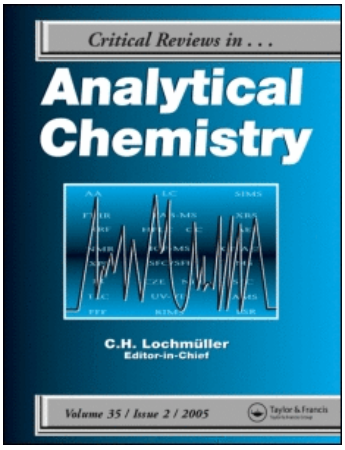

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The Role of the Liquid Chromatography-Mass Spectrometry in Pesticide Residue Determination in Food

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The use of liquid chromatography (LC) in pesticide residue determination was usually limited to groups of compounds or single compounds for which no suitable gas chromatographic (GC) conditions were available. However, recent developments have significantly enlarged the LC scope in this field of analysis. One of the most important advances was the on-line coupling of efficient LC separation with mass spectrometry detectors (LC-MS and LC-MS/MS) that makes this technique an excellent method for the determination of pesticides and their transformation products in complex matrices such as food. This review considers the application of LC-MS/MS in this field. Emphasis is placed on the tandem MS applications: advantages of the technique; the sensitive and unequivocal confirmation of the presence of pesticides in food; and, important factors affecting the performance of LC-MS/MS instruments, like the type of mass analyzer or the ionization source design which would be discussed on the particular framework of pesticide and their metabolite analysis. This review also highlights a number of problems associated with the LC-MS/MS analysis of pesticides such as the matrix effects that make quantification difficult.

Keywords liquid chromatography, tandem mass spectrometry, atmospheric pressure ionization sources, pesticides, food

INTRODUCTION

Since its introduction in the late 1950s, gas chromatography (GC) (1) has been one of the most important and widely applied analytical techniques in modern chemistry based on a favorable combination of high selectivity and resolution, good accuracy and precision, wide dynamic concentration range and extraordinary sensitivity (2, 3). Traditionally, GC has been the ultimate "standard" analytical technique in research, development, and quality control in many industries, especially petrochemical manufacturing, in environmental (4), food (5), and drug quality control and in forensic analysis (6, 7). Within these fields, the preservation of human health from exposure to pesticide residues in food remains a major objective of food safety. Pesticide analysis in food samples has been, following the above-mentioned trend, usually carried out by means of multi-residue methods that apply GC as the preferred techniques because many of these compounds are low polar, thermally stable and volatile.

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However, this bucolic situation was awkward when new active ingredients that, because of their physicochemical properties such thermal instability and polarity, were not amenable to GC started to appear in the industry of pesticide formulations. Most of these novel compounds can be efficiently separated by liquid chromatography (LC) (8) without a preceding laborious derivation step. This fact, in addition with some remarkable developments, has turned this technique into a conventional and routine determination method (9–11).

Currently, pesticides comprise more than 1000 active ingredients, which have been and are currently formulated in thousand of different commercial products (12). They include a variety of compounds, mainly insecticides, herbicides and fungicides, with very different physico-chemical characteristics and large differences in polarity, volatility and persistence. For this reason, GC and LC have always been considered complementary techniques (13–16).

Most LC methods use common ultraviolet (UV), diode array detection (DAD), fluorescence, or electrochemical detection, which are occasionally combined with postcolumn derivatization. However, these procedures may not be selective or sensitive because of the variety and complexity of matrices and low concentrations of pesticide residues present in them (17). The

introduction into the market of robust and easy-operating LC-mass spectrometry (MS) instruments provides a new way for analyzing pesticides more efficiently (18). There is a clear trend to increase the number of applications of LC in pesticide residue analysis, both in specific/individual and in multiresidue methods (19).

This trend was caused by the recent improvements in LC-MS/MS instrumentation that have increased sensitivity, selectivity and robustness of the methods enabling the increase in the number and diversity of pesticides that can be included in a single analysis (20). The use of this technique allows, in many cases, one to reduce the sample pre-treatment, even facilitating the direct injection of the extracts (21).

Although the LC-MS, advantages such as the simplification of sample preparation steps and the high sensitivity and selectivity that facilitates the analysis of target analytes at low concentrations, the use of single MS still has severe drawbacks. The most important is the limited fragmentation of the molecules that can induce to uncertainty in pesticides confirmation. The next one is the matrix effect caused for coeluting undetected components causing suppression or enhancement in analyte ionization that affect both quantitation and detectability of the pesticide residues in 9 real sample (22, 23).

This article reviews several recent applications of LC-MS and LC-MS/MS techniques for the determination of pesticide residues in food. A brief account of the various liquid separation, ionization sources and MS techniques, as applied to pesticides is presented. Important aspects such as the occurrence of pesticides, and their transformation products, and the difficulties of analyzing complex matrices are discussed, with emphasis on the quantification, identification and confirmation of the analytes in food.

LIQUID CHROMATOGRAPHIC SEPARATION

Pesticides can be classified according to their polarity in neutral and ionic pesticides. The simultaneous multi-residue determination of the former pesticides is commonly feasible. On the contrary, ionic pesticides require specific and particular methods.

Neutral pesticides are mainly separated by reversed-phase liquid chromatography (RP-LC), which covers more than 95% of applications. In RP-LC, medium polarity and polar pesticides are separated according to the differences in hydrophobicity by partitioning between an apolar stationary phase and a polar mobile phase.

The mobile phase is a critical issue because it influences both the chromatographic separation and the mass spectrometric response of the analyte. The most common solvents used as mobile phase in LC/atmospheric pressure ionization (API)-MS are gradient mixtures water, methanol and/or acetonitrile. The amount of organic modifiers can increase or decrease the ionization efficiency depending on the interface. The efficiency of the electrospray ionization (ESI) process depends on the conductivity and surface tension of the liquid being nebulized. When the conductivity, directly related to the dielectric constant of the solvent, is too high (i.e., highly aqueous) it is difficult to produce a stable spray and to vaporize the droplets formed by the action of the high voltage and nebulizing gas. Because the surface tension of water is much higher than the surface tension of methanol or acetonitrile, the sensitivity is reduced when using more than 70–80% of aqueous mobile phase. The aqueous-organic ratio is more significant when working at high flow rates since there is more solvent to be nebulized and vaporized. A very high organic content may also decrease the sensitivity, especially if an additive is not used, because the conductivity of pure organic solvent is lower. A small percentage of water in the mobile phase aids the droplet formation. Commonly, higher organic modifier percentages improve ionization in ESI and worsen it in atmospheric pressure chemical ionization (APCI). Although solution chemistry is not as critical in APCI as in ESI, the solvent properties still need to be considered for the best performance. Protic solvents, such as methanol, improve positive ionization.

The effect of methanol and acetonitrile as modifiers in the mobile phase has been tested by Jansson et al. (24) for more than 50 pesticides in a wide variety of fruits and vegetables. For all pesticides included in this study the signal was normally much higher in methanol than in acetonitrile. Similar results have been reported earlier for different groups of pesticides (9, 25). For most compounds the signal in acetonitrile was in the range of 10–40% compared to the signal in methanol, but for some pesticides the signal was even lower than 10%. Because gas phase basicity or acidity (proton affinity) of the organic solvent can favor positive ionization or negative ionization modes, the use of methanol instead of acetonitrile is encouraged. In the study reported by Jansson et al. (24) all the pesticides can not be detected at a concentration equivalent to 0.01 mg/kg when using acetonitrile instead as the modifier.

The pH of the mobile phase determines the ionization state of the analytes when working with acids, bases or amphoteric species, and therefore affects the response in LC/API-MS. The mobile phase pH also determines chromatographic selectivity for ionizable compounds.

Buffers incorporated in to the mobile phase for chromatographic purposes should be volatile to avoid problems with the MS interface; buffers usually consist of acetic acid, formic acid, ammonium acetate, ammonium formate or ammonia (26–28). The signal of analytes in LC-MS is normally affected by the ionic strength, so that the highest signal is achieved at the lowest ion strength. However, because the characteristics of the samples analyzed can influence the signal by altering the ionic strength it is important to use a buffer with sufficient buffering capacity to stabilize the system. Most of the reported mobile phase to separate and determine pesticide residues achieves a compromise in this sense using up to 10 mM ammonium formate. The higher ionic strength contributes to a more stable system both for retention and signal. In addition, the addition of volatile buffers, such as ammonium formate-formic acid or

acetic acid-ammonium acetate, improve the signal intensity of the basic analytes and the presence of the ammonium cation helps to reduce the formation of the sodium adduct in favor of the protonated molecule or the ammonium adduct, which are more easily ionizable than the sodium adduct.

The specifity of LC-MS decreases the interest in obtaining good chromatographic separation because of the mass selectivity and distinctive fragmentation patterns that achieve proper identification of the compound, even if it is co-eluted with other compounds. Most stationary phases are based on silica that has been chemically modified with octadecyl (C_{18} or ODS) or octyl (C_8 or OS) (29–31) chains. However, chromatographic separation is still important in some cases, which in turn are directly related to the vast number (approximately 600) of different stationary phases with available. This facilitates the solution of many separation problems simply by selecting appropriate stationary phases with different selectivities to allow polar analyte separations or to improve peak shape of basic compounds, on the

