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TIME OF FLIGHT MASS SPECTROMETRY APPLIED TO THE LIQUID CHROMATOGRAPHIC ANALYSIS OF PESTICIDES IN WATER AND FOOD

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Liquid chromatography coupled to mass spectrometry (LC-MS) is an excellent technique to determine trace levels of polar and thermolabile pesticides and their degradation products in complex matrices. LC-MS can be equipped with several mass analyzers, each of which provides unique features capable to identify, quantify, and resolve ambiguities by selecting appropriate ionization and acquisition parameters. We discuss in this review the use of LC coupled to (quadrupole) time-of-flight mass spectrometry (LC-(Q)ToF-MS) to determine the presence of target and non-target pesticides in water and food. This technique is characterized by operating at a resolving power of 10,000 or more. Therefore, it gives accurate masses for both parent and fragment ions and enables the measurement of the elemental formula of a compound achieving compound identification. In addition, the combination of quadrupole-ToF permits tandem mass spectrometry, provides more structural information, and enhances selectivity. The purpose of this article is to provide an overview on the state of art and applicability of liquid chromatography time-of-flight mass spectrometry (LC-ToF-MS), and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QToF-MS) for the analysis of pesticides in environmental matrices and food. The performance of such techniques is depicted in terms of accurate mass measurement, fragmentation, and selectivity. The final section is devoted to describing the applicability of LC-(Q)ToF-MS to routine analysis of pesticides in food matrices, indicating those operational conditions and criteria used to screen, quantify, and identify target and "suspected" pesticides and their degradation products in water, fruits, and vegetables. The potential and future trends as well as limitations of LC-(Q)ToF-MS for pesticide monitoring are highlighted. © 2006 Wiley Periodicals, Inc., Mass Spec Rev 25:866–880, 2006

Keywords: *liquid chromatography; time-of-flight mass spectrometry; pesticides; water; fruit; vegetables*

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I. INTRODUCTION

In the last 5 years, high performance liquid chromatography (HPLC) or liquid chromatography (LC) has become popular for the analysis of not GC amenable pesticides (non-volatile, thermolabile, and polar) (Picó et al., 2000; Careri, Bianchi, & Corradini, 2002). HPLC coupled to mass spectrometry (MS) based methods have permitted to achieve the sensitivity needed to meet European Union (EU) legislation for the analysis of pesticides in water (Directive 60/2000/EU, 2000) and food samples, which have been set through maximum residue levels (MRL) (European Commission, 1999; WHO, 2000).

Quadrupole (Q) mass analyzers are extensively used mass spectrometers for their easy use and calibration, and many laboratories rely on their use for pesticide analysis in different food matrices (Lacorte & Barceló, 1996; Blasco et al., 2002). Their main limitation is that single quadrupole mass analyzers work at unit resolution and lack the accuracy needed to trace determination and identification of pesticides in samples that contain large amounts of co-extracted compounds. As a result, co-elutions with matrix interferences occurring in the ion source may affect the quantitative response and confirmation cannot be assured in the case that a single ion is produced at the MS interface. The identification capabilities of quadrupole MS are very low due to the low sensitivity in full scan mode. On the other hand, selected ion monitoring (SIM) lacks structural information needed to identify suspected compounds. LC-tandem mass spectrometry (MS/MS) overcomes this drawback by fragmenting a selected precursor ion and analyzing the product ions. The performance of MS/MS analysis depends on the type of mass analyzer used, which can perform several (generally 1–3) fragmentation steps.

Ion trap mass analyzers (IT-MS) have been used to determine pesticides in water and food samples (Hiemstra & de Kok, 2002; Liapis, Aplada-Sarlis, & Kyriakidis, 2003; Blasco, Font, & Picó, 2005; Soler, Mañes, & Picó, 2005). Its main advantages are the high sensitivity in the scanning mode and the possibility to perform MS^n that enables the identification of

unknowns in a sample by the interpretation of the successive spectra obtained. However, spectra interpretation might be difficult when no well-known pesticide moieties and only C, H, O, N fragments are present. Furthermore the presence of co-extracted compounds can introduce extra difficulties in the correct selection of the diagnostic ions.

Triple quadrupole instruments (QqQ) should, nowadays, be regarded as the most widely used technique for the routine multi-residue screening of pesticides in water and food (Hau et al., 2000; Hogenboom et al., 2000; Riediker et al., 2002; Taylor et al., 2002; Agüera et al., 2004; Picó, Blasco, & Font, 2004; Blasco, Font, & Picó, 2005). Different acquisition options based in scanning parent ions, neutral losses, daughter ions and multiple reaction monitoring (MRM) provide high selectivity and quantitative capabilities to the technique and make identification more plausible but still limited to elucidate a structure. With QqQ, limits of detection (LOD) at the μg/kg level, necessary to determine pesticides in food, can be achieved. In addition, LC-MS/MS in MRM presents excellent sensitivity and selectivity but does not permit structural elucidation of non-target compounds (Bobeldijk et al., 2001). However, full scan acquisition with MS or MS/MS produces a great abundance of peaks difficult to identify because, besides the loss in sensitivity (Steen, Bobeldijk, & Brinkman, 2001), the lack of libraries with LC-MS/MS spectra prevents identification of unknowns. As a result, the use of other analyzers such as quadrupole ion trap (QIT) or time-of-flight instruments (ToF) has increased (Picó, Blasco, & Font, 2004).

ToF-MS instruments are capable of 10,000 or more resolving power expressed in terms of FWHM (full peak width at one-half maximum). ToF-MS has a high acquisition speed and provides accurate mass measurement (possibility to yield mass accuracy <2 ppm with an adequate calibration range) as well as full scan spectral sensitivity. Accurate mass measurement gives the elemental composition of parent and fragment ions, used to identify unknown species and a greater differentiation of isobaric species (two different compounds with the same nominal mass but different elemental composition, and thus, different exact masses). When coupled to a quadrupole mass filter, QToF-MS permits MS/MS analysis and provide accurate masses for both precursor and product ions, which constitutes a higher order mass identification than those afforded by nominal mass measurements obtained by other types of mass analyzers.

Table 1 compares sensitivity, selectivity, accuracy, and dynamic range of ToF instruments with other MS analyzers (Ferrer & Thurman, 2003). In general, we can consider the sensitivity of ToF in scan mode higher than for QqQ or IT instruments, but lower than QqQ in SRM, Q or IT-MS in SIM. With LC-QToF-MS, better signal to noise ratios should be obtained although the ion collection of the quadrupole filter does not have a 100% efficiency and some ions are lost, resulting in a sensitivity similar to or worse than LC-ToF-MS. Nevertheless, sensitivity is a parameter that changes a lot depending on the mass spectrum generation and so, this comparison can be considered theoretical and not real.

On the other hand, based on the tandem MS capabilities, the selectivity of QqQ and IT MS/MS is high. LC-ToF-MS is less selective than LC-Q-ToF but very accurate since all ions are collected in the analyzer. The selectivity of precursor ion scans is very high on QToF instruments because the high resolving power of the reflectron-ToF mass analyzers provides high accuracy fragment ions without compromising sensitivity.

Accuracy of ToF mass analyzers is much higher than for any other instrument due to the excellent ion separation and detection in the flight tube (Eckers, Haskins, & Langridge, 1997; Pergantis et al., 2000). Accurate mass spectra achieve a much better identification of target analytes in complex matrices such as food. As for quantitative purposes, QqQ instruments permit the identification and confirmation of target compounds at very low concentrations (ppt) showing a considerable high dynamic range of three orders of magnitude typically, whereas (Q)ToF instruments have higher LOD and lower dynamic range two orders of magnitude typically, compromising in some cases quantification of target pesticides at ultra-trace level. This lower dynamic range is a consequence of ion saturation at the upper part of the concentration range.

Given the specific features of ToF instruments and comparing to other MS analyzers, the scope of this review is to indicate the performance of the different types of LC-(Q)ToF-MS instruments and their applicability in the field of pesticide analysis. Although the use of (Q)ToF is still emerging, mainly due to their high acquisition cost, the unique performance of LC-(Q)ToF-MS instruments provides a valuable tool for routine monitoring of target pesticides, screening purposes and to identify pesticide degradation products in water and food matrices.

II. TYPES OF LC-(Q)ToF-MS INSTRUMENTS

Throughout the years, ToF mass analyzers have undergone several technical advances that have transformed these instruments in valuable tools for pesticide analysis. In a first stage, the narrow linearity response of ToF-MS did not enable accurate quantitative analysis, and it was not till 1999 that the quantitative potential of a Q-ToF for residue analysis was reported (Clauwaert

TABLE 1. Comparison of LC/MS systems (Ferrer & Thurman, 2003, with permission)

	Sensitivity in full scan	Selectivity	Accuracy	Dynamic range	Unique features
Triple quadrupole	Medium	High	Low. Unit resolution	High	Neutral loss
IT MS/MS	High	High	Low. Unit resolution	Medium	MS^n
LC-ToF-MS	High	Low	High	Low	Accurate mass and sensitivity
LC-QToF-MS	High	High	High	Medium	Accurate mass and selectivity

et al., 1999). These instruments suffered from narrow dynamic ranges, requiring mathematical algorithms, such as the ''time to digital correction'' to attain a longer linear dynamic range, and this limited the usability of the ToF-MS (Ferrer & Thurman, 2003). New instruments offer a dynamic linear range of about two to three orders of magnitude, which is somehow lower than those offered by other MS analyzers but enough for pesticide analysis.

One of the main advantages of LC-ToF instruments is that atmospheric pressure ionization (API) interfaces are used to couple LC with (Q)ToF-MS, similarly to other types of analyzers, with the possibility to perform ionization and in-source fragmentation of target compounds. Two main types of ToF instruments can be found in the market, the LC-ToF-MS and LC-QToF-MS, which differ mainly in the capacity or not to perform MS/MS experiments.

