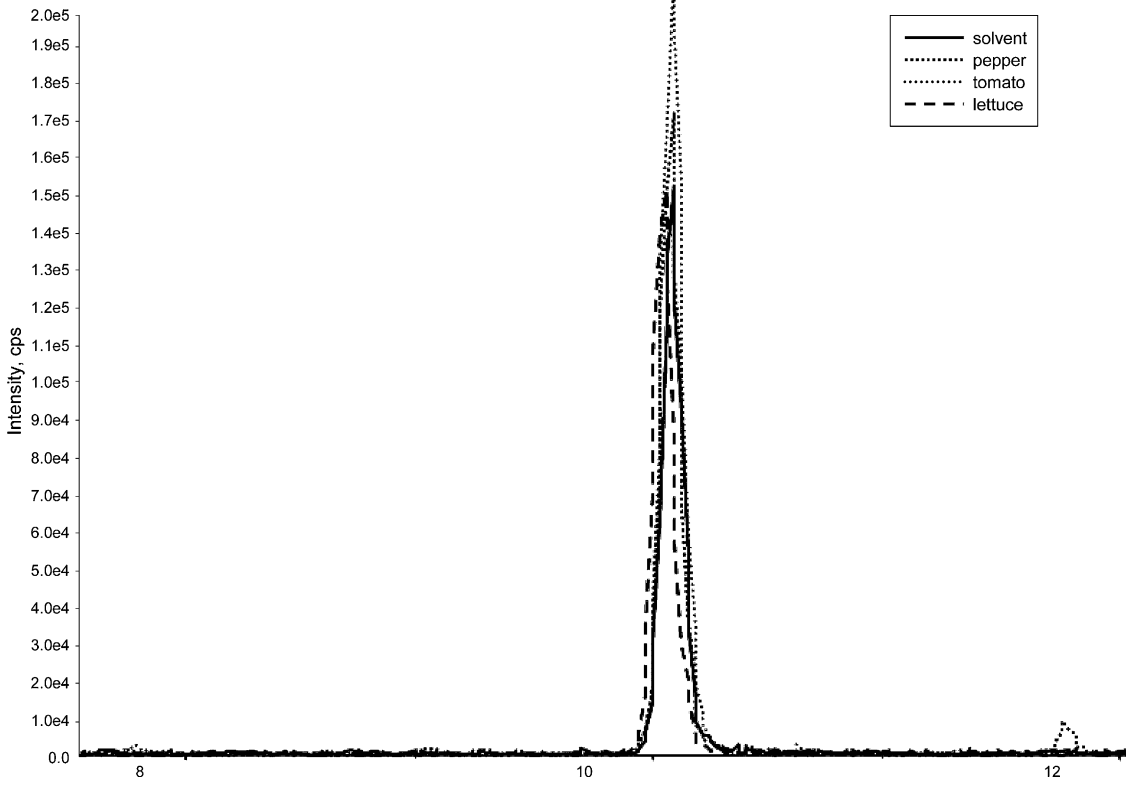

Figure 5. Effect of different vegetable matrices (pepper, lettuce, tomato) on the extracted ion chromatogram (XIC) of imidacloprid (*m/z* 256) at a concentration of 0.1 mg/kg.

Table 5. Application of liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS) to the analysis of a selected group of pesticides in two fruit extracts used in a proficiency test

Sample/pesticide	TestQual Value[a]	LC/TOF/MS[a]	z score	LC/Q/MS[a]	z score
Strawberry					
Carbendazim	0.30	0.25	−0.7	0.27	−0.4
Hexaflumuron	0.22	0.27	0.9	0.26	0.7
Imidacloprid	0.09	0.10	0.4	0.12	1.3
Methomyl	0.58	0.53	−0.3	0.45	−0.9
Spinosad	–	0.13	–	0.15	–
Azoxystrobin	–	0.14	–	0.17	–
Apple					
Carbendazim	0.32	0.21	−1.4	0.17	−1.9
Methomyl	0.27	0.32	0.7	0.24	−0.4

[a]Assigned value (μg kg^{-1}) from a proficiency test (23 laboratories).

necessary to carry out detailed matrix-characterization studies (e.g., water percentage, acid content, dryness, sugar and fat content) [8].

3.3. Quantitative analyses of pesticide residues in vegetable samples

The effectiveness of LC-TOF-MS for routine quantitative analyses of pesticide residues has been reported in environmental or drinking waters for rotenone [34], bayrepel (insect repellent) [35], quaternary ammonium herbicides [36] or lyophilized meat extracts (heterocyclic amines) [37]. Furthermore, the use of TOF-MS for quantifying pesticide residues in vegetables samples has been investigated [22,25].

As an example, Table 5 shows the results obtained from the analysis of two samples from a proficiency test (with 23 laboratories) for pesticide-residue analysis in fruits organized by TestQual (www.TestQual.com). All the target compounds covered by the comparison test were properly identified by LC-TOF-MS (carbendazim, methomyl, imidacloprid and hexaflumuron). Moreover, two additional non-target pesticides studied in a multi-residue method developed previously [25] were also identified (spinosad and azoxystrobin) in one of the samples. The results obtained with the LC-TOF-MS method compared well with those obtained with an LC quadrupole MS method in SIM mode. Values reported for the two methods were significantly very close, thus verifying the feasibility of the LC-TOF-MS method for the quantitative analyses of vegetable samples. Furthermore, the z score values obtained from the quantitation by TOF-MS were below 2 in all cases, which means that the values obtained were significantly ''acceptable''. The data from real samples, therefore, demonstrated that LC-TOF-MS is suitable for analyzing pesticides at low concentrations, together with additional information of accurate mass measurements as mentioned in the previous section.

4. Conclusions and future trends

The application of LC–MS in routinely analyzing pesticides in food is relatively new in multi-residue pesticide control it is clear and it is likely to increase exponentially in the near future. Currently, the first option is typically triple quadrupole MS for target-pesticide analysis. However, the introduction of new TOF-MS will greatly improve pesticide analysis in food. This is a consequence of the relatively low price of bench-top TOF spectrometers, yielding mass accuracy lower than 2–5 ppm with an adequate linear calibration range. These analyses can tremendously enlarge the range of detection of the analysis from target to unknown pesticide residues, which will be an important issue in the near future. The resolving power, accurate mass-measurement capability and full spectral sensitivity then makes LC-TOF-MS attractive as a tool for identifying non-target ''unknown'' compounds in complex vegetable matrices.

In addition, the predicted increase in sensitivity in the future by factors of 10–100, which is a general trend in MS, will also contribute to the suitability of LC-TOF-MS in achieving lower LODs, and that will avoid many of the difficulties related to matrix effects. Moreover, the use of hybrid TOF-MS instruments, such as Q-TOF-MS, will combine the capabilities of accurate mass measurement with highly useful structural information provided by characteristic fragmentation of the compounds of interest.

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