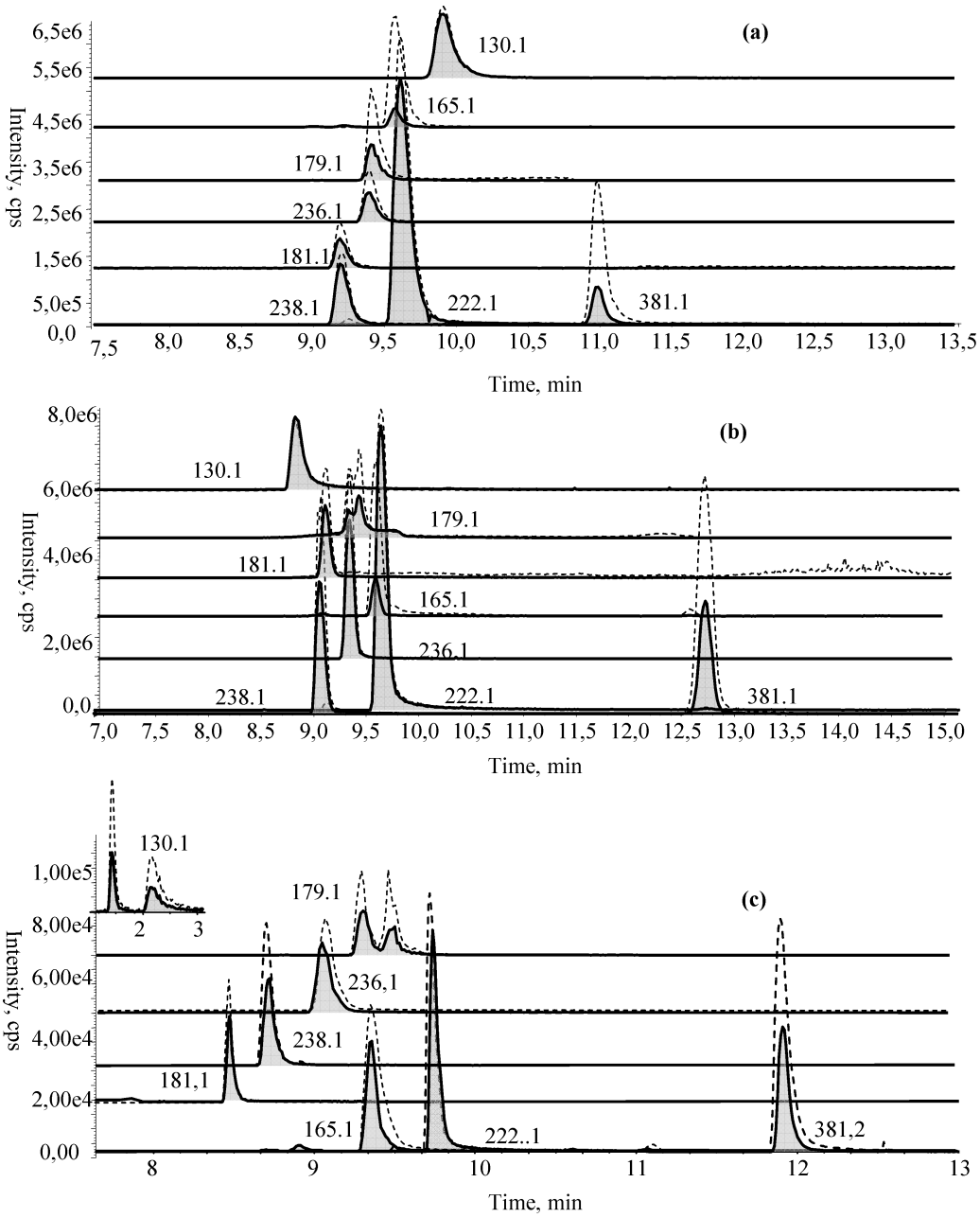

FIG. 1. Chromatograms obtained employing (a) Jupiter C_5, (b) Max RP and (c) Zorbax Bonus RP analytical column for the determination of carbosulfan and its main metabolites. Reproduced from (32), with permission from Elsevier B.V., Copyright 2006.

other hand, identifying the most suitable column for a specific purpose can be far from straight forward. A further complicating factor is the large groups of nominally identical materials that often show very different chromatographic properties.

New alternatives to conventional non-polar alkyl bonded phases have been introduced; the most popular are the embedded and encapped phases. These are modifications of the traditional C_{18} phase, with the addition of a functional non-polar or polar group, usually an amide or carbamate group, either within (embedded) or at the end (endcapped) of the alkyl chain, thus modifying the selectivity in comparison to conventional alkyl bonded phases.

An interesting example can be found in the case of two metabolites of the pesticide carbofuran, 3-ketocarbofuran and 3-keto-7-phenolcarbofuran, because in-source fragmentation of the former produces the precursor ion of the latter. Soler et al. (32) compared the performance of six different columns to choose the most appropriate. Poor peak shapes and deficient resolution were observed using most of the column checked. Figure 1 depicts the most outstanding examples of this study obtained using the Jupiter C_5, Max RP and Zorbax Bonus RP analytical columns. Figure 1a illustrates the complete peak overlap obtained for most of the tested columns, which were designed for the analysis of polar analytes. Figure 1b shows the incomplete resolution of both peaks obtained using the MAX RP column, which was designed for sharp peak shape of basic compounds at neutral pH. The extracted ion chromatogram (XICs) show in Figure 1c presents resolved peaks for the 3-keto metabolites: 9.12 minutes (3-ketocarbofuran) and 9.68 minutes (3-keto-7-phenolcarbofuran) using the analytical column Zorbax Bonus-RP. This column, with an embedded amide linkage in the C14-alkyl chain, is also targeted to improve the peak shape for challenging basic compounds. It should be noted that both LC columns are able to provide some separation of the 3-keto metabolites, MAX-RP and Zorbax bonded RP are prepared to reduce the interactions of the basic analytes with the bonded phases.

Ionic pesticides are mainly separated by two mechanisms — RP-LC using an ionic pair and ion-exchange liquid chromatography (IELC).

The ion pair reagents are added to the mobile phase for improving chromatographic behavior and peak shape of the molecules ionized in solution (33, 34). For analytes present as anionic forms, such as fosetyl, tributyl- or triethylamine are used as counter ions; for those existing as the cationic forms, such as diquat, paraquat, chlormequat, or cyromazine, heptabluorobutiric acid is added. The ion pair reagent added to the mobile phase must also be volatile.

IELC has also been used to separate ionic pesticides such as ammonium quaternary herbicides. An example of this is the determination of chloromequat residues in pears (35) employing a cation-exchange column. The ionic nature of chloromequat makes it an excellent candidate for IELC and for detection by MS. In developing the chromatographic procedure, the main goal is to achieve a good retention of selected compound while separating it from matrix constituents. For this purpose, for the determination of chloromequat in pears different eluents were tested to obtain well-resolved signals; 20 mM H_2SO_4 in 4% acetonitrile provided complete separation with elution of chlormequat at 8 minutes. There are three samples shown in Figure 2: one pear sample free of chlormequat, one spiked with 3 mg/kg and one naturally contaminated with 7 mg/kg. Some minor peaks appear in the chromatogram before and after the chloromequat

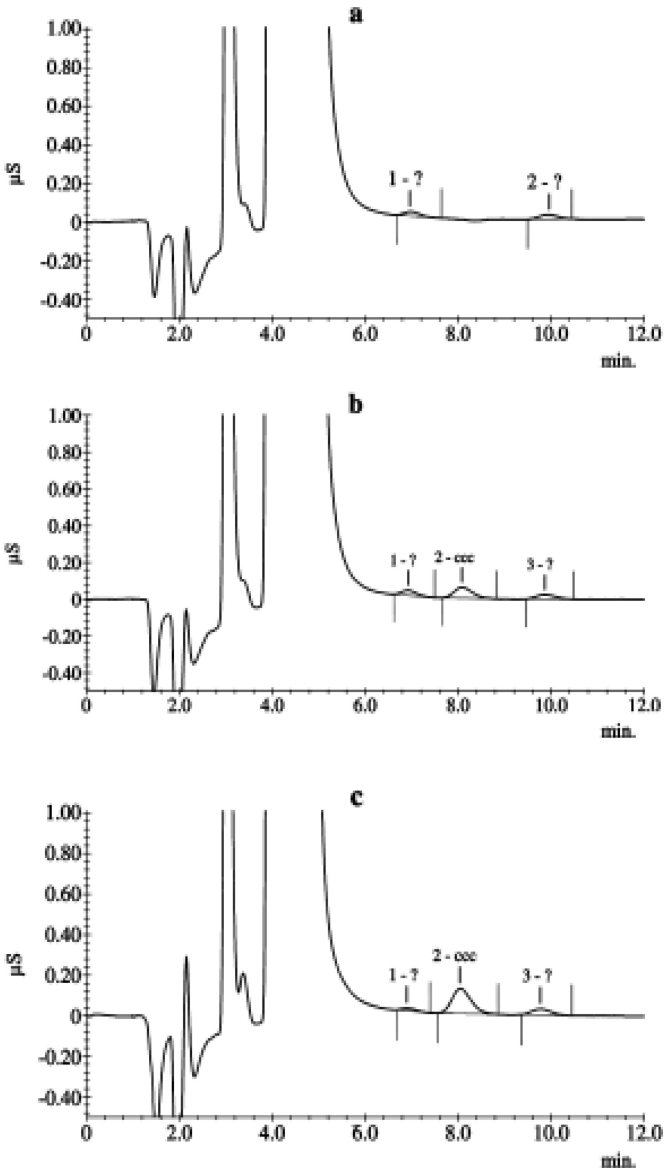

FIG. 2. IC determination of chlormequat (ccc) in blank pear matrix (a), pear sample fortified with chlormequat (3 mg/kg) (b) and pear contaminated with chlormequat (7 mg/kg) (c). Eluent: 20 m*M* H_2SO_4 in 4% acetonitrile, 1.0 ml/min; detection, conductivity (mS); columns, IonPac CG12A and CS12A. *Reproduced from (35), with permission of Elsevier B.V. Copyright, 2001.*

peak, even in a sample free of pesticides, but these peaks do not disturb the target compound analysis becoming a convenient method for the analysis of this ammonium quaternary pesticide in pears.

INTERFACING SYSTEMS

Actually, the large majority of pesticides analyses are carried out using either ESI or APCI interfaces. Atmospheric pressure photoionization (APPI) was commercialized just few years ago but is not yet largely used.

ESI is a soft ionization technique, appropriated for pesticides that are ionized in solution or that have a high molecular weight (up to 600 u). The spray is formed by electrically charging the liquid to a very high voltage. The charged liquid in the nozzle becomes unstable as it is forced to hold more and more charge until it reaches a critical point at which it can hold no more electrical charge; at the tip of the nozzle, the droplets explode by coulomb repulsion into highly charged molecules. The capillary is held a at high voltage in atmospheric pressure to generate the spray. Sometimes the spraying is supported by a make-up flow, which allows the use of higher flow rates. Liquid-phase chemistry plays a key role in the ion formation on ESI (36–41).

In APCI, a stable spray is generated by heating an aerosol from the liquid effluent of the LC with a sheathflow of gas at atmospheric pressure. Ions are generated by a corona discharged in the spray, forming chemical ionization plasma. APCI is better suited for non-ionic pesticides of moderate molecular weight. Chemical ionization occurs in the vapor state to form the $[M+H]^+$ ion. The corona-discharge needle in the APCI source produces a stream of electrons, which ionizes the solvent of the mobile phase. According to the current theory on APCI ionization, in the positive ionization (PI) mode the $CH_3OH_2^+$ and H_3O^+, present in the vapor state, transfer protons to the weakly basic pesticides in the vaporized state in line with their proton affinity, whereas in the negative ionization (NI) mode, the electronegative compounds attach an electron and become negatively charged. APCI interface can produce thermal degradation of thermolabile compounds.