Figure 1 shows a scheme of a ToF instrument. ToF-MS measures the time an ion needs to travel through a field-free region, differing from quadrupole or ion trap (IT) systems, which use an electric field to separate the ions with different *m*/*z* ratios. The ions generated in the ion source are accelerated as discrete packages into the field-free flight tube by using a pulsed electrical field. Flight times, which are proportional to the square root of the *m*/*z* of an ion, are in the order of microseconds. Consequently, ToF-MS can operate at very high repetition rates, typically 5–30 kHz, that is, 5,000–30,000 raw mass spectra are generated per second. Of course, fast detector electronics (which have been available only recently) are required to record the arrival times of the ions at the end of the flight tube. A number of the raw mass spectra are added or averaged, and typically 10–500 spectra/sec are stored on the computer system (Dallüge, Roose, & Brinkman, 2002). In ToF-MS, there are two possible approaches, instruments that have very high resolution (5,000–10,000) but moderate scan speed (10 Hz) and instruments that have speed of 100–500 spectra/sec but provide unit resolution. In the frame of non-target pesticide identification in complex matrices, the former approach is best suited.

ToF-MS can record accurate full scan spectrum throughout the acquisition range, and has resulted in an excellent tool for the unequivocal non-target identification and for compound confirmation. With respect to standard monitoring practices which use SIM or MRM, ToF-MS offers identification and structural elucidation of target and non-target compounds in a sample. Nonetheless, recent improvements in ToF analyzers in relation to number of scans per second and new digital sampling techniques have overcome initial limitations leading to instruments capable to perform both qualitative and quantitative analysis. At present, ToF mass analyzers offer high selectivity under full scan conditions, resolution of around 10,000 and high mass accuracy as a result of a very stable calibration (Ferrer, Thurman, & Fernández-Alba, 2005a). ToF mass analyzers permit structure elucidation which has been applied for the analysis of pesticides (Ferrer & Thurman, 2003; Ferrer, García-Reyes, & Fernandez-Alba, 2005b) and identification of suspected compounds or their degradation products in environmental and food matrices (Thurman et al., 2005a). With a LC-ToF-MS instrument, it is possible to:

1. collect data across a wide mass range without decrease in sensitivity, so that full scan spectra is achieved;
2. resolve interferences with high resolving power; and
3. achieve accurate mass measurements for the estimation of elemental composition.

Ultimate confirmation of target analytes is achieved with hybrid quadrupole time-of-flight mass spectrometer (QToF) which consists of (i) a MS1 and collision region adapted from a triple quadrupole instrument and (ii) a reflectron-type orthogonal acceleration time-of-flight analyzer for MS2 (Fig. 2). It can be seen as a triple quadrupole where the last quadrupole has been replaced by a ToF analyzer. In a QToF, the sample is introduced through the interface and ions are focused using the hexapole ion bridge into the quadrupole MS. The introduction of ions is such that the flight path of the ions changes 90°, which is called orthogonal ToF. This permits to optically focus the kinetic energy of the ions to avoid shifts among the different ions. The ions are then accelerated and travel towards the reflectron. The reflectron slows down ions of equal mass but higher kinetic energy and then focuses this beam of ions at the detector such that ions of the same exact mass but slightly different energies arrive at the detector at exactly the same moment. This process results in the mass accuracy of the QToF-MS (Ferrer & Thurman, 2003).

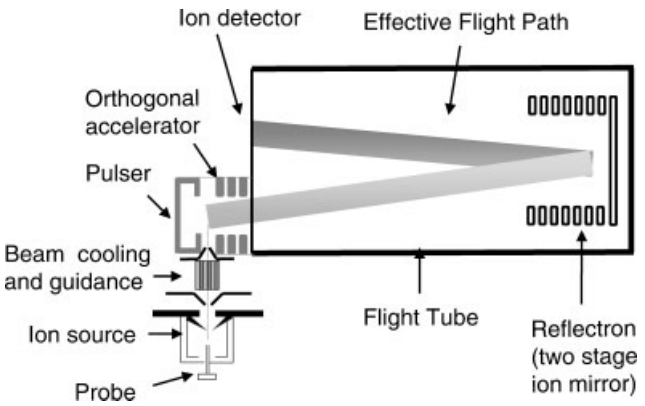

FIGURE 1. Schematic overview of the ToF mass spectrometer.

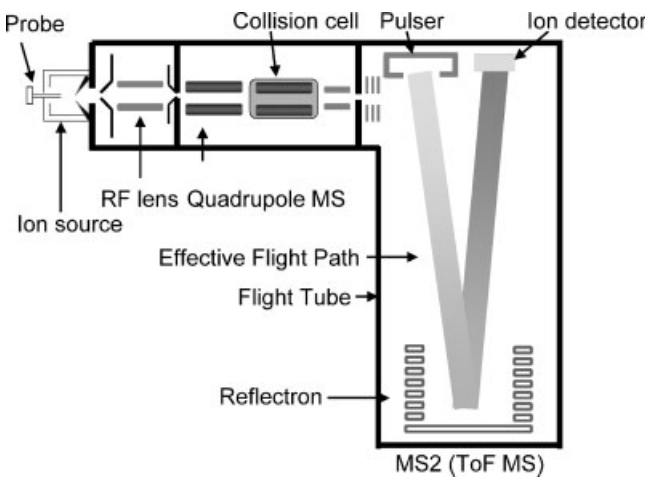

FIGURE 2. Schematic overview of the QToF mass spectrometer.

On the other hand, QToF has the capability for single MS as well as MS/MS operation modes. In the former, the first quadrupole is operated in band pass mode and the analysis is performed on the ''high end'' ToF analyzer (Van Bocxlaer et al., 2005). For MS/MS, a precursor ion is selected in the first quadrupole, the second produces collision-induced dissociation and the mass analysis of the fragment ions is performed in the ToF analyzer. By virtue of its tandem MS capabilities, full scan product ion spectra are obtained and any ion can be selected to reconstruct an ion chromatogram. Contrarily to QqQ instruments, the high resolution analysis allows the construction of accurate, sub-unit mass interval ion chromatogram, which results in a better signal to noise ratio (Van Bocxlaer et al., 2005).

QToF-MS is a very attractive tool due to the combination of high sensitivity, high resolution, and high mass accuracy for both precursor and fragment ions, which is performed when the instruments are used in information-dependent acquisition (IDA) experiments. IDA acquisition does not compromise on scan speed, mass range, or resolution (Decaestecker et al., 2000). In an IDA experiment, the data itself direct the kind of mass analysis function which is used. Its main advantage is that within a single run, product ion spectra can be generated for precursor ions which are unknown beforehand. In addition, data acquisition is so fast that multiple precursors from co-eluting peaks can be monitored simultaneously.

III. GENERAL FEATURES OF LC-(Q)ToF

A. Accurate Mass Measurements

(Q)ToF instruments permit the measurement of accurate masses within 5 ppm, value accepted for the verification of the elemental composition (Ferrer & Thurman, 2003). To achieve such accurate mass measurement, ToF-MS instruments require frequent tuning and calibration of the spectrometer. Accurate mass measurement for target pesticides may involve a single point correction of the base calibration (to compensate for the slight drift of the calibration because of temperature fluctuations in the flight tube, changes in the accelerating fields, and instabilities of the power supplies) utilizing a reference compound or lockmass (Benotti et al., 2003).

In early instruments, mass calibration was external and, thus, accuracy was much lower than that achieved in modern instruments. Calibration in positive electrospray mode can be conducted with PEG mixture containing PEG200, PEG400, and PEG600 in equimolar quantities (Bobeldijk et al., 2001) attaining a resolution of 7,000. Sulfadimethoxine, which ionizes in positive (m/z 311.0814) and negative (m/z 309.0658) electrospray ionization modes, can also be used. Other possible lock mass are leucine enkephaline and 3,5-diiode-L-tyrosinase. The lock mass can be added post-column at a flow rate of 1-2 μL/min to allow for internal mass calibration. However, the addition of a lockmass to the LC mobile phase or post-column via a T-piece can produce (i) ion suppression for compounds which are difficult to ionize in ESI, (ii) interferences between lockmass and analyte, and (iii) the possibility to cluster the analyte with the reference compound. As a result, newer generation of ToF-MS instruments are equipped with dual nebulizer ion source to perform accurate mass calibration automatically, introducing a reference compound at a very low flow rate along with the output of the LC system. The ''on-line'' calibration is vital to adjust the mass differences for drifts occurring during the course of the measurement. In practice, calibration depends on the masses used for calibration of each system, which is internal and specific of each instrument, together with algorithm mechanism of calibration, which depends on the software.

To obtain an accurate response, detection is performed using a multi-channel plate (MCP) time-to-digital convertor (TDC). The ''stop time'' of TDC is the size of the pulse needed to register as being an ion. Decreasing the ''stop time,'' of the TDC from 150 to 100 mV can cause an increase of the ion signal. Another parameter that affects accuracy is the ''scan time'' which is the time interval during which ToF spectra acquired are integrated. As the ''scan time'' increases, the ion intensity (as well as the noise) increases exponentially; an increase in peak area reduces integration errors and leads to an improvement in accuracy of the analysis at very low concentrations.

Table 2 reports the accuracies obtained in the mass measurement of the protonated molecule of several pesticides in a tomato extract fortified at 0.05 mg/kg. The errors obtained are for all compounds <2 ppm. Figure 3 illustrates the mass accuracy for carbendazim. Using a mass window of 10 ppm, seven elemental compositions of the ion 192.0767 are possible. Two formulas are found with an error <3 ppm, and among them, carbendazim was identified according to retention time match against a standard. The accepted accuracy threshold for confirmation of elemental compositions is established at 5 ppm, so mass measurement accuracy, along with specific retention time, usually provides highly reliable identification of target species. In addition, mass accuracy is also achieved for all characteristic ions, thus providing two sets of information for unequivocal identification. Also, the effect of different concentration levels and matrix complexity on the accuracy of mass measurement showed no significant differences in the accuracy obtained in the various matrix-matched standards compared to those prepared with pure solvent, the error being kept <5 ppm, with an average of 1 ppm for most pesticides (Ferrer et al., 2005c). In principle, if ions can be measured with sufficient accuracy, it is possible to assign unique elemental compositions to peaks observed during the course of an analysis.