The selection of the most appropriate ionization source for the analysis of pesticide residues depends on the types of pesticides investigated as well as on the evolution of source geometry for different instruments, brands and types. When ESI-MS is interfaced to LC, the interface is designed to work with high flow rates.

Thurman et al. (42) evaluated APCI and ESI for the LC-MS determination of over 75 pesticides and degradation products. A diagram called the "ionization continuum" shows that proton affinity in the gas phase and polarity in solution (pKa) are useful for selecting APCI or ESI (Figure 3). The various classes of pesticides are plotted on the diagram according to whether they ionize in PI, in NI, or in both modes. Neutral and basic pesticides (carbamates, phenylureas, triazines) are more sensitive using APCI (especially in positive ion mode) while cationic and anionic herbicides (bipyridilium ions, sulfonic acids, phenoxy acids, nitrophenols and bentazone) are best ionized with ESI (specially in negative ionization mode). There are a number of studies using the same interface design as that of Thurman et al. that confirm and corroborate the conclusions reported by these authors.

In contrast, in a study of a liquid LC-MS/MS multi-method for more than 200 pesticides analysis, the ESI interface was on average 20 times more sensitive compared to the APCI interface

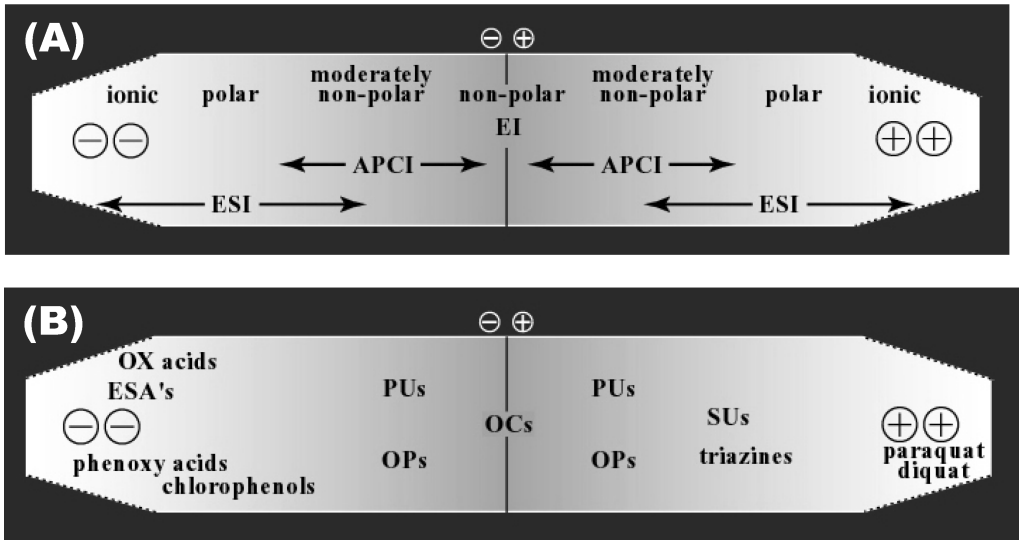

FIG. 3. Ionization-continuum diagram (A) showing the regions of effective usefulness of the various interfaces for LC-MS including APCI, ESI, and electron impact ionization (EI) and (B) for pesticides. Key: ESA, ethanesulfonic acid degradate of the chloroacetanilide herbicides; PU, phenylurea herbicides; OP, organophosphate insecticides; Carb, carbamate insecticides; OC, organochlorine insecticides; SU, sulfonylurea herbicides. *Adapted from (42), with permission from American Chemical Society, Copyright 2000.*

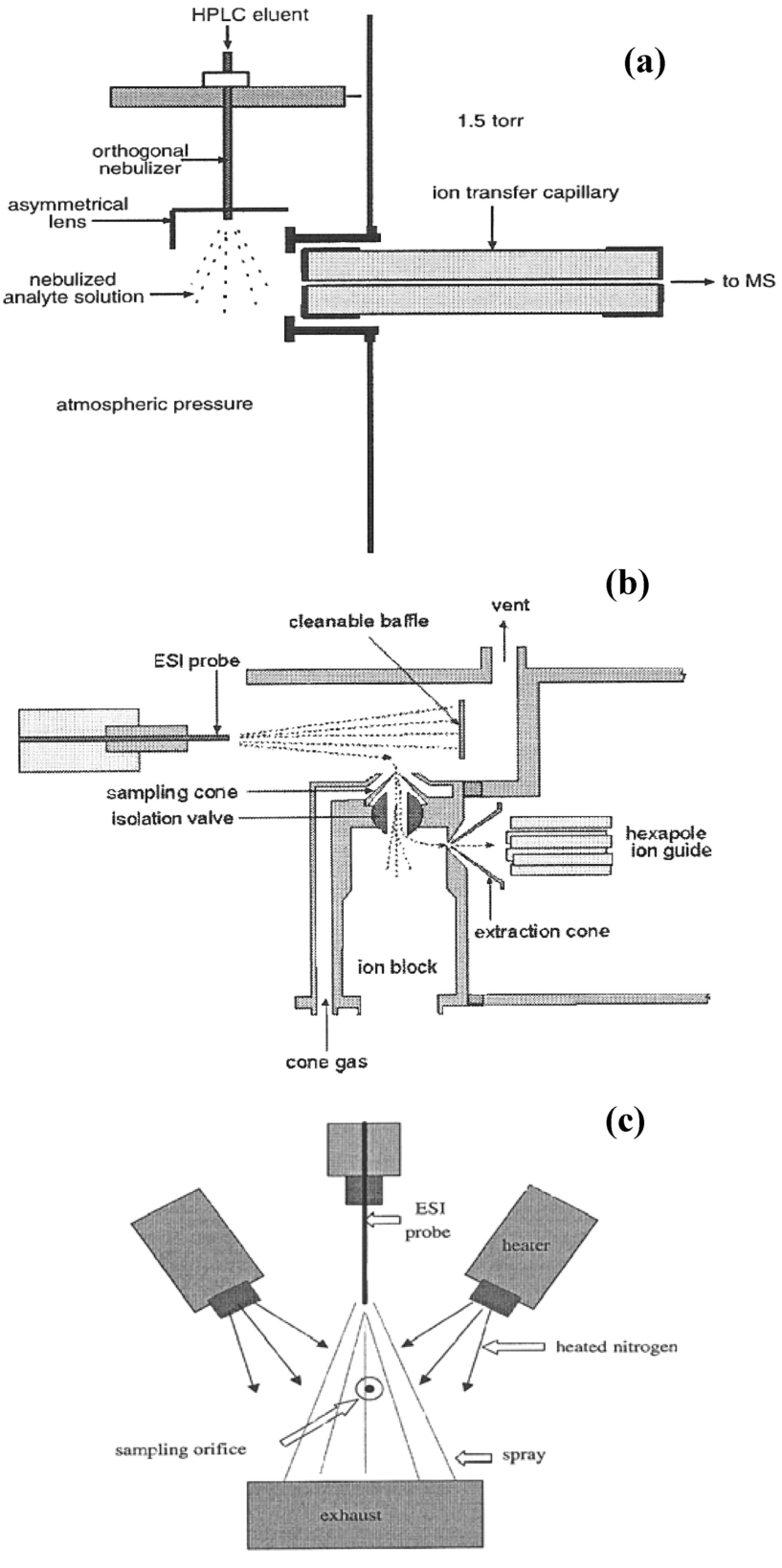

FIG. 4. Schematics of the configuration of three different design sources (a) ESI by Agilent technologies, (b) Zspray^{TM} by Water/Micromass, and (c) Turbo V^{TM} by MDS SCIEX.

(43). This study is also corroborated by that of Jansson et al. (24), who in a search for the most appropriate conditions to optimize the MS systems for analysis of 57 pesticide residues, examined different ionization techniques. The comparison resulted in 10–20 times higher response in ESI than in APCI for all of the tested pesticides. Similar results are reported by Hernndez et al. (19). Of the initially 60 selected pesticides, two (formetanate and dithianon) were found to be non-easily ESI ionizable. Only in the case of dithianon, the use of APCI in negative mode was found to be much more appropriate than ESI.

These divergences can be explained by the differences in the source design of the MS manufacturers involved in those studies (Agilent technologies, Water/Micromass, and MDS SCIEX). The schematics of the configuration of the three different sources are shown in Figure 4. The three sources sample orthogonally from the spray plume to minimize contamination. The study reported by Thurman et al. (42) was carried out with the Agilent Technology source, which incorporated an additional asymmetrical lens. Their purpose is to help initiate and sustain electrospray. The study of Klein and Alder (43) uses the TurboIonSpray source developed by MDS SCIEX, in which heated nitrogen gas that is released from a unit external to the sprayer is used to assist evaporation of the spray droplets at atmospheric pressure. This allows it to vaporize the large amounts of solvent emerging from the sprayer as efficiently as possible. Finally, the other two reported studies using the Water/Micromass Zpray interface. The spray is first sampled orthogonally through the sampling cone into a low pressure chamber. An extraction cone (skimmer) is oriented at a right a angle relative to the axis of the spray to sample for a second time the next differentially pumped vacum stage. The double orthogonally sampling systems prevent solvent and neutral molecules from entering the analyzer, resulting in reduced chemical background. These differences can explain the contradictory results reported by the different authors.

APPI has been scarcely applied to the determination of pesticide residues. All of the studies compared it with the APCI interfaces because fundamentals of both sources are similar. The difference is that APPI uses photons in the vacuum UV region instead of a discharge of electrons. The principal mechanism of APPI of a molecule (M) is photo absorption and electron ejection to form the molecule radical $[M]^+$, which extract an H- atom from the water vapor or protonic solvent to form a protonated molecule $[M + H]^+$. Pesticides, which show lower first ionization potential than the energy of the photons, are ionized.

Takino et al. (44) optimized APPI parameters for the determination of 22 carbamates including their metabolites in grapes and onions. As show in Figure 5, both sample matrices led to alterations in the chromatograms, including some additional peaks and rise in the base line. However, these additional peaks caused no interference, because they were well separated for the peaks of all carbamates. Futhermore, average changes in retention time and peak intensity of all carbamates obtained from matrix-matched standard solutions prepared from analyte-free sample were less than 1 and 10%, respectively. These results pointed out that APPI is a very useful technique for high-throughput

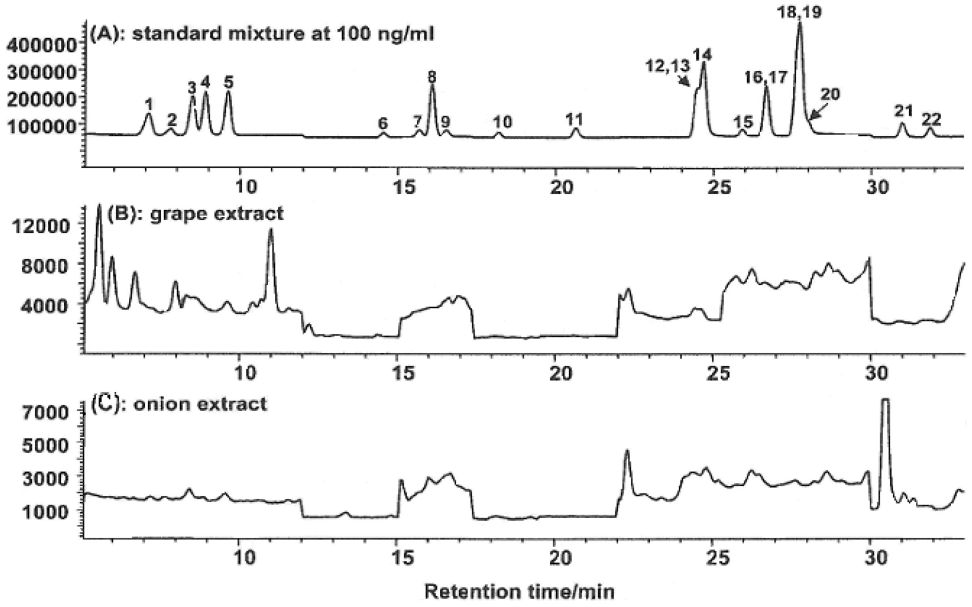

FIG. 5. Total ion chromatograms of standard mixtures at 100 ng/mL (A), a grape extract (B), and an onion extract (C) by SIM mode. Peaks: 1, butocarboximsulfoxide; 2, aldicarbsulfoxide; 3, butocarboximsulfone; 4, aldicarbsulfone; 5, oxamyl; 6, thiofanoxsulfoxide; 7, thiofanoxsulfone; 8, methiocarbsulfoxide; 9, dithiocarb; 10, methiocarbsulfone; 11, aldicarb; 12, carbofuran; 13, bendiocarb; 14, aminocarb; 15, carbaryl; 16, ethiofencarb; 17, XMC; 18, thiodicarb; 19, pirimicarb; 20, isoprocarb; 21, fenobucarb; 22, methiocarb.*Reproduced from (44), with permission of American Chemical Society, Copyright 2004*.

applications because it minimizes the need to prepare matrix-matched standards. Sensitivity is similar in both interfaces. However, an advantage of using APPI for carbamate determination in fruits and vegetables is the low matrix effect observesd that makes the use of matrix-matched standards unnecessary (44, 45).

LC-MS specificity can simplify sample preparation. However, the sample complexity and the number of compounds that co-eluted with the analytes generate a very important problem in LC-MS—the matrix effect caused by the co-extractants present in the injected sample, which may cause enhancement or suppression of the analyte signal (46). These compounds influence the effectivity of the ionization processes. The matrix effect is related to the ionization source in the API interfaces. ESI interface is more susceptible than APCI to matrix signal-suppression effects.

There are different theories on the origin of matrix effect. The most instituted one states that the organic compounds present in the sample at concentrations greater than 10^5 M may compete with the analyte for access to the droplet surface for gas-phase ion emission, and that the gas-phase basicity or acidity of co-eluting matrix components may be higher than that of the analyte of interest. Thus, the basic or acidic character of the matrix components could promote the formation of the protonated or deprotonated molecular ions of the analytes during the ionization process, causing enhancement of the signal. There is still a lot to be done to understand the mechanisms and to properly predict matrix effects.

These effect demonstrate that the response of an analyte in pure solvent standard varies significantly from that in matrix samples. They can be easily detected by comparing the response obtained from a standard solution and that from a spiked pretreated sample (post-extraction spike). The matrix effects differ from matrix to matrix to and from pesticide to pesticide being very variable. An example of matrix suppression is illustrated for the pesticide triflumisol in different matrices (Figure 6). Triflumisol peaks acquired by LC-ESI-MS in selected reaction monitoring (SRM) mode in solvent or post-extraction spiked extracts of eggplant, lettuce, and pepper are compared. Co-eluting matrix components in the pepper extracts almost completely suppress the analyte response, while for eggplant and lettuce extracts less, but still significant, suppression is observed (22). Furthermore, it is not possible to use the matrix effect for one pesticide in a specific matrix to predict the matrix effect of other pesticide in the same matrix. Jansson et al (24) report more than 2000 tests of matrix effect on pesticides, which showed that, in general, the measured matrix effect is quite small, with a mean value of 104% and relative standard deviation of 23%. Although the mean value is very nearly 100%, within this value there is a variation, mainly depending on different values. Ortelli et al. (47) also showed that matrix effect is generally low. Lemon was the only matrix that gave a significantly different response for several substances, probably due to signal suppression or stability problems in very acidic samples.

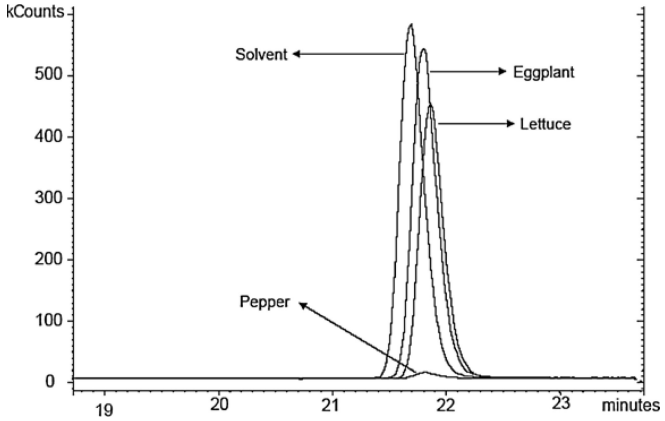

FIG. 6. Matrix suppression in the LC-ESI-MS analysis of triflumisol in different matrices. The compound was isolated by liquid extraction with ethyl acetate, evaporation to dryness, and reconstitution in methanol. *Reprinted from (22), with permission from Elsevier.*

Different actions can be taken to overcome the matrix effects in the accuracy and/or precision of the method. The sample constituents responsible for the matrix effects can be reduced or eliminated by improving the sample pre-treatment and/or the chromatographic separation (efficiency and/or resolution). This would be the best approach but it could be impracticable, especially in multi-residue analysis, where a variety of interferences may be involved. The mass spectrometric conditions can be modified by changing ESI to another ionization method (APCI or APPI) less sensitive to matrix components. However, the ideal and simplest action is to use an appropriate calibration technique that compensates for the matrix effects. The most recommended approach is the addition of isotopically labelled analytes as an internal standard in isotope dilution based methods. The merit of this approach is that both species behave identically but they can be quantified independently because each can be distinguished by its nominal mass. Although it would be the best solution, isotopically labelled standards are rather expensive (especially in multicomponent analysis) and are not always commercially available. A recently proposed alternative is the Eco-Peak technique, which involves the injection of the sample and a conventional standard in the same chromatographic run but displaced by a few seconds in time. The inconvenience is that the double peaks appear in the chromatogram, causing some problems, especially with structural isomers.

Although it is not considered the best solution, the use of matrix-matched standards is the most reported approach to compensate for the matrix effects because it is economical and easy to apply (2, 43, 47–61). These standards are prepared by spiking blank matrices (same matrix as the sample) for confirmation. In this case, the calibration takes into account the matrix effect and the final result is corrected. The drawback of this method is that there is not a general regression curve for different food

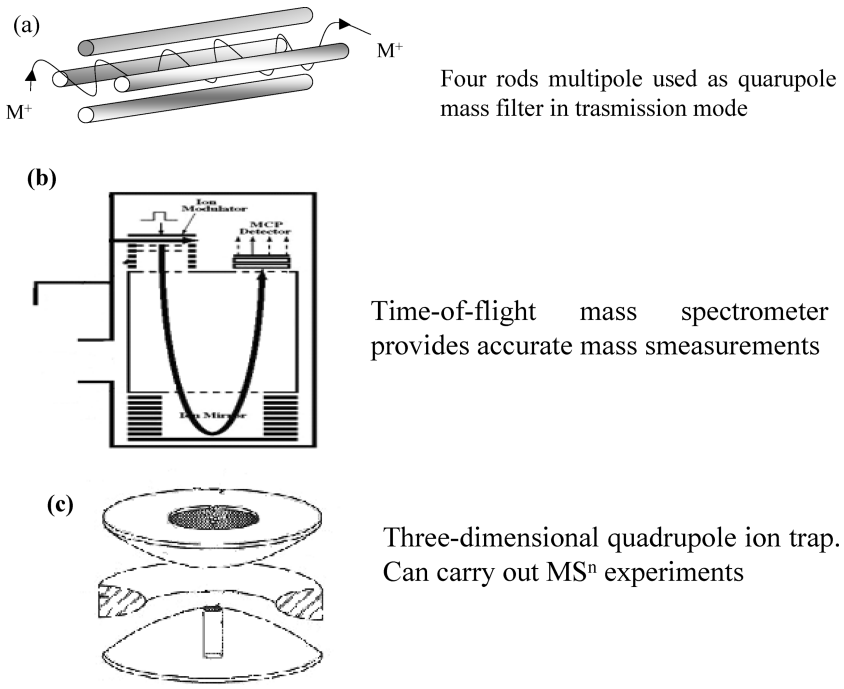

FIG. 7. Common mass spectrometers.

matrices; a particular one should be made for each matrix, which is extremely time-consuming.

MASS ANALYZERS

Single quadrupole mass analyzer has been the traditional approach to couple chromatography with MS. However, the innovations in the last year have diversified the mass analyzers available for analysis. These recently introduced mass analyzers have been divided into two different groups: the first group comprises high resolution techniques, such as time-of-flight mass spectrometry (TOF-MS), and the second group includes techniques capable of carrying out tandem mass spectrometry, such as triple quadrupole (QqQ), quadrupole ion trap (QIT) or the hybrid quadrupole time-of-flight (QqTOF). Figure 7 illustrates similarities and differences of the these analyzers.