B. Fragmentation

Using electrospray (ESI) or atmospheric pressure chemical ionization (APCI) interfaces, an increase of extraction potential accelerates the ions and the collisions between them induce fragmentation via collision-induced dissociation (CID). However, CID mass spectra can be to some extent comparable to MS/MS spectra but selectivity can be affected in complex samples, making the spectral interpretation difficult due to the presence of multi-background ions. This often occurs in the case of food analysis. In those cases, a clean-up step to remove impurities which would interfere with target analytes is required to obtain a qualitative CID mass spectrum using quadrupole mass analyzers. However, the use of ToF-MS permits an enhanced selective mass measurement of CID fragment ions, thus avoiding the problem of sample interferences. In multi-residue analysis, the fragmentor voltage cannot be optimized for each single pesticide because of

TABLE 2. LC-ToF-MS accurate mass measurements in a tomato extract fortified with a pesticide mixture

Compound	Formula	Selected ion	m/z experimental	m/z calculated	Error (ppm)	RSD (%)		LOD
						Intra day (0.05 mg/kg)	Inter day (0.25 mg/kg)	ug/kg (pepper)
Cyromazine	$C_6H_{10}N_6$	$[M + H]^+$	167.1040	167.10397	0.17	7.1	5.9	5
Carbendazim	$C_9H_9N_3O_2$	$[M + H]^+$	192.0767	192.07675	0.27	2.7	6.0	5
Thiabendazole	$C_{10}H_7N_3S$	$[M + H]^+$	202.0430	202.04334	1.7	2.2	3.6	10
Methomyl	$C_5H_{10}N_2O_2S$	$[M + Na]^+$	185.0355	185.03552	0.11	6.0	10.0	30
Imidacloprid	$C_9H_{10}N_5O_2Cl$	$[M + H]^+$	256.0597	256.05957	0.47	3.1	6.1	10
Acetamiprid	$C_{10}H_{11}N_4Cl$	$[M + H]^+$	223.0742	223.07450	1.3	0.8	7.7	5
Thiacloprid	$C_{10}H_9N_4ClS$	$[M + H]^+$	253.0308	253.03092	0.48	2.1	10.0	4
Spinosyn A	$C_{41}H_{65}NO_{10}$	$[M + H]^+$	732.4668	732.46812	1.81	2.6	2.6	1
Spinosyn B	$C_{42}H_{67}NO_{10}$	$[M + H]^+$	746.4832	746.48770	0.77	n.a.	n.a.	n.a.
Dimethomorph	$C_{21}H_{22}NO_4Cl$	$[M + H]^+$	388.1310	388.13101	0.03	2.9	10.4	2
Azoxystrobin	$C_{22}H_{17}N_3O_5$	$[M + H]^+$	404.1243	404.12409	0.50	3.1	5.7	0.3
Triflumizol	$C_{15}H_{15}N_3OF_3Cl$	$[M + H]^+$	346.0925	346.09285	1.0	3.8	9.4	0.9
Hexaflumuron	$C_{16}H_8N_2O_3F_6Cl_2$	$[M + H]^+$	460.9885	460.98889	0.85	2.6	11	10
Teflubenzuron	$C_{14}H_6N_2O_2F_4Cl_2$	$[M + H]^+$	380.9816	380.98152	0.20	5.8	10	10
Lefunuron	$C_{17}H_8N_2O_3F_8Cl_2$	$[M + H]^+$	510.9854	510.98570	0.58	5.6	8.0	10
Flufenoxuron	$C_{21}H_{11}N_2O_3F_6Cl$	$[M + H]^+$	489-0440	489.04351	1.0	5.3	9.2	10

Intra- and inter-day relative standard deviation (% RSD) and limits of detection (LOD) are also indicated.
n.a., not available.

the proximity of other target analytes, so a compromise between sensitivity and fragmentation has to be achieved. It has been reported that fragmentation voltages of 250 V or higher lead to extensive fragmentation, even of the reference masses (Ferrer, García-Reyes, & Fernandez-Alba, 2005b). Figure 4 shows the accurate identification of chlorotoluron in a tomato extract at 190 and 230 V of fragmentation voltage and using ToF-MS in full scan. For many pesticides, voltages of 120 V provide minimal fragmentation. Fragmentor voltage window programs along the chromatographic run permit to optimize the fragmentation and sensitivity but, as a consequence, they limit the number of target compounds that can be included in each run. When pesticides undergo CID, the presence of inspected organic residues can be confirmed in food using the accurate mass of the protonated molecule along with that of the characteristic fragment ions. This feature enlarges the number of positive findings which otherwise could be reported as ''non detected.''

When using a QToF, tandem MS acquisition produces a product ion spectrum in full scan, which can resolve isobaric analytes and enhance identification. Fragmentation is performed by resonance in the quadrupole, leaving the fragmentor voltage at low values to obtain high intensity of high molecular weight ions. Those ions are fragmented in the quadrupole by resonance which is more efficient and faster than CID collision (milliseconds versus seconds) and yields more structural information. A main drawback is that the accuracy might drop due to the fragmentation of calibration ions.

C. Selectivity

The selectivity of LC-(Q)ToF-MS relies on the resolving power of the instrument on the *m/z* axis. The higher the resolution provided by the instrument is, the better the selectivity for unequivocal identification. Taking into account that the resolving power of a ToF instrument is in the range of 5,000–10,000, it can discriminate between ''isobaric'' interferences within 0.05 Da mass difference (Ferrer & Thurman, 2003). An isobaric interference in LC-ToF-MS would therefore arise only for co-eluting interfering species at the same exact mass. This selectivity is significantly higher than that provided by any other LC-MS instrument, which generally works at unit resolution. Figure 5 shows how selectivity can be enhanced by analyzing an olive extract by LC-ToF-MS. When a wide mass window (e.g., ±0.5 Da) is selected in the extracted ion chromatogram (*m/z* 233.0735), interferences might be present (Fig. 5B). When the same window is narrowed (e.g., ±0.05 Da), a more selective identification of target compounds can be achieved and an enhanced signal to noise ratio for diuron. The chromatogram shows the total ion chromatogram of a 0.01 mg/kg matrix-matched standard from a tomato sample together with the extracted ion chromatogram (XIC) used for quantification of diuron.

Compared to triple quadrupole (TQ) instruments, (Q)ToF instruments with accurate mass measurement of target compounds enhance selectivity since matrix interferences which might take place when analyzing complex samples are avoided. For example, quantitative errors can occur due to the contribution of an unknown species in the surrogate or internal standard (^{13}C or deuterated), which cannot be avoided when using LC-MS(MS) either in SIM or SRM. Due to the high resolving power of LC-ToF-MS, these isobaric interferences can be avoided since 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 benchtop ToF mass spectrometers for the trace analysis of food residues.

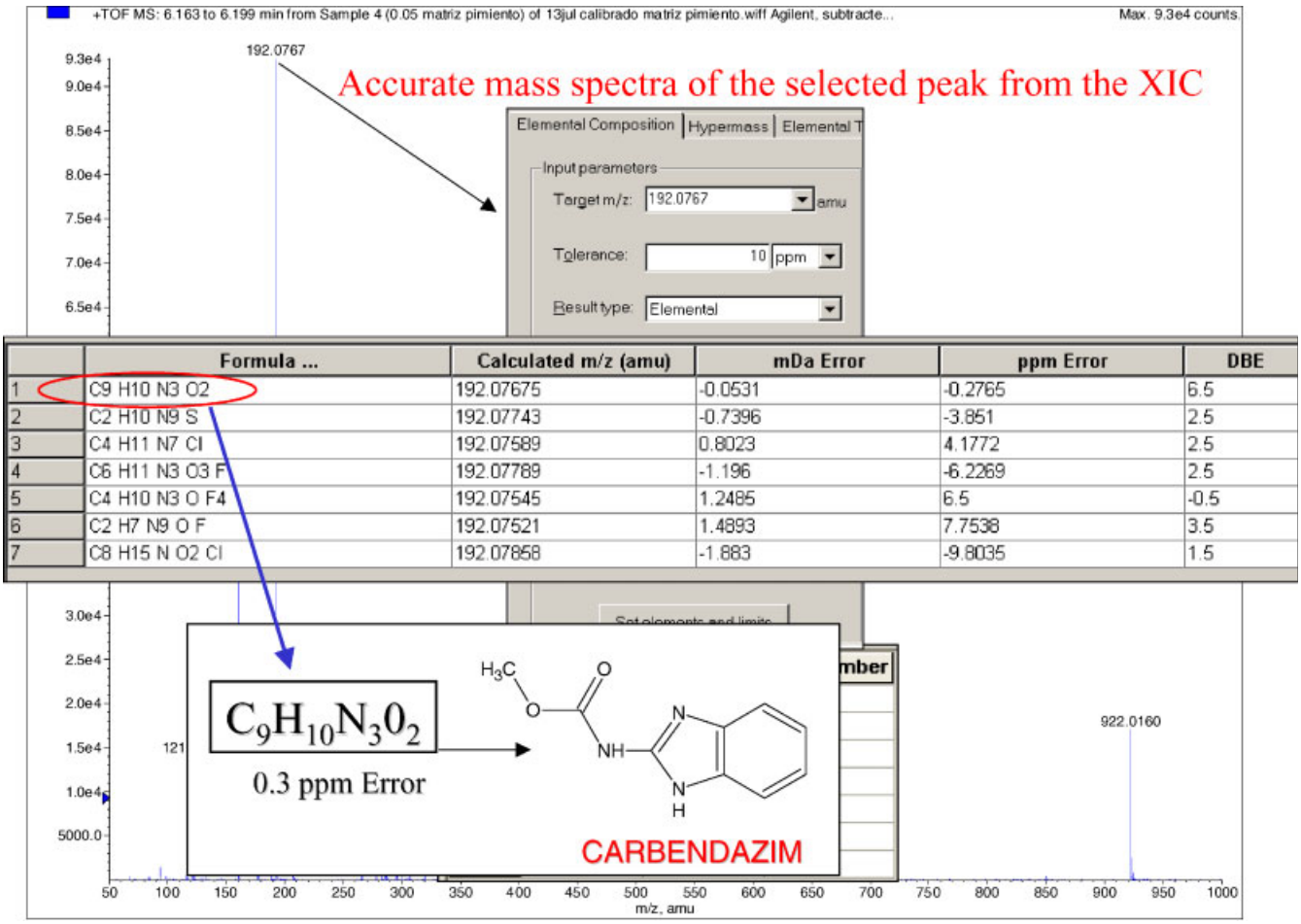

FIGURE 3. Identification of carbendazim in a pepper sample by LC-ToF-MS.