SINGLE QUADRUPOLE

Quadrupoles are not used only as mass analyzers; they also are implemented frequently as ion transfer optics and collision cells. The quadrupole consists of four parallel metal rods. When used as mass analyzers, each opposing rod pair is connected together electrically and a radio frequency (RF) voltage is applied between one pair of rods, and the other. A direct current voltage is then super-imposed on the RF voltage. Only ions of a certain m/z will reach the detector for a given ratio of voltages; other ions have unstable trajectories and will collide with the rods. This allows selection of a particular ion, or scanning by varying the voltages.

In full-scan experiment, the DC and RF components are ramped at a constant ratio, and ions entering from the ion source are enabled to pass through the rod assembly successively, resulting in low sensitivity that makes this mode inappropriate to determine pesticides because they are commonly at trace levels in the sample.

The sensitivity is much higher in the selected ion monitoring (SIM) mode of the quadrupole (close to 100%). In this case, the DC and RF potentials are held constant, so only a specific m/z ratio can pass through. More than one m/z ratio can be detected sequentially by jumping between the different voltages. The drawback of this mode is that only a limited number of ions can be monitored for each compound because ESI and APCI were designed to provide a soft-ionization process that leads to a mass spectrum with only a few ions. The poor fragmentation of molecules is translated in deficient specificity because isobaric interferences (compounds with the same m/z relation) or multiple-component spectra are frequently observed in extracts of complex matrices such as food.

Single quadrupole instruments are usually limited to measuring intact species generated by the ionization source, resulting in less selectivity. In any case, this mass analyzer has been the most applied in pesticides analysis for many decades in different food matrices because it is relatively inexpensive, rugged, particularly sensitive in SIM mode, and ideal for trace target applications. Table 1 summarizes a large number of examples. However, either the lack of fragment ions or that those fragment ions could come from several compounds which co-eluted in the same peak in LC–MS can make structure assignments difficult (similarly,

TABLE 1
Applications of single quadrupole in pesticides analysis in food

Pesticide	Matrix	Extraction	Determination	Recovery (%)	LOD (ng/ml)	LOQ (mg/kg)	Ref.
6 pesticides of different groups	Citrus fruits	Comparison MSPD and SLE. 0.5 g sample by MSPD (C18 + dichloromethane-methanol) and 5 g sample by SLE (ethyl acetate)	• ESI positive; Luna C_{18} (150 × 4.6 mm, 5 μm); gradient MeOH-water at flow rate 0.6 ml/min. • SIM was separated in four time-windows. • Confirmatory analysis by QqQ and QIT.	57–97	5–10	0.02–0.4	(9)
Carbamates	Peaches and nectarines	50 g sample by SLE (ethyl acetate + sodium sulphate anhydrous)	• APCI positive; Luna C_{18} (150 × 4.6 mm, 5 μm); gradient MeOH-water at flow rate 0.8 ml/min. SIM of protonated molecule was used for 3 pesticides and a fragment for the last one. • For confirmation of the compounds, second ion was used.	14–108		0.02	(25)
Imidacloprid and benzimidazoles	Fruits and Vegetables	15 g sample by SLE (ethyl acetate + sodium sulphate)	• ESI positive; Zorbax SB-C_8 (150 × 4.6 mm, 5 μm); gradient MeOH-50 mM ammonium formate in water at flow rate 1 ml/min. • For confirmation of the compounds, second ion was used.	89–110	0.5–1	0.05–0.5	(62)
4 post-harvest pesticides	Citrus fruits	50 g sample by PLL (water and diethyl eter)	• APPI positive and negative; Inertsil ODS-3 (150 × 3 mm, 5 μm); gradient MeOH-water at flow rate 0.5 ml/min. Second ions obtained at high fragmentator vales were used as qualifier ion to confirm the identity.	67–100	10–50		(45)

(Continued on next page)

TABLE 1
Applications of single quadrupole in pesticides analysis in food *(Continued)*

Pesticide	Matrix	Extraction	Determination	Recovery (%)	LOD (ng/ml)	LOQ (mg/kg)	Ref.
1 herbicide, 1 fungicide and 3 fungicides	Fruits and vegetables	0.5 g sample by MSPD (C_{18}+ diclorometh ane)	• APCI positive; Luna C_{18} (2) (150 × 2 mm, 4.6 μm); gradient MeOH-ammonium formate (50 mmol/l) at flow rate 1ml/min. • SIM of most abundant ion was used for quantification. Second fragments were used for confirmation.	70–110	(5–50) × 10^3	0.02–0.2	(63)
Carbamates	Bovine milk	3 ml sample PLE (sand + water, 90°C, 5 min)	• ESI positive; Alltima C_{18} (7.5 × 4.6 mm, 5 μm); gradient MeOH-water (10 mM formic acid) at flow rate 1 ml/min. • SIM of the protonated molecule and two additional ions separed in five time windows. • Use of an IS for calibration.	85–105	1–5	3–8	(64)
Carbamates	Fruits and Vegetables	1 g sample by MSPD (Bondesil C_8+ diclorometh ane-acetonitrile)	• APPI positive; Zorbax Eclipse XDB C_{18} (150 × 3 mm, 5 μm); gradient MeOH-water (10 mM ammonium acetate) at flow rate 0.5 ml/min. • SIM of the most abundant ions ($[M+H]^+$, $[M+NH_4]^+$ or /and $[M+H\text{-}CH_3NCO]^+$).	82–106	0.5–5		(44)
Carbamates	Fruits and vegetables	2 g sample PLE (sand + water, 50°C, 5 min).	• ESI positive; Alltima C_{18} (7.5 × 4.6 mm, 5 μm); gradient MeOH-water (10 mM formic acid) at flow rate 1 ml/min. SIM of the protonated molecule and two additional ions separed in five time windows. • Use of an IS for calibration.	84–110	2–7	2–10	(65)

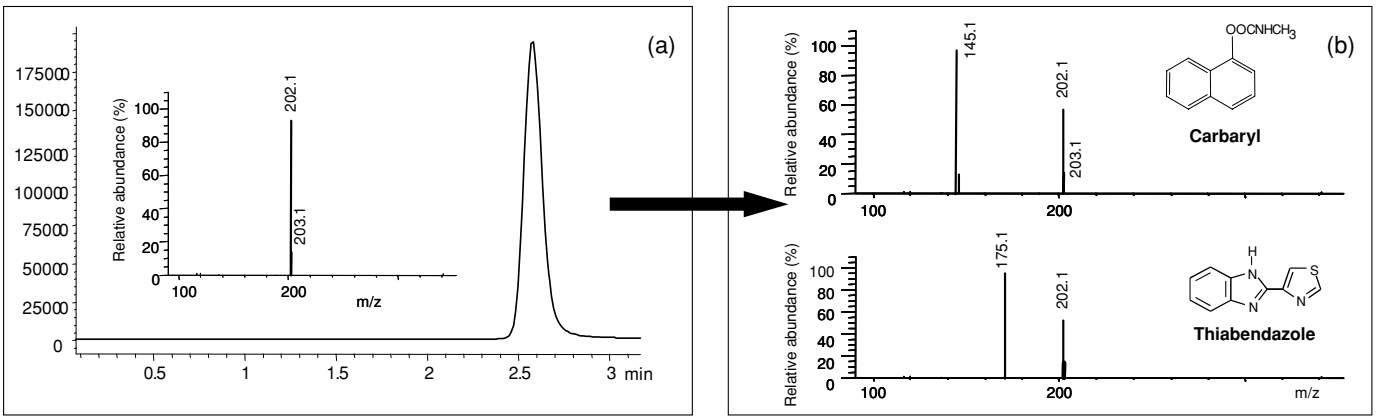

FIG. 8. (a) LC-MS chromatogram of a mixture of carbaryl and thiabendazole using LC-MS and appliying a cone voltage (known also as fragmentor voltage) of 20 V. (the mass spectrum is shown as an insert) and (b) the mass spectra of carbaryl and thiabendazole obtained using a cone voltage of 120 V.

quantitative analysis in the SIM mode would be difficult when isobaric interferences contribute to the chromatographic peaks). Figure 8a shows a typical example of isobaric interferences. Carbaryl and thiabendazole are two pesticides that have a very different structure but the same molecular weight. These pesticides generally co-eluted in isocratic LC conditions. In a single-quadrupole instrument, operating with an ESI or APCI source, selectivity can be increased when fragmentation is induced in the ion source region "in-source collision induced dissociation (CID)". This procedure refers to the activation of ions in the region between the ion source and the analyzer, in which fragmentation can be initiated by collisions with residual gas molecules at intermediate pressures. The generated fragment ions can be used for limited structure elucidation or confirmation purposes. Figure 8b illustrates that by increasing these fragmentor or cone voltages thiabendazole and carbaryl can be distinguished by obtaining a characteristic fragment ion of each molecule, 175 m/z for thiabendazole and 145 m/z for carbaryl (52). The drawback of CID is that it is normally detrimental to sensitivity. A compromise should be achieved between fragmentation and sensitivity, which taken into account the low maximum residue limit (MRLs) established implies that is not possible to obtain high fragmentation.

TOF

TOF mass spectrometer is basically composed of an ion-accelerating region, a flight tube, and a detector. In theory, all ions experience the same potential difference during acceleration and, consequently, have the same kinetic energy at the start of the flight tube, and thus different velocities depending upon their mass. Therefore, their arrival time at the detector is proportional to their mass and they reach the detector in order of increasing mass. TOF instruments commonly employ reflectons to enhance resolution, which focus ions of the same m/z but of different kinetic energy. The reflecton is located after the drift tube, creating a retarding field that the ions penetrate. Depending upon their kinetic energy, they enter this field at different depths and then are reflected back into the flight tube, where they drift to the detector, which is placed close to the ion source.

The pulsed nature of TOF analysis makes the coupling with electrospray —a continuous source of ions— difficult. The necessary focusing of the ions coming from the ESI source is usually carried out by an orthogonal acceleration (oa) TOF type. The ions are focused into the orthogonal accelerator as a narrow ion beam and a slice of it is pushed down into the flight tube.

LC-TOF-MS instruments present several advantages. Such as the high mass range that can be analyzed (with a linear TOF instrument) and the high ion transmission attained by the quasis-imultaneous detection of all ions resulting in high sensitivity "full spectrum" analyses (TOF-MS is not a scanning instrument). These characteristics make this instrument just right in qualitative applications such as the identification of non-targeted and/or unknown compounds, in which the acquisition of an entire mass spectrum is required. In addition, LC-TOF-MS offers improved selectivity due to the high-resolution power linked to the capability to provide exact mass chromatograms over nominal mass chromatograms (1 Da mass range), which attains identification of mass interferences with analytes having the same nominal mass and chromatographic retention time.