IV. APPLICATIONS OF LC-(Q)ToF-MS FOR THE ANALYSIS OF PESTICIDES IN WATER AND FOOD

After a thorough bibliographic search, it has been observed that the application of LC-(Q)ToF-MS instruments for pesticide residue determination is still scarce mainly due to the still high costs of the technique and to the initial poor quantitative capabilities of QToF instruments, which fall behind other more commonly used MS such as quadrupole or triple quadrupole (Van Bocxlaer et al., 2005). However, several applications have been reported which will, in the near future, revolute the field of pesticide residue analysis.

A. Routine Monitoring of Target Pesticides

Monitoring of target pesticides require a quantitative determination. Quantification with LC-MS depends on multiple parameters, such as the choice of the analytical column, mobile phase, flow rate, ionization and acquisition conditions. For LC-(Q)ToF-MS, we should ensure that pesticide analysis meet EU standards for quantification and sensitivity. With (Q)ToF-MS, quantification is performed by integration of the extract ion chromatogram, obtained from a single MS data for any particular ion, as reviewed by Thurman, Ferrer, & Fernandez-Alba (2005b).

Table 3 summarizes the different studies performed regarding the analysis of pesticide residues in water and food using LC-(Q)ToF-MS. The exact mass measurement of 10 non-volatile or thermally unstable carbamate, urea, and thiourea pesticides was determined by LC-ToF-MS using positive electrospray (Maizels & Budde, 2001). With a benchtop ToF equipped with an electrostatic mirror and a resolving power of 3,500–5,000 and acquisition over a mass range of 10–10,000 Da with individual spectra accumulated over 2 sec each, the mean errors from three replicate exact mass measurement were in the range of 0–5.4 ppm. Analyte confirmation was achieved by exact mass measurement which can resolve ambiguities, when an ion of the same nominal mass is used to identify two co-eluting analytes. Large volume injection and LC-UV-ToF-MS was used to determine the pesticide rotenone in river waters (Holm et al., 2003). LOD of 100 ng/L were obtained with a inter-day precision <8.9% relative standard deviation for levels between 0.5 and 5 µg/L and the system behaved linear over a concentration range of 0.5–50 ng. Recoveries were estimated to be 95% and the proposed method was highly time efficient since extraction and clean-up steps were eliminated and quantification was performed precisely.

Within the field of food analysis, a multi-residue methodology using LC-ToF-MS was used for the quantitative routine

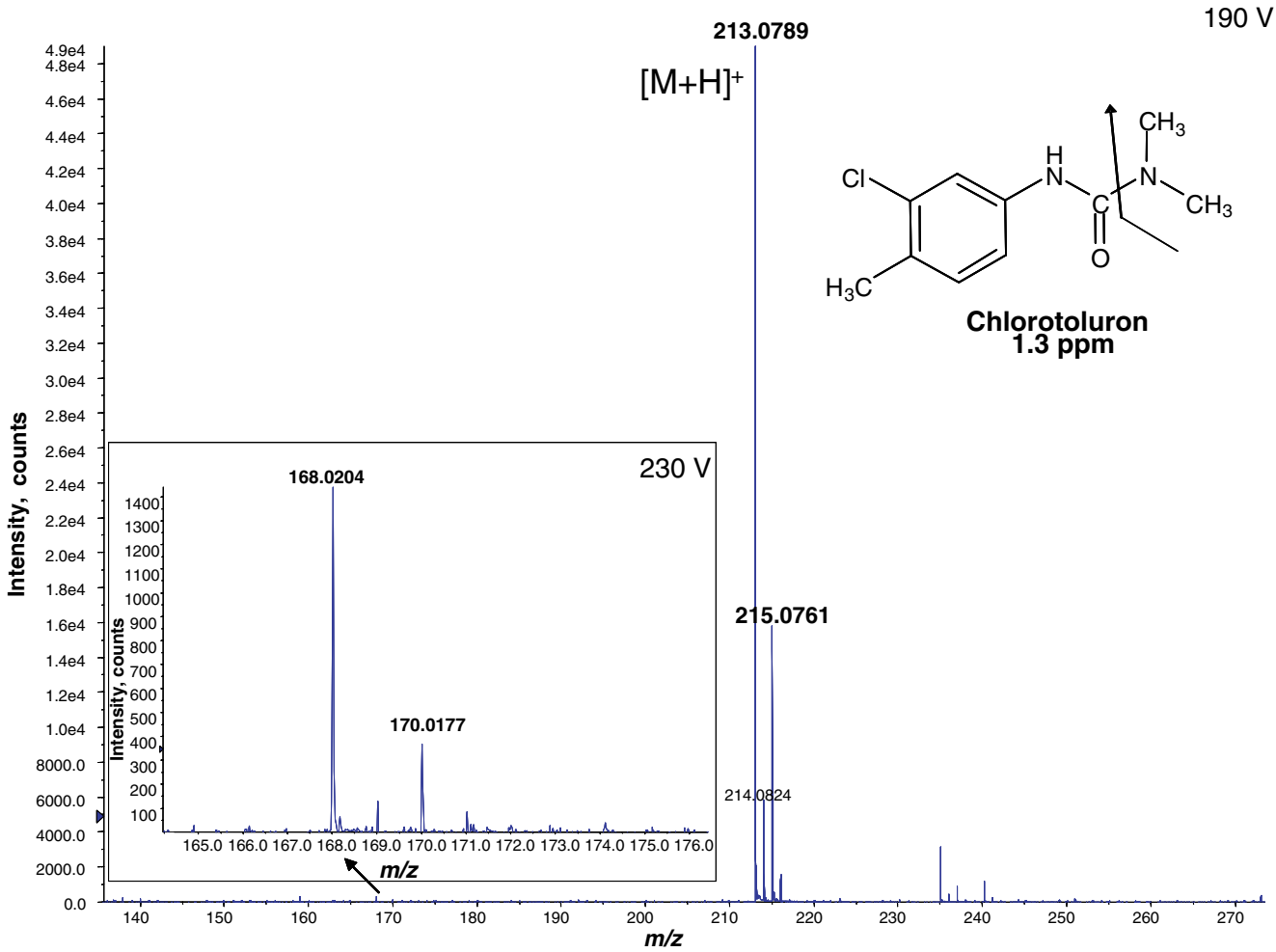

FIGURE 4. LC-ToF-MS full-scan spectrum of chlorotoluron in a tomato extract at 190 V and at 230 V, showing the typical fragments obtained and the corresponding accurate masses. (Reproduced from García-Reyes et al., 2005, with permission from John Wiley Ltd., copyright 2005.)

analysis of 15 pesticides in several types of fruits and vegetables (Ferrer et al., 2005c). Accurate mass measurements below 2 ppm were obtained in different matrices at 0.01–0.5 mg/kg concentrations and the linearity of the analytical response across the studied range was excellent, with correlation coefficients higher than 0.992. This article reports the quantitative analysis of pesticides in food, and LOD between 0.0005 and 0.03 mg/kg were obtained. The study concludes that LC-ToF-MS can be used for the quantitative analysis of pesticides in fruits and vegetables and reports the usefulness of this technique to obtain structural information for unequivocal identification of target compounds provided by elemental composition formula information. The same research group evaluated the accuracy of quantitative analysis of LC-ToF-MS by participating in a proficiency test (with 23 participating laboratories) organized by TestQual (www.TestQual.com) (Ferrer, Thurman, & Fernández-Alba, 2005a). Target compounds (carbendazim, hexaflumuron, imidacloprid, methomyl, spinosad, and azoxystrobin) were identified in tomato, lettuce, pepper, and cucumber obtaining similar values to those obtained with LC-Q-MS (Table 4). In addition, the Z-scores obtained with LC-ToF-MS were below 2, indicating that the calculated levels were acceptable. In this exercise, target compounds were detected at 0.1–0.5 μg/kg, thus proving the efficiency of ToF instruments to analyze low pesticide levels.