A nice example of identification of non-targeted pesticides and their metabolites was reported by Thurman et al. (53) who use this technique to identify (without the initial use of standard), different post-harvest fungicides and their metabolites. In summary, the method involves accurate mass identification of the molecule and its isotopic clusters, database searches, and MSn pathway elucidation, followed by standard identification when possible. Figure 9 displays the total ion chromatogram of a lemon extract. A chlorine-containing suspected specie was found in this total ion chromatogram (TIC) at the retention time of 22.7 min. As can be seen in Figure 9a, in the accurate mass

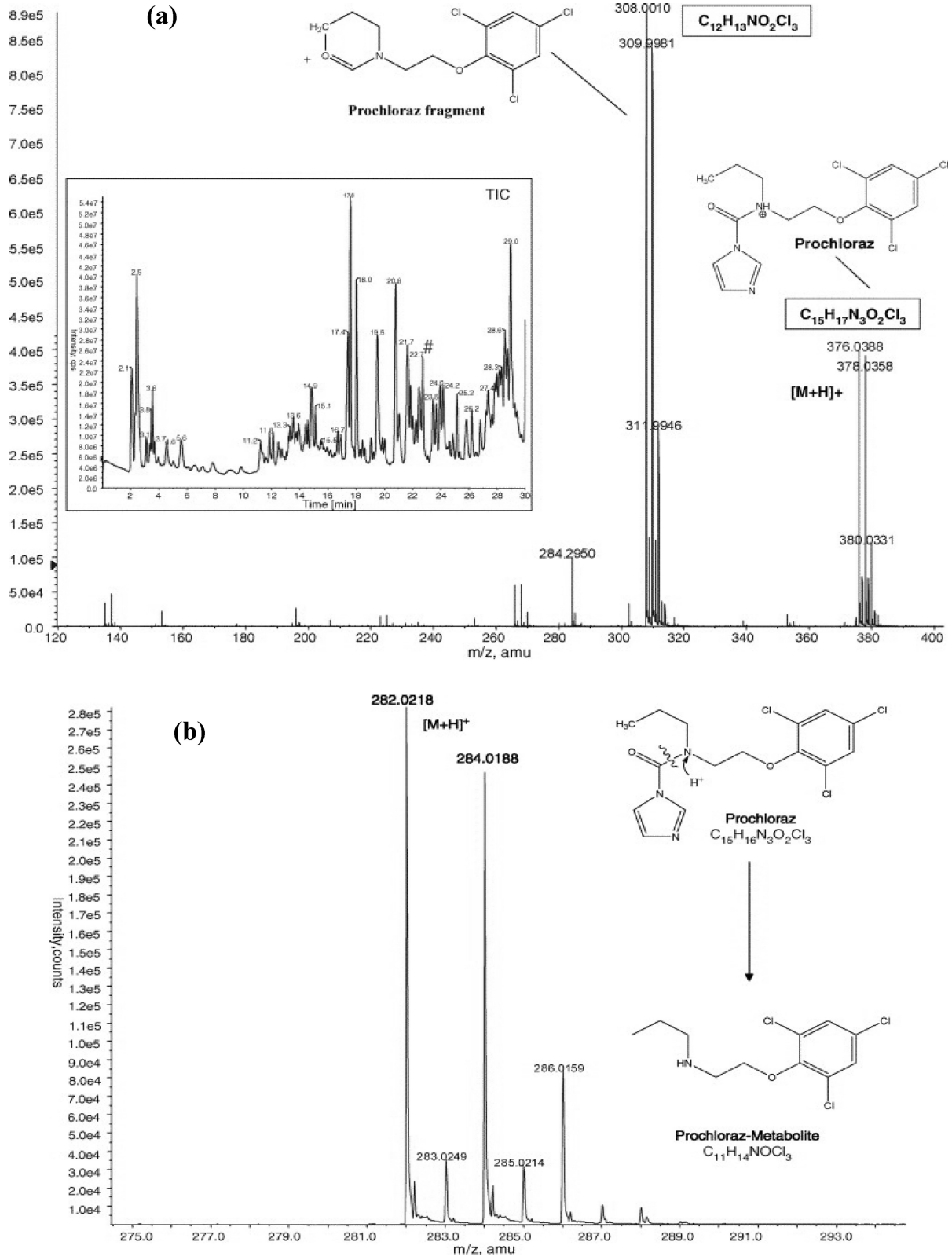

FIG. 9. (a) Accurate mass spectrum of prochloraz, identified in the studied lemon extract at t_R 22.7 min; (insert) TIC of the lemon extract and (b) Accurate mass spectrum of the proposed prochloraz degradation product identified in the studied lemon extract at t_R 16.9 min. *Adapted from (53) with permission of Elsevier B.V., Copyright 2005*.

spectrum, there are two main peaks: 376 and 308 m/z. The isotopic pattern and the difference between both signals evidence the presence of three chlorine atoms in the studied specie. Using a large accuracy error threshold and including a minimum and a maximum number of three chlorine atoms in the elemental composition calculator tool, only two elemental compositions matched with the m/z input: $C_{15}H_{17}N_3O_2C_{l3}$ and $C_8H_{17}N_9SC_{l3}$. Using "The Merck Index" data base, a unique match with the second formula, corresponding to prochloraz, was found. In the same lemon extract, an ion with the same isotopic pattern (three chlorine atoms) at the retention time of 16.9 minutes (Figure 9b) appears.

The disadvantage of TOF is its limited dynamic range; that is, the ratio of the maximum to the minimum observable ion intensities or concentrations over which a linear response is obtained from the detector. This is the direct result of the types of detectors that are employed for handling the large number of high-resolution spectra, making the application of TOF instruments to quantitative analyses less attractive, but not impossible as demonstrated by the number of applications of LC-TOF-MS to quantitative determination of pesticides, shown in Table 2.

Tandem Mass Analyzers

MS/MS involves two or more stages of mass analysis separated by a reaction or fragmentation step. The most important advantage of MS/MS is the reduction of "chemical noise" due to the high specificity of the instrument.

There are two fundamentally different approaches to MS/MS: tandem in space and tandem in time. Tandem in space instruments have different and independent mass analyzers in physically different locations of the instrument. Examples of these instruments includ, but are not limited to, QqQ and QqTOF. Tandem in time instruments are, in general, ion-trapping mass spectrometers which comprise 2-D and 3-D quadrupole ion traps and Fourier transform ion cyclotron. The various stages of MS are conducted within the same physical trapping volume but at different times during the experiment. Of all the instruments described here, the three-dimensional 3-D trap is the only one applied to pesticide residue determination.

QqQ

The most common mass analyzer for quantitative analyses is the QqQ. This MS/MS instrument consists of three consecutive quadrupoles (Q_1-q_2–Q_3; *Q* refers to a mass-resolving quadrupole, *q* to an RF-only quadrupole). This configuration achieves additional ion activation in q_2, after the target ion has been selected in Q_1. The second quadrupole q_2 is operated in the RF-only mode, in which only an RF voltage is applied to the quadrupole rods (no DC component), thus effectively becoming a wide-band pass for the ions. It can be filled with a neutral gas such as N_2 or Ar, acting as a collision gas. The ions leaving Q_1 are accelerated into q_2 with offset voltages between 0 and 100 V. The results of the CID can be analyzed with the mass analyzer Q_3. The common mode of operation in pesticide residue analysis is SRM, in which the two resolving quadrupoles monitor specific precursor-to-product ion transitions. SRM enhances the detection limit in analytical procedures. The advantage is that QqQ can perform this operation for multiple precursor and product ions with high sensitivity, and selectivity, that is commonly now as multiple reaction monitoring (MRM). The disadvantage is that the spectrum can not be obtained.

Figure 10 shows a significant example of the application conducted with QqQ instruments for the quantitative analysis of pesticides and their metabolites in fruits and vegetables (54). This study highlights the large number of pesticides that can

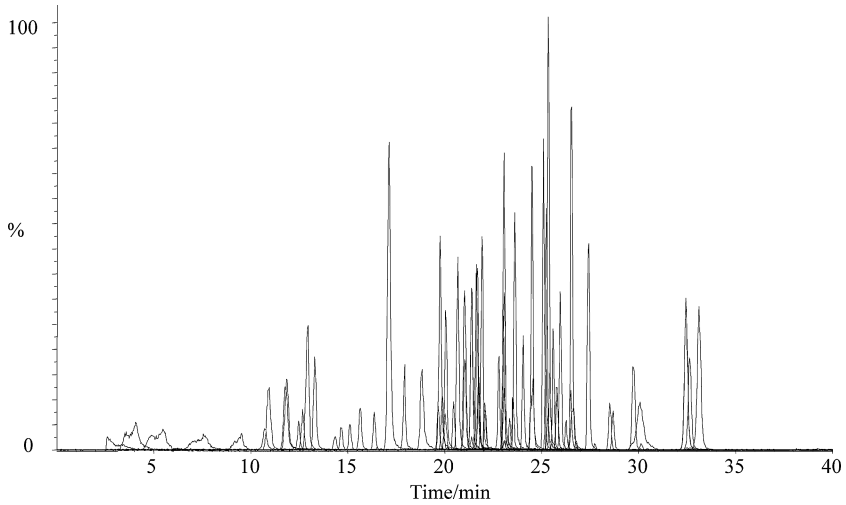

FIG. 10. Combined MRM chromatogram of a matrix-matched standard at 0.05 mg/kg prepared in orange. *Reproduced from (54) with permission from Wiley InterScience, Copyright 2004.*