One of the main problems in the quantitative determination of pesticide residues is that its extraction easily carries away interferences (sugars, cellulose, lipids, etc.) in the final extract (Reynolds, 2005). Therefore, quantitative analysis can be severely affected by matrix effects, the most common being the suppression or enhancement of analyte ionization in the mass spectrometer, which lead to unacceptable results if no correction is being made. Signal suppression or enhancement is related to the ionization procedure rather than the analyzer used (Ferrer, Abián, & Fernández-Alba, 2005d) and depends on the type of pesticides being analyzed and the type of matrix. This effect is more important when using electrospray interfaces, and the effect is more intense under positive ionization mode. The extent of suppression or enhancement of the signal is typically 0–30% but in some cases, it can be total (Klein & Alder, 2003; Jansson et al., 2004). For this reason, procedures optimized with standards in

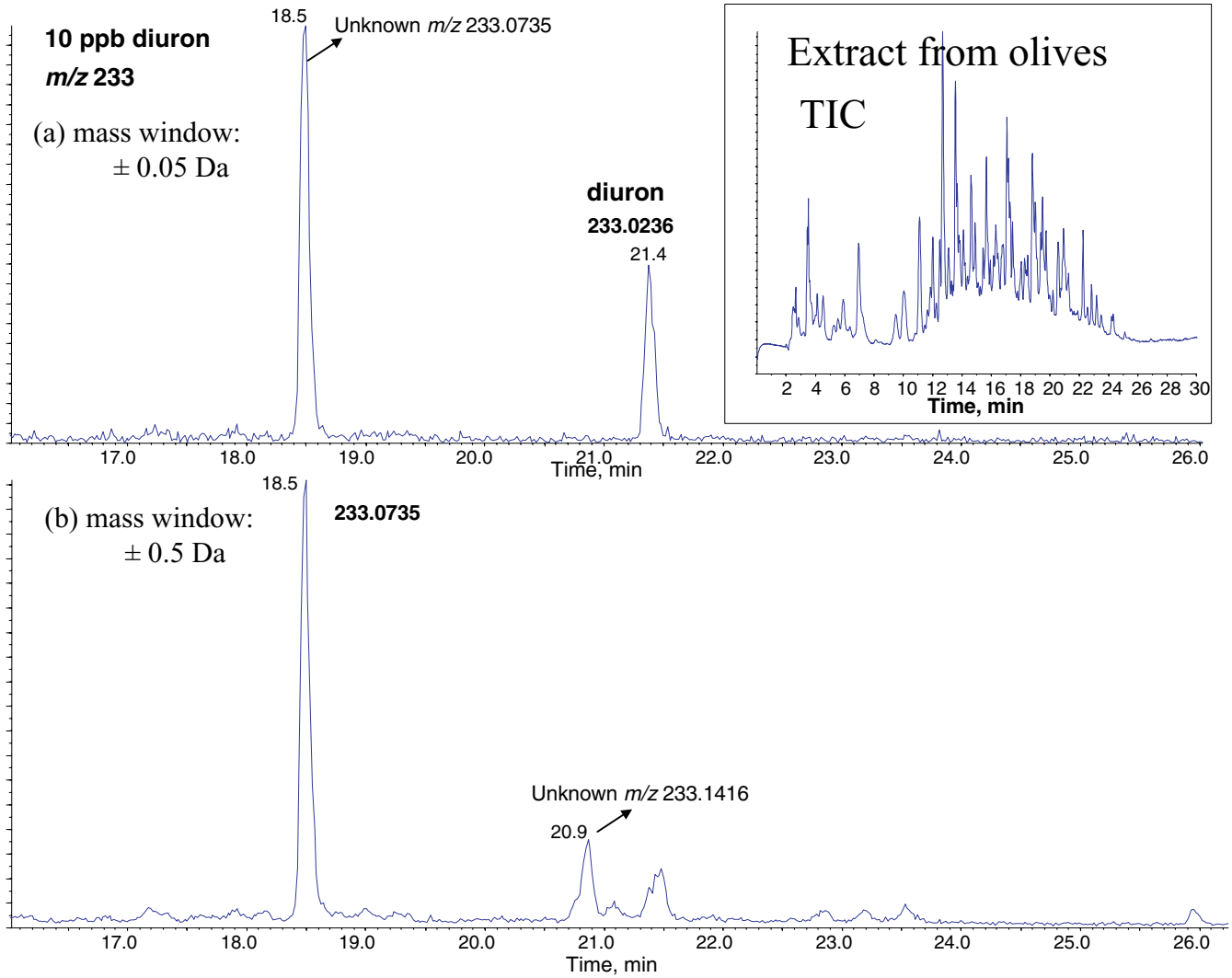

FIGURE 5. Analysis of an extract from olives. Figure shows the extracted ion chromatograms for *m/z* 233 (diuron) at two different accurate mass windows (**a**) 0.05 Da and (**b**) 0.5 Da. Total ion chromatogram is also shown (top right).

pure solvent by adjusting MS parameters can lead to wrong conclusions. To evaluate signal suppression, it is a good practice to perform a matrix-matched calibration (standards in identical or similar matrix than sample to be analyzed) or to use appropriate labeled surrogate and internal standards or application of an efficient clean-up step (Hernández et al., 2005a). Another way would be by comparing the signals obtained for a specific compound in solvent or in food matrices. Ferrer et al. demonstrate the linearity of a LC-ToF-MS over a concentration range from 0.01 to 0.5 mg/kg for several pesticides (thiabendazole, azoxystobin, carbendazim, and spinosad), obtaining correlation coefficients of 0.98–0.99 in both pure solvents and matrix-matched standards (pepper, broccoli, lemon, orange, and melon) with <10% inter-day variation (Ferrer, García-Reyes, & Fernandez-Alba, 2005b). Table 5 shows the linearity for three pesticides (imidacloprid, acetamiprid, and thiacloprid) in solvent and four vegetable extracts. It can be observed that the contribution of the independent factor varies depending on the extracted matrix. This study reports that linearity of LC-ToF-MS can be compared to that of quadrupole or QqQ instruments. Table 2 reports the relative standard deviation obtained from run to run ($n = 5$) and day to day (five consecutive days) of the same solution. Values lower than 11% were found at different spiking levels. These precisions are comparable to other analyzers and acceptable for routine quantitative purposes.

Sage et al. (2002) explored the quantitative performance of ToF-MS comparing it with LC-MS/MS with MRM for 15 pesticide residues in fruit extracts. MRM was 4 to 25 times more sensitive than ToF although LC-ToF-MS could also be used to quantify pesticide residues. This technique provided elevated spectral resolution allowing exact mass measurement and full mass spectral sensitivity for low level analyte detection. Analysis was performed within 15 min, the reporting levels were between 0.05 and 0.2 mg/kg, and due to the inherent high specificity of the technique, the need for sample clean-up was removed (Sage et al., 2002).

TABLE 3. LC-(Q)ToF-MS methods used to analyze pesticides in standard solutions, water or food matrices

Compounds	Matrix	Technique	Identification	Accuracy	Linearity	LOD	RSD	Reference
Carbamate Urea Thiourea	Standards	LC-ESI-ToF Rs:3500-5000 Calibrant: morpholine, azobenzene, 3,3'-dimethylbenzidine	Accurate mass Elemental composition	0-8.9 ppm	n.a.	n.a.	1.6-35%	Maizels et al., 2001
Pirimicarb Metramitron Metribuzin Atrazine Isoproturon Diuron	Water	LC-QToF Z-spray ESI (+) Rs: 7000 Lock mass: PEG	Accurate mass Elemental composition Isotopic clusters Retention time UV spectra CID fragmentation MS/MS	< 8.8 ppm	n.a.	n.a.		Bobeldijk et al., 2001
Rotenone	River water	LC-UV-ToF-MS Z-spray ESI	Retention time UV spectra CID fragmentation	n.a.	0.5-50 ng	10 ng/L	< 8.9%	Holm et al., 2003
Triazine Urea Organophosphorus	Water	LC-QToF-MS Orthogonal Z-spray ESI (+/-) 60-600 Da Lock mass: 3,5-diiode-L-tyrosine	Accurate mass Identification points Isotope clusters Fragment ions	<14.6mDa	n.a.	0.1 µg/L	n.a.	Hernández et al., 2004
27 pesticides	Water	LC-QToF-MS ESI(+/-)	Accurate mass Identification points Total product ion spectra	0.6-2.9 mDa	n.a.	n.a.	n.a.	Hernández et al., 2005 b
Diclofenac phototransformation products	Water	LC-ToF-MS ESI (+/-) M/z 50-1000 Lock mass Rs. 9500	Accurate mass Elemental composition Empirical formula Isotopic clusters	< 1ppm	n.a.	n.a.	n.a.	Agüera et al., 2005
Terbuthylazine Simazine Terbutryn Terbumeton transformation products	Water	LC-QToF-MS Orthogonal Z-spray ESI (+) 50-500 Da Rs. 5000 Lock mass: 3,5-diiode-L-tyrosine	Accurate mass Identification points Isotope clusters Fragment ions	<3.5 mDa	n.a.	n.a.	n.a.	Ibáñez et al., 2004
15 pesticides	Peach	LC-oa-ToF-MS ESI(+) Dual ion source Lock mass:Leucine enkephaline	Accurate mass	<6.9 ppm	0.025-0.5 µg/mL	0.02-0.2	n.a.	Sage et al., 2002
15 pesticides	Pepper Broccoli Tomatoe Orange Lemon Apple Melon	LC-ToF ESI (+) Ortogonal sprayer 50-1000 Da Rs 9500+-500	Accurate mass Elemental composition Isotopic clusters	< 2ppm	0.01-5 mg/kg	0.5-30 µg/kg	< 11%	Ferrer et al., 2005c
Carbendazim Hexaflumuron Imidacloprip Methomyl Spinsad Azoxystrobin	Tomato Lettuce Pepper Cucumber	LC-ToF-MS ESI (+) Orthogonal spayer Rs 9500+-500	Accurate mass Elemental composition Isotopic clusters	< 2ppm	0.005-1 mg/kg	1-10 µg/kg	< 5%	Ferrer et al., 2005a
16 pesticides	Solvent Pepper Broccoli Lemon Orange Melon	LC-ToF-MS	Accurate mass CID fragmentation Elemental composition Isotopic clusters	< 5.2 ppm	0.01-0.5 mg/kg	0.3-30 µg/kg	<11%	Ferrer et al., 2005b
Imazalil Prochloraz TPs	Citrus	LC-ToF ESI (+) Rs: 9500 Dual nebulizer	Accurate mass Elemental composition Isotopic clusters	< 9.0 ppm	n.a.	n.a.	n.a.	Thurman et al., 2005a
Carbendazim Buprfezin Thiophanate	Tomatoes	LC-ESI-ToFv(+) LC-IT-MS	Accurate mass Elemental composition ChemIndex database Merck Index database	< 3ppm	n.a.	n.a.	n.a.	Thurman et al., 2005c

(*Continued*)

TABLE 3. (*Continued*)

Compounds	Matrix	Technique	Identification	Accuracy	Linearity	LOD	RSD	Reference
Non target chlorinated pesticides	Tomato, apple, grapes	LC-ToF-MS	No standards Full scan spectra Accurate mass Isotopic clusters Elemental composition Database	< 2ppm	n.a.	n.a.	n.a.	Garcia-Reyes et al., 2005
Carbosulfan Metabolites	Citrus	LC-QToF-MS Turbo ion spray source 50-500 Da	Accurate mass Elemental composition Fragment ion	n.a.	n.a.	4-23 µg/kg	7-16%	Soler et al., 2006
Diazinon, metabolites, transformation products	Standards	LC-QToF-MS ESI (+/-) Rs. 5000 60-600 Da Lock mass: 3,5-diiode-L-tyrosine	Retention time Accurate mass Elemental composition Fragment ions	< 2.9 mDa	n.a.	n.a.	n.a.	Ibáñez et al., 2005

n.a., information not available.

More selective is LC-Q-ToF-MS because the accurate mass measurement of product ions allows to remove ambiguities. Soler et al. compare four LC-MS systems, equipped with single quadrupole, QqQ, QIT, QToF to evaluate the performance for the analysis of carbofuran and its metabolites. Although quantitative results were best with QqQ, QToF was the most selective technique because the accurate mass of product ions allowed ambiguities to be removed (Soler et al., 2006). The LODs obtained in this study were in the range of 0.04–0.4 µg/kg for QqQ and from 4 to 23 µg/kg for QToF-MS with a relative standard deviation below 7% and 16% for QqQ and QToF, respectively. Using matrix-matched standards and LC-ToF-MS, Ferrer et al. reports LOD (using a signal to noise ratio of 3) between 0.9 and 30 µg/kg for several pesticides (Ferrer, García-Reyes, & Fernandez-Alba, 2005b; Ferrer et al., 2005c). Table 2 specifically reports the LOD calculated from pepper extracts and varied from 0.3 to 30 µg/kg.

Confirmation of pesticides in water by QqQ and QToF were also compared according to the number of identification points earned (Hernández et al., 2004). The QqQ allowed the confirmation of pesticides at ng/L level with four and five identification points whereas with ToF instruments, confirmation was only possible for those compounds that are sensitive enough, have a typical isotopic pattern or provide easy in-source fragmentation.

B. Screening of Non-Target Pesticides

For food quality controls and to provide a safe fruit and vegetable market, it is necessary to screen and identify non-target

TABLE 4. Comparison of LC-ToF-MS and LC-MS results for the analysis of pesticide residues in certified fruit samples (Ferrer et al., 2005, with permission)

SAMPLE / pesticide	TestQual Value[a]	LC/TOF/MS[a]	LC/Q/MS[a]
APPLE			
Carbendazim	0.32	0.21	0.17
Methomyl	0.27	0.32	0.24
STRAWBERRY			
Carbendazim	0.30	0.25	0.27
Hexaflumuron	0.22	0.27	ESI(-)?
Imidaloprid	0.09	0.10	0.12
Methomyl	0.58	0.53	0.45
Spinosad	-	0.13	0.15
Azoxystrobin	-	0.14	0.17

[a] mg Kg^{-1}

TABLE 5. Calibration data for imidacloprod, acetamiprid, and thiacloprid in different matrices. Comparison with a solvent matrix.

	Matrix	Calibration Equation	R^2
Imidacloprid	Solvent	$y = 1.0 \cdot 10^7 x + 18867$	0.9993
	Tomato	$y = 1.0 \cdot 10^7 x + 172000$	0.9995
	Pepper	$y = 8.7 \cdot 10^6 x + 453000$	0.9980
	Lettuce	$y = 9.6 \cdot 10^6 x + 143000$	0.9962
	Cucumber	$y = 9.0 \cdot 10^6 x + 19100$	0.9996
Acetamiprid	Solvent	$y = 2.0 \cdot 10^7 x + 464616$	0.9974
	Tomato	$y = 2.2 \cdot 10^7 x + 444000$	0.9974
	Pepper	$y = 1.9 \cdot 10^7 x + 678000$	0.9918
	Lettuce	$y = 2.0 \cdot 10^7 x + 503000$	0.9944
	Cucumber	$y = 2.0 \cdot 10^7 x + 433000$	0.9958
Thiacloprid	Solvent	$y = 2.0 \cdot 10^7 x + 758528$	0.9953
	Tomato	$y = 2.0 \cdot 10^7 x + 1150000$	0.9936
	Pepper	$y = 1.9 \cdot 10^7 x + 1040000$	0.9922
	Lettuce	$y = 1.9 \cdot 10^7 x + 858000$	0.9904
	Cucumber	$y = 1.8 \cdot 10^7 x + 678000$	0.9930

pesticides, many times unexpected or not included in routine monitoring protocols because of the different speeds on introduction and approval of new substances in each country. Screening methods are aimed to quickly detect the presence of one or more compounds in a quantitative or semiquantitative manner at a specified concentration limit (Malato et al., 2003; Hernández et al., 2005a). Screening methods should be highly efficient and capable to discriminate samples containing pesticide residues. For screening purposes, the principles of identification of chemical compounds using mass spectrometry are provided by the European Community (EU Commission, 2002), FDA (US FDA, 2003), and WADA (WADA, 2003), which are summarized in a recent review (Milman, 2005). For pesticide residue analysis in food, the EU guideline SANCO 10476/2003 which describes the analytical quality control requirements should also be taken into account. For the analysis of pesticides regulated in Directive 96/23/EC, the identification criteria set up by the European Commission (Decision 2002/657/EC, 2002) should be considered. This document indicates the need to obtain three identification points to confirm the presence of organic drugs in food (four if they are banned substances). Most LC-MS techniques are able to meet this EU criterion. When using single quadrupole MS, three or four ions are needed for confirmation whereas with MS^n (IT or MS/MS), the choice of one precursor and two or three product ions would be needed (Careri et al., 2002; Blasco, Font, & Picó, 2005). In the case of ToF-MS, accurate mass of the (de)protonated molecule along with characteristic fragment ions would also follow EU rules as regards to identification criteria. In addition, the ToF instruments offer the possibility to screen non-target compounds present in a sample without performing additional analysis since compared to QqQ, pre-selection of analytes is not a requisite. However, ToF provides lower sensitivity than QqQ in SRM, so identification and quantification of ultra-trace pesticide levels cannot be achieved (Hernández, Sancho, & Pozo, 2005b).

Non-target contaminants were identified in surface waters by LC-QToF-MS at 7,000 resolution, enabling the detection and identification at levels <0.25 μg/L (Bobeldijk et al., 2001). Based on accurate mass measurement, the elemental composition of precursor and product ions was calculated. Then, the calculated chemical formulae were searched against a Merck index, the Nist library, and an own database containing around 2,500 water contaminants as well as CI-CID library containing tandem MS spectra of 100 water contaminants. Target compounds were identified with errors below 8.8 ppm, and several unknown compounds were detected in the survey scan and MS/MS mode. However, it is reported that exact mass alone is not sufficient for unambiguous identification of contaminants in surface water and additional information such as retention time of standards, UV spectra, fragmentation upon CID, or in-source fragmentation is required.

LC-ToF-MS with positive ESI and working at a resolution of 9,500 using two reference masses at 121.0509 and 922.0098 *m/z* introduced at a constant flow rate of 100 μL/min was used to search empirical formulas generated through accurate mass and it was possible to identify the pesticides carbendazim, buprofezin, and thiophanate in tomato skin (Thurman, Ferrer, & Rodriguez Fernandez-Alba, 2005c). The possibility to relate the data

obtained by LC-ToF-MS and LC-IT-MS has permitted to develop a new identification scheme to detect unknown pesticides from market-place vegetables. Accurate mass was used and empirical formulas were generated with LC-ToF-MS. Following, these structures were identified with the aid of the Merck and Chemindex databases which contain 10,000 and 77,000 compounds, respectively. Then, LC-IT-MS(MS) was used to identify fragment ions and finally verification was performed with authentic standards. This procedure of unknown identification was useful to identify pesticides in complex matrices but could also be extended to other compounds.

Non-target chlorinated pesticides were determined in food using LC-ToF-MS without the use of analytical standards (García-Reyes et al., 2005). Full scan spectra and accurate mass measurements (better than 2 ppm accuracy) of the protonated molecule, together with isotope clusters lead to 1–2 elemental compositions. Together with the characteristic fragment ions of suspected species, chlortoluron, iprodione, and procymidone were identified in tomato, apples, and grapes, respectively. Confirmation and quantification was finally performed with standards.

Criteria to achieve unequivocal identification of unknowns in food extracts using LC-(Q)ToF-MS (Thurman, Ferrer, & Parry, 2002; Ferrer et al., 2004; Thurman et al., 2005a) are as follows:

1. Based on accurate mass, the elemental composition of an unknown peak is calculated using the ''elemental composition'' tool.
2. According to the isotopic pattern, the number of halogenated atoms in the molecule is calculated.
3. Search is performed in a pesticide database, obtaining identification of unknowns with a mass deviation of less than 5 ppm.
4. When a Q-ToF is used, MS^2 provide extra chemical characterization of a given molecule, increasing the identification capabilities compared to a ToF-MS.

Based on accurate mass measurements, it is obvious that several elemental compositions of pesticides are possible. The combination of different types of information obtained from Q-ToF mass analyzers such as accurate mass, neutral loss, and fragment ions yield conclusive data for compound identification. Other information such as LC retention time, characteristic halogen isotope clusters, and ultraviolet spectra from the diode array detector (DAD) can be also needed.

C. Identification of Pesticide Transformation Products and Metabolites

Within regular monitoring programs, another important aspect that should be dealt with is the identification of ''unknowns'' present in the sample which might correspond to pesticide degradation products which are of interest because of their potentially toxic properties. The use of MS and especially MS/MS permit to identify unknown pesticide transformation products (Tops) and metabolites in water and food samples to elucidate their degradability and final fate. The inherent advantage of Tofu-MS is that the sensitivity in scan mode is superior to that of quadrupoles, facilitating the detection of less abundant metabolites. The mass accuracy provided by ToF allows the assignment of a highly probable empirical formula for each compound and the differentiation between nominal isobaric compounds and the possibility of performing MS/MS spectra with accurate mass measurements for the final characterization of TPs/metabolites (Ibáñez et al., 2005).