TABLE 2
Applications of TOF in pesticides analysis in food

Pesticide	Matrix	Extraction	Determination	Recovery (%)	LOD (ng/ml)	LOQ (mg/kg)	Ref.
3 chloronicotinyl pesticides	Vegetables	15 g sample SLE (ethyl acetate + sodium sulfate anhydrous)	• ESI positive; Zorbax Eclipse XBD C_8 (150 × 4.6 mm, 5 μm); gradient of acetonitrile-0.1% formic acid in water at flow rate 0.6 ml/min. • Between one and three fragments obtained since the protonated molecule for each pesticide. • The errors were always less than 2 ppm for protonated ions and less than 3 ppm for the fragment ions.	77–102	2–10		(66)
15 different pesticides	Fruits and vegetables	15 g sample SLE (ethyl acetate + $WNaSO_4$)	• ESI positive; Zorbax Eclipse XBD C_8 (150 × 4.6 mm, 5 μm); gradient of acetonitrile-0.1% formic acid in water at flow rate 0.6 ml/min. • Fragmentation at fragmentator voltages of 190 and 230 V of the protonated molecule, except or methomyl, which gave the sodium adduct were carried out. • The errors in a tomato extract fortified with the pesticide mixture were < 2 ppm for all the analytes.		0.5–30	0.01–0.5	(67)
Non-target chlorinated	Fruits and vegetables	15 g sample by SLE (acetonitrile + NaCl + $MgSO_4$) and SPE (PSA + $MgSO_4$)	• ESI positive; Zorbax Eclipse XBD C_8 (150 × 4.6 mm, 5 μm); gradient of acetonitrile-0.1% formic acid in water at flow rate 0.6 ml/min. • TIC was scrutinized in order to find unambiguous structural information. • The proposed elemental composition of each fragment ion was compared with the structure of the suspected species proposed.				(68)
Identification of imazalil and prochloraz	Citrus fruits	15 g sample by SLE (acetonitrile + NaCl + $MgSO_4$) and SPE (PSA + $MgSO_4$)	• ESI positive; Zorbax Eclipse XBD C_8 (150 × 4.6 mm, 5 μm); gradient of acetonitrile-0.1% formic acid in water at flow rate 0.6 ml/min. • Identification and confirmation of parent compounds were based on the accurate mass measurement of molecular and fragments ions. Fragmentation was cheked by LC-IT-MS.				(53)

be simultaneously determined (more than 73 in this case). In these analyses, the MRM mode is almost always applied, allowing enhanced sensitivity and selectivity by circumventing isobaric interferences from food. LC-MS/MS with QqQ in SRM has become so far the most widely used technique overall in the multi-residues methods for the quantitation of pesticides in food (Table 3) because, under these conditions, a high sensitivity is achieved. Nowadays, a QqQ mass spectrometer is able to detect approximately 100 analytes simultaneously with sufficient sensitivity for determination at the μg/kg level. The use of time windows programm (periods) is not necessary unless the number of analytes to be analyzed within one run is significantly increased, or the compound presents a very low response. Because of the high sensitivity achieved by MS/MS, gradient elution on a small RP analytical column is usually sufficient for identification. However, for confirmation purposes, at least two transitions must be recorded, and then an increase in the limits of detection (LODs) occurs because the second transition is less abundant. Usually, transitions from the most abundant precursor to the most abundant product ions are selected. Small fragments with m/z ratios of < 80 were generally omitted if alternative product ions are available.

QIT

The QIT is the 3- D version of the linear quadrupole mass filter. In this device, ions are subjected to forces applied by an RF field but the forces occurs in the three dimensions instead of just two (55, 56). This mass analyzer consists of three electrodes: one ring electrode between two hyperbolic endcap electrodes, which form a 3-D trap. The oscillating potential difference established between the ring and endcap electrodes forms a substantial quadrupolar field (up to 6 kV of RF voltage can be applied to produce the trapping and the scanning). The ions pass in and out of the traps by holes in the endcaps (56, 57).

The QIT instrument has the advantage, over other tandem mass analyzers, that it multiplies the stages of mass analysis numerous times by preselecting an ion and analyzing the induced fragments, which helps to elucidate fragmentation pathways (58). This is designated as multi-stage tandem mass spectrometry (MS^n), analysis, where $^{n-1}$ indicates the number of fragmentation steps used (55).

The principal advantages of the QIT in chemical analysis are: high sensitivity of full-scan in MS and MS/MS modes and MS/MS experiments are available by performing sequential mass analysis measurements. Precursor ion isolation, fragmentation and product ion analysis all take place in the trapping volume by separating the events in time, rather than in space. Supplementary frequencies applied to the end cap electrodes are used both to eject unwanted ions during the precursor ion isolation and to excite precursor ions to carry out CID.

These features make QIT an attractive option to detect pesticides in food; some more relevant examples are related in Table 4. For the determination of pesticides that are completely unknown, data dependent full-scan MS and MS^n are proposed by some authors (53, 59).

QIT also suffers from some drawbacks, such as: (i) low resolution and mass shift, (ii) limited dynamic range (i.e., it can not handle samples in which the ion abundances vary greatly and the range of ion traps is limited to $\sim$106—when there are too many ions in the trap, space charge effects led to diminish performance), (iii) the inability to trap product ions below 50 m/z and the existence of an upper limit on the ratio between the precursor mass and the lowest trapped fragment ion mass, which is approximately 0.3 depending on the qz value. and (iv) the limited number of ions that can be simultaneously isolated and fragmented.

Figure 11 shows an example of MS^3 determination of 10 pesticides. Chromatograms correspond to tangerine samples (non-spiked and spiked at limit of quantification (LOQ) levels). The chromatographic resolution and the peak performance were satisfactory for the studied pesticides in the spiked samples. The sample that contains none of the studied pesticides show the lack of interfering peaks that can give a false positive sample (60).

Qq TOF

The implementation of a quadrupole mass filter prior to the TOF tube is another tandem mass spectrometer that is used in the field of food toxicant analysis. In fact, this can be taken one step further by adding a second, radio frequency RF-only quadrupole as a collision cell (Q_1-q_2-TOF). The TOF analyzer then is used to provide accurate mass data for fragment ions formed in q_2.

QqTOF can work in 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. For MS/MS, a precursor ion is selected in the first quadrupole, the second produces CID and the mass analysis of the fragment ions is performed in the TOF analyzer. By virtue of its MS/MS capabilities, full-scan product ion spectra are obtained and any ion can be selected to reconstruct an ion chromatogram. Contrary 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.

However, its applications in the field of pesticides analysis in food are still very scarce due to several disadvantages attributed to the QqTOF that, in addition to its high cost, are the same reported for the TOF: low efficiency in obtaining quantitative information at trace levels, narrow dynamic ranges, little robustness and lack of accuracy for quantitative purposes. The only reported application has been to determine carbosulfan and its main metabolites in citrus (61). The comparison of this mass analyzer with QqQ and QIT demonstrated that the sensitivity for QqTOF was worse than for QqQ and similar to QIT. In spite of this disadvantage, the exact mass measurements improves identification capabilities very much.

TABLE 3
Applications of triple quadrupole in pesticides analysis in food

Pesticide	Matrix	Extraction	Determination	Recovery (%)	LOD (ng/ml)	LOQ (mg/kg)	Ref.
Carbamates, benzimidazoles and organophosphates.	Fruit juices	1 g sample by MSPD (diatomaceous earth + ethyl acetate)	• TIS positive and negative (iprodione); Alltima C_{18} (250 × 4.6 mm, 5 μm); gradient of MeOH-water at flow rate 1 ml/min. Post-column addition of 20 mM formic acid at flow rate 0.1 ml/min. • SRM of the one most sensible transition • Data acquisition was divided into 4 time-windows.	77–102	8–120	0.0005–0.01	(69)
74 pesticides (carbamates, conazole, benzimidazole and pyrimidine)	Fruits and vegetables	20 g sample SLE (ethyl acetate)	• ESI positive; Nucleosil 100 C_{18} (70 × 2 mm, 5 μm); gradient MeOH-water (0.1% formic acid) at flow rate 0.3 ml/min. • SRM of two abundant MS/MS transition for each substance was used divided in 11 acquisition groups. • For suspected samples, two confirmation analyses were conducted.	63–133		0.01	(47)
32 multi-class pesticides	Fruits and vegetables	10 g sample by SLE (ethyl acetate + Na_2SO_4)	• ESI positive; Polaris C_{18}-A (150 × 2 mm, 5 μm); gradient MeOH-2 mM ammonium formate at flow rate 0.2 ml/min. • MRM of protonated molecule and the most abundant and characteristic fragment ion was used for quantitation separated in individual time-windows for each analyte. • Two selected fragment ions were selected for confirmation.	74–105	<1	0.009–0.025	(70)
8 triazolic and pyrimidine fungicides	Tomato puree and lemon juice	20 g tomato puree and 10 g lemon juice by LLE (acetone + NaCl, ethyl acetate-cyclohexane + Na_2SO_4)	• ESI positive; Zorbax SB-18 (50 × 2.1 mm, 3.5 μm); gradient 2% aqueous formic acid-MeOH at flow rate 0.2 ml/min. • For all compounds, the protonated molecular ion was the most abundant inthe full mass spectrum. • Two MS/MS transitions were monitored for each pesticides. • For Penconazole, the monitorization of a third transition was possible.			0.005–0.01	(49)

32 pesticides and 25 TPs benzimidazoles and carbamates	Fruits and vegetables	g sample by SLE (ethyl acetate + Na_2SO_4)	• ESI positive and negative mode, Genesis C_{18}(100 × 3 mm, 4μm), gradient MeOH-10 mM ammonium formate in MeOH-water at 0.3 ml/min. • The fragmentation of the protonated molecule gave two abundant ions. • Use of an internal standard for confirmation.	70–100		0.01–0.5	(24)
57 pesticides and 16 TPs mainly carbamates	Fruits and vegetables	10 g sample SLE (acetonitrile + $MgSO_4$ + NaCl)	• ESI positive, HyPURITY C_{18} (150 × 2.1 mm, 5 μm), gradient 10 mM aqueous ammonium acetate-MeOH at flow rate 0.2 ml/min. • The MS/MS transition with highest MRM response was used for each analyte to build a screening method. • To confirm the residues, the sample were re-analyzed monitoring two transitions for the residues found.	77–124		0.01–0.1	(52)
20 carbamates and other relatively polar pesticides	Fruits, vegetables and cereals	10 g sample (fruits and vegetables) and 3 g cereals SLE (20 mM ammonium acetate-acetic acid in MeOH-water)	• ESI positive, Genesis C_{18} (100 × 3 mm, 4 μm), gradient water-MeOH/water (20 mM ammonium acetate-acetic acid) at flow rate 0.3 ml/min. • MRM detection of the most sensitive MS/MS transition separated in 4 time-windows were used. • Secondary transition was possible for confirmation of only 8 pesticides. • Use of an internal standard ($^{13}C_6$ I.S. carbaryl)	70–120	0.01–0.02 mg/kg	0.04–2	(13)
24 new pesticides	Lemon juice, tomato and apple puree	10 g sample LLE (acetone + ethyl acetate-ciclohexane + Na_2SO_4 + NaCl)	• ESI positive; Synergy Polar-RP (150 × 2 mm, 4 μm); gradient 0.1% aqueous formic acid-acetonitrile at flow rate 0.25 ml/min. • 55 MRM transitions were carried out (2 tansitions for almost all analytes and 3 in the case of 7 pesticides) in a single injection using only one retention time window.	76–106		0.001–0.01 and 0.002–0.02	(50)

TABLE 4
Applications of ion trap in pesticides analysis in food.