Thurman et al. (2005a) reported the identification of prochloraz and imazalil and their main degradation products in citrus extracts using LC-ToF with an electrospray interface. Identification of parent compounds was based on the elemental composition obtained from accurate mass measurements and additional qualitative information from the high-resolution chlorine isotopic clusters of both the protonated molecules and their characteristic fragment ions. Ion trap MS/MS provided complementary information, which permitted to elucidate the structures of the degradation products. In this study, ToF accurate mass measurements were obtained using an automated calibrant delivery system using a dual-nebulizer electrospray source which introduces the flow from the outlet of the chromatograph together with a low flow of a calibrating solution which contains the internal reference masses at m/z 121.0509 and 922.0098. With such a procedure, several possible elemental composition of pesticides gave errors lower than 9 ppm.

Soler et al. used LC-QToF-MS to identify six transformation products of carbosulfan by means of identifying fragment ions and comparing the mass spectra with other mass analyzers (Soler, Mañes, & Picó, 2005). Figure 6 shows a LC-QToF-MS chromatogram corresponding to carbosulfan and its metabolites indicating that although some co-elutions appeared, they could be resolved by their differential mass. In this study, a turboIonSpray source was used using a ternary mobile phase with water, methanol, and acetonitrile with ammonium acetate, which shortened the chromatographic run in comparison to binary phases. Compared to other mass analysers, QToF was the most selective and was especially useful for the determination of metabolites in citrus fruits.

LC-ToF-MS in both positive and negative ionization modes was used to elucidate a complete range of diclofenac photo-transformation products (Agüera et al., 2005). Accurate mass spectra were acquired over a mass range of m/z 50–1,000 and at a 9,500 resolution (according to the reference mass at 922.0098 m/z). The elemental composition of seven compounds permitted to elucidate their structure with an error <1 ppm. With that information, the phototransformation pathways of diclofenac were established.

LC-QToF-MS was applied to study the transformation products of the pesticides terbuthylazine, simazine, terbutryn, and terbumeton in water (Ibáñez et al., 2004). Due to the high sensitivity in full scan, minor metabolites $<2\%$ of the total peak area were identified. The mass errors were <2 mDa and with the use of empirical formula, three to five degradation products were identified per compound and the degradation curves could be established over a period of 7 days.

The photodegradation and metabolism of diazinon was studied sing a LC-QToF-MS at 5,000 resolution and acquisition over a mass range of 60–600 with a multi-channel plate detector

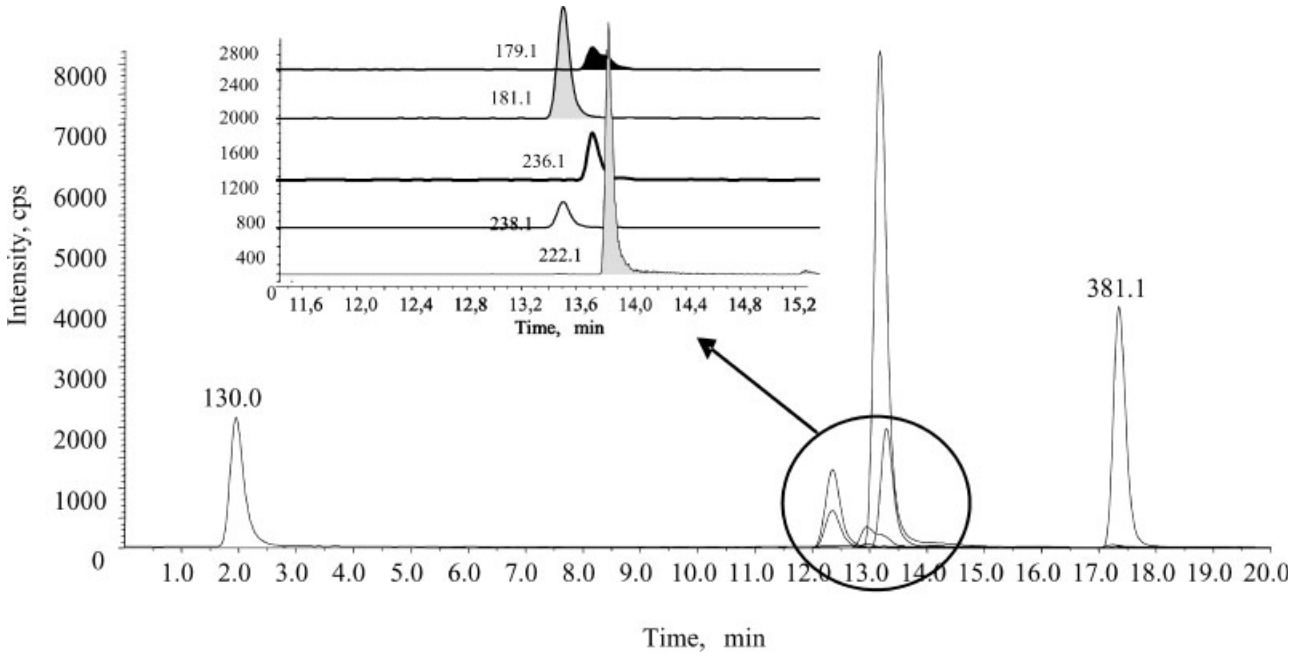

FIGURE 6. Chromatogram obtained by LC-QToF-MS corresponding to carbosulfan and its metabolites.

potential set at 2,700–2,800 V in positive and negative electrospray ionization modes, (Ibáñez et al., 2005). Nine TPs were identified by means of exact mass measurements which had an error <2.7 ppm and MS/MS spectra, and the kinetics of parent and main TPs were established. One of the main disadvantages indicated in the study of Ibañez is that unequivocal elucidation of the structure of some compounds is not feasible due to the limited understanding of the fragmentation rules in MS/MS of (de)protonated molecules. This drawback can be overcome if standards are available or otherwise, the combination of several analytical techniques might be needed.

V. CONCLUSIONS

LC-ToF-MS is not yet a well-established technique used in the field of pesticide analysis, given the scarce number of scientific papers available in the literature. The success and future applicability of LC-ToF-MS instruments for trace determination of pesticides in food matrices is dependant on the development of accurate and precise quantification procedures using appropriate internal standards and on the availability of compound databases and mass spectral libraries that can be searched. However, the possibility of exact mass (elemental composition) of unknown compounds in combination with the fragmentation pattern is a very valuable tool for screening polar non-target pesticides or pesticide degradation products in fruits and vegetables. In addition, the high increase in selectivity permits to achieve LOD at the low ppb level, and that will avoid many difficulties related to matrix effects and will meet the EU terms regarding identification and MRL of pesticides in food.

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REFERENCES

Agüera A, López S, Fernández-Alba A, Contreras M, Crespo J, Piedra L. 2004. One year routine application of a new method based on liquid chromatography-tandem mass spectrometry to the analysis of 10 multiclass pesticides in vegetable samples. J Chromatogr A 1045:125–135.

Agüera A, Pérez Estrada LA, Ferrer I, Thurman EM, Malato S, Fernández-Alba AR. 2005. Application of time-of-flight mass spectrometry to the analysis of phototransformation products of diclofenac in water under natural sunlight. J Mass Spectrom 40:908–915.

Benotti M, Fergusson PL, Rieger LA, Iden CR, Heine CE, Brownawell BJ. 2003. Chapter 7 in Liquid Chromatography Mass Spectrometry/Mass Spectrometry, MS/MS and Time-Of-Flight MS: Analysis of emerging contaminants, Americal Chemical Society Symposium Series 850. New York, USA: Oxford University Press, 2003.

Blasco C, Font G, Picó Y. 2005. Analysis of pesticides in fruits by pressurized liquid extraction and liquid chromatography–ion trap–triple stage mass spectrometry. J Chromatogr A 1098:37–43.

Blasco C, Fernández M, Picó Y, Font G, Mañes J. 2002. Simultaneous determination of imidacloprip, carbendazim, methiocarb and hexythiazox in peaches and nectarines by liquid chromatography-mass spectrometry. Anal Chim Acta 461:109–116.

Bobeldijk I, Viessers JPC, Kearney G, Major H, van Leerdam JA. 2001. Screening and identification of unknown contaminants in water with liquid chromatography and quadrupole-orthogonal acceleration-time-of-flight tandem mass spectrometry. J Chromatogr A 929:63–74.

Careri M, Bianchi F, Corradini C. 2002. Recent advances in the application of mass spectrometry in food related analysis. J Chromatogr A 970:3–64.

Clauwaert K, Van Bocxlaer J, Major H, Claereboudt J, Lambert W, Van den Eeckhout E, Van Peteghem C, De Leenheer A. 1999. Investigation of the quantitative properties of the quadrupole orthogonal acceleration time-of-flight mass spectrometry with electrospray ionization using 3,4-methylendi-oxymethamphetamine. Rapid Commun Mass Spectrom 13:1540–1545.

Dallüge J, Roose P, Brinkman UATh. 2002. Evaluation of a high resolution time-of-flight mass spectrometer for the gas chromatographic determination of selected environmental contaminants. J Chromatogr A 970: 213–223.

Decaestecker T, Clauwaert K, Van Bocxlaer J, Lambert W, Van den Eeckhout E, Van Peteghem C, De Leenheer A. 2000. Evaluation of automated single mass spectrometry to tandem mass spectrometic function switching for comprehensive drug profiling analysis using quadrupole-time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 14:1787–1792.

Directive 60/2000/EU of the European Commission. 2000. European Parliament and Council, 23rd October 2000.

EC, Commission Decision 2002/657/EC, August 12, 2002. Off J Eur Commun (17.8.2002) L 221/8.

Eckers C, Haskins NJ, Langridge JL. 1997. The use of liquid chromatography combined with quadrupole-time-of-flight analyzer for the identification of trace impurities in drug substance. Rapid Commun Mass Spectrom 11(17):1916–1922.

European Commission. 1999. Commisssion Directive (EC) 1999/39 of 6 May 1999. Off J Eur Commun L124:8.

Ferrer I, Abián J, Fernández-Alba A. 2005d. LC-MS II: Applications for pesticide food analysis in Chromatographic-mass spectrometric food analysis for trace determination of pesticide residues (Fernandez Alba A, editor). Wilson & Wilson' Comprehensive analytical chemistry (Barceló D, editor). pp 403–434.

Ferrer I, García-Reyes JF, Fernandez-Alba A. 2005b. Identification and quantitation of pesticides in vegetables by liquid chromatography time-of-flight mass spectrometry. Trends Anal Chem 24:671–682.

Ferrer I, Thurman EM, Fernández-Alba AR. 2005a. Quantitation and accurate mass analysis of pesticides in vegetables by liquid chromatography/time of flight mass spectrometry. Anal Chem 77:2118–2125.

Ferrer I, Thurman EM. 2003. Liquid chromatography/time-of-flight/mass spectrometry (LC/ToF/MS) for the analysis of emerging contaminants. Trends Anal Chem 22:750–756.

Ferrer I, Mezcua M, Gómez MJ, Thurman EM, Agüera A, Hernando MD, Fernández-Alba AR. 2004. Liquid chromatography/time of flight mass spectrometric analyses for the elucidation of the photodegradation products of triclosan in wastewater samples. Rapid Commun Mass Spectrom 18:443–450.

Ferrer I, García-Reyes JF, Mezcua M, Thurman EM, Fernández-Alba AR. 2005c. Multi-residue pesticide analysis in fruits and vegetables by liquid chromatography-time-of-flight mass spectrometry. J Chromatogr A 1082:81–90.

García-Reyes JF, Ferrer I, Thurman M, Molina-Díaz A, Fernandez-Alba A. 2005. Searching for non-target chlorinated pesticides in food by liquid chromatography/time of flight mass spectromatry. Rapid Commun Mass Spectrom 19:2780–2788.

Hau J, Riediker S, Vatga N, Stadler R. 2000. Determination of plant growth regulator in food by liquid chromatography-electrospray ionisation tandem mass spectrometry. J Chromatogr A 878:77–86.

Hernández F, Sancho JV, Pozo OJ. 2005b. Critical review of the application of liquid chromatography/mass spectrometry to the determination of pesticide residues in biological samples. Anal Bioanal Chem 382:934–946.

Hernández F, Ibañez M, Sancho JV, Pozo OJ. 2004. Comparison of different mass spectrometric techniques combined with liquid chromatography for confirmation of pesticides in environmental waters based on the use of identification points. Anal Chem 76:4349–4357.

Hernández F, Pozo OJ, Sancho JV, López FJ, Marín JM, Ibáñez M. 2005a. Strategies for quantification and confirmation of multi-class polar pesticides and transformation products in water by LC-MS2 using triple quadrupole and hybrid quadrupole time of flight analyzers. Trends Anal Chem 24:596–612.

Hiemstra M, de Kok A. 2002. Determination of propamocarb in vegetables using polymer based high-performance liquid chromatography coupled with electrospray mass spectrometry. J Chromatogr A 972:231–239.

Hogenboom AC, Hofman MP, Kok SJ, Niessen WMA, Brinkman UATh. 2000. Determination of pesticides in vegetables using large volume injection column liquid chromatography-electrospray tandem mass spectrometry. J Chromatogr A 892:379–390.

Holm A, Molander P, Lundanes E, Greibrokk T. 2003. Determination of rotenone in river water utilizing packed capillary column switching liquid chromatography with UV and time of flight mass spectrometric detection. J Chromatogr A 983:43–50.

Ibáñez M, Sancho JV, Pozo OJ, Hernández F. 2004. Use of quadrupole time of flight mass spectrometry in environmental analysis: Elucidation of transformation products of triazine herbicides in water after UV exposure. Anal Chem 76:1328–1335.

Ibáñez M, Sancho JV, Pozo OJ, Hernández F. 2005. Use of liquid chromatography time of flight mass spectrometry in the elucidation of transformation products and metabolites of pesticides. Diazinon as case study. Anal Bioanal Chem (in press).

Jansson C, Pihlström T, Österdahl BG, Markides KE. 2004. A new multiresidue method for analysis of pesticide residues in fruit and vegetables using liquid chromatography with tandem mass spectrometry detection. J Chromatogr A 1023:93–104.

Klein J, Alder L. 2003. Applicability of gradient liquid chromatography with tandem mass spectrometry to the simultaneous screening for about 100 pesticides in crops. J AOAC Int 86:1015–1037.

Lacorte S, Barceló D. 1996. Determination of parts per trillon levels of organophosphorus pesticides in groundwater by automated on-line liquid-solid extraction followed by liquid chromatography/atmospheric pressure chemical ionization mass-spectrometry using positive and negative modes of operation. Anal Chem 68(15):2464–2470.

Liapis KS, Aplada-Sarlis P, Kyriakidis N. 2003. Rapid multi-residue method for the determination of azinphos methyl, bromopropylate, chlorpyrifos, dimethoate, parathion methyl and phosalone in apricots and peaches by using negative chemical ionization ion trap technology. J Chromatogr A 996:181–187.

Maizels M, Budde WL. 2001. Exact mass measurements for confirmation of pesticides and herbicides determined by liquid chromatography/time-of-flight mass spectrometry. Anal Chem 73:5436–5440.

Malato S, Albanis L, Piedra A, Aguera A, Hernando D, Fernandez Alba A. 2003. Chapter 5 in Liquid Chromatography Mass Spectrometry/Mass Spectrometry, MS/MS and Time-Of-Flight MS: Analysis of emerging contaminants, Americal Chemical Society Symposium Series 850, New York, USA: Oxford University Press, 2003.

Milman B. 2005. Identification of chemical compounds. Trends Anal Chem 24:493–508.

Pergantis SA, Wangkarn S, Francesconi KA, Thomas-Oates JE. 2000. Identification of arsenosugars at the pg level using nano-electrospray quadrupole-time of flight. Anal Chem 72:357–366.

Picó Y, Blasco C, Font G. 2004. Environmental and food applications of liquid chromatography-tandem mass spectrometry in pesticide residue analysis: An overview. Mass Spectrom Rev 23:45–85.

Picó Y, Font G, Moltó JC, Mañes J. 2000. Pesticide residue determination in fruit and vegetables by liquid chromatography-mass spectrometry. J Chromatogr A 882:153–173.

Reynolds SL. 2005. Sample handling and clean up procedures I in Chromatographic-mass spectrometric food analysis for trace

determination of pesticide residues (Fernandez Alba A, editor). Wilson & Wilson's Comprehensive analytical chemistry (Barceló D , editor). pp 75–106.

Riediker S, Obrist H, Varga N, Stadler RH. 2002. Determination of chlormequat and mepiquat in pear, tomato, and wheat flour using on-line solid-phase extraction (Prospekt) coupled with liquid chromatography-electrospray ionization tandem mass spectrometry. J Chromatogr A 966:15–23.

Sage AB, McCullagh M, Taylor MJ, Hunter K. 28th-31st May 2002. Multiresidue screening of pesticides in fruit extracts bt LC/MS—A comparative study using OA-ToF and tandem quadrupole mass spectrometry. Rome, Italy: poster presented at EPRW.

Soler C, Mañes J, Picó Y. 2005. Comparison of liquid chromatography using triple quadrupole and quadrupole ion trap mass analyzers to determine pesticide residues in oranges. J Chromatogr A 1067:115–125.

Soler C, Hamilton B, Furey A, James KJ, Mañes J, Picó Y. 2006. Comparison of four mass analyzers for determining carbosulfan and its metabolites in citrus by LC/MS. (submitted to J Chromatography A).

Steen RJCA, Bobeldijk I, Brinkman UATh. 2001. Screening for transformation products of pesticides using tandem mass spectrometry scan modes. J Chromatogr A 915:129–137.

Taylor MT, Hunter K, Hunter KB, Lindsay D, Le Bouchllec S. 2002. Multiresidue method for rapid screening and confirmation of pesticides in crude extracts of fruits and vegetables using isocratic liquid chromatography with electrospray tandem mass spectrometry. J Chromatogr A 982:225–236.

Thurman EM, Ferrer I, Fernandez-Alba A. 2005b. LC-MS I. Basic Principles and technical aspects of LC-MS for pesticide analysis in Chromatographic-mass spectrometric food analysis for trace determination of pesticide residues (Fernandez Alba A, editor). Wilson & Wilson's, Comprehensive analytical chemistry (Barceló D, editor). pp 369–400.

Thurman EM, Ferrer I, Parry RJ. 2002. Accurate mass analysis of ethenesulfonic acid degradates of acetolachlor and alachlor using high performance liquid chromatography and time-of-flight mass spectrometry. J Chromatogr A 957:3–10.

Thurman EM, Ferrer I, Rodriguez Fernandez-Alba A. 2005c. Matching unknown empirical formulas to chemical structure using LC-MS ToF accurate mass and database searching: Example of unknown pesticides on tomato skins. J Chromatogr A 1067:127–134.

Thurman EM, Ferrer I, Zweigenbaum JA, García-Reyes JF, Woodman M, Fernández-Alba AR. 2005a. Discovering metabolites of post harvest funguicides in citrus with liquid chromatography/time-of-flight mass spectrometry and ion trap mass spectrometry. J Chromatogr A 1082: 71–80.

US FDA. 2003. Mass Spectrometry for confirmation of the identity of animal drug residues. Guideance for industry. Rockville, MD, USA: US FDA Center for Veterninary Medecine.

Van Bocxlaer JF, Vande Casteele SR, Van Poucke CJ, Van Peteghem CH. 2005. Confirmation of the identity of residues using time-of-flight mass spectrometry. Anal Chim Acta 529:65–73.

WADA. 2003. Technical Document TD2003IDCR, version 1.2.

WHO. Residues of pesticides in foods and animals feeds,Vol. 2, 2nd edition. Codex Alimentarius Commission, 2000.

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