Pesticide	Matrix	Extraction	Determination	Recovery (%)	LOD (ng/ml)	LOQ (mg/kg)	Ref.
Postharvest fungicides	Fruits	1 g sample by SPME (acetone + NaCl + fiber CW/TPR)	• APCI negative; Luna C_{18} (250 × 4.6 mm, 5 μm); gradient of MeOH-water at flow rate 0.6 ml/min. • SIM of the most abundant ion (commonly a fragment fo the molecule) was subjected to the first stage of MS^2 • The MS^2 analysis provided product ions for all of them being employed for the quantification and identification in MRM mode.	10–60	8–120	0.0005–0.01	(72)
17 polar pesticides	Aples and apricots	12.5 g sample SPE (acetonitrile + MCX and HBL SPE cartridges).	• ESI positive and negative; Discovery C_{18} (150 × 3 mm, 5 μm); gradient MeOH-water (2 different groups) at flow rate 0.5 ml/min. • SIM of (de)protonated molecule divided in time-windows for fragmentation. • Only tebuconazole did not provide a stable ion product.	75–122	0.1–10 μg/kg	0.002–0.024	(73)
2 insecticides, 3 fungicides and 1 acaricide	Oranges	5 g sample by SLE (ethyl acetate + Na_2SO_4)	• APCI positive; Luna C_{18} (150 × 4.6 mm, 5 μm); gradient MeOH-water at flow rate 0.8 ml/min. • SIM of protonated molecule was used in 6 different time-windows (one for each analyte). • A MS^3 fragment ions were reached for all compounds. • MRM of MS, MS^2 and $MS^3$was used to identification and quantification	72–94		0.001–0.3	(74)

Chlormequat	Pears	10 g sample by LE (MeOH-100 mM ammonium formate) and SPE (Envi-18 cartridge)	• ESI positive; Kromasil C_8 (200 × 21 mm, 5 μm); gradient 15 mM HFBA water-acetonitrile at flow rate 0.15 ml/min. • The fragmentation of the protonated molecule gave two abundant ions. • Use of an internal standard for confirmation.	86–92	0.03 mg/kg	0.08	(31)
Daminozide	Apple and apple leaves	25 g sample by LE (methanol) and SPE (Supelclean Envicarb)	• APCI positive; Hypersil APS-2 (100 × 3 mm, 5 μm); gradient MeOH-water (10 mM ammonium acetate) at flow rate 0.5 ml/min. • First MS gave as abundant ion the protonated and dehydrated molecule. • The MS^2 analysis provided 2 product ions employed for the quantification and identification in SRM mode.	98–112	0.008–0.02 mg/kg		(26)
Benzimidazoles, azoles, OPs, carbamates, neonicotinoids and acaricides.	Fruits	2.5 g sample PLE (ethyl acetate + acidic albumina; 75^{o}C, 1500 psi)	• APCI positive; Luna C_{18} (150 × 4.6 mm, 5 μm); gradient MeOH-water at flow rate 0.8 ml/min. • Only methidation did not give a MS^3 mass spectrum. The rest of the compounds were quantified in citrus fruits employing the triple-mass stage spectrum.	48–98		0.025–0.25	(60)
9 different pesticides	Fruits	Comparison MSPD and SLE. 0.5 g sample by MSPD (C18 + dichloromethane-methanol) and 5 g sample by SLE (ethyl acetate)	• ESI positive; Luna C_{18} (250 × 2 mm, 5 μm); gradient MeOH-water at flow rate 0.6 ml/min. • The pyrethroids gave the sodium adduct that not fragment. • Two compounds reached the MS^3 fragmentation. • MRM of MS and MS^2 was used for the quantification of the compounds in fruit samples.	52–108 (MSPD) 59–101 (SLE)		0.05–2 (MSPD) 0.01–0.4 (SLE)	(51)

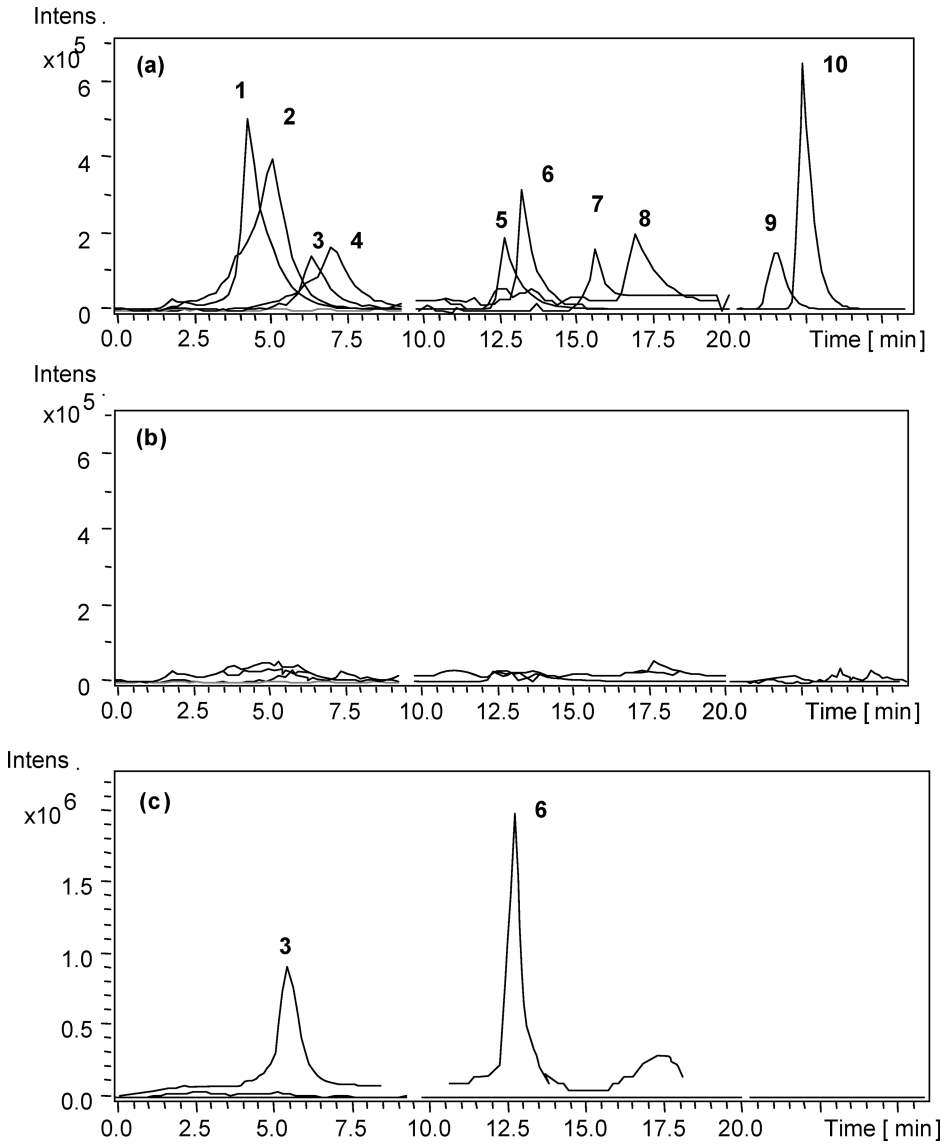

FIG. 11. LC-IT-MS chromatograms obtained after PLE for (a) spiked orange; (b) unspiked orange; and (c) sample no. Peak identification: (1) imidacloprid, (2) trichlorfon, (3) carbendazim, (4) thiabendazole, (5) methidation, (6) methiocarb, (7) imazalil, (8) bitertanol, (9) pyriproxyfen and (10) hexythiazol. *Reproduced from (60) with permission of Elservier B.V. , Copyright 2005.*

APPLICATIONS

Tables 1–4 summarize the applications of the different mass analyzers in pesticide residue analysis classified according to the mass analyzer employed. An important issue is sample preparation. The low MRLs established by the different national and international legislation makes it necessary for an appropriate extraction, isolation and concentration of pesticides.

In this way, it should be commented and remarked that selectivity and specificity of LC-MS has allowed the extraction techniques to evolve from very complicated techniques to simpler ones. Nowadays, extraction of pesticide from food samples is mainly performed by a plain organic solvent extraction with acetonitrile, methanol, ethyl acetate or dichloromethane or by using matrix solid-phase dispersion (MSPD). The last technique presents the advantage of carrying out simultaneous extraction and clean-up. Other alternative techniques, such as pressurized liquid extraction (PLE), solid-phase microextraction (SPME) or stir-bar sorptive extraction (SBSE), have also been tested in combination with the different LC-MS techniques showing good capability but they are not generalized in this field of analysis yet.

Applications of LC-MS to pesticide residue determination are almost restricted to fruit and vegetable samples. Other applications to food of animal origin, such as milk, honey, meat or fish, are really scarce and treated like a punctual aspect. This is contradictory with the fact that MRLs are established also for

this type of food and the analysis of these samples is frequently required.

The "boom" of LC-MS application has been exploited in the last five years because of the strict regulations in this field. Among the different mass analyzers, QqQ is the most widely used because of its high sensitivity and because the number transition required for confirmation are easily attained. In addition, until the moment, Q_qQ is the most suitable mass spectrometer to determine up to 50 pesticides in the same injection.

CONCLUSIONS AND FUTURE TRENDS

The common feature of different approaches to pesticide chemistry is the appearance of compounds increasingly more polar. For this reason, LC techniques, especially RP, are useful in their separation without any pre-treatment. Moreover, the contribution of MS, with its high sensitivity and considerable diagnostic power, is fundamental. The relatively high number of publications on the analysis of pesticides in food samples by LC coupled to MS/MS shows that this technique has become a powerful tool in the quality control of food and food safety issues.

Although reported results are controversial, ESI is the most frequently utilized ionization source because of its higher sensitivity for most important group of pesticides. Deeper studies into the different source designs are required to find a relation between sensitivity and ionization source.

The benefits of LC-M for pesticide residue analysis in food are widely recognized. However, some critical aspects need to be taken into account in relation to quantification, especially the matrix effect is an unresolved question yet. Some approaches to overcome this effect have been commented on and discussed thoroughly in this paper. The tendency is to perform an appropriate quantification with matrix-matched standards.

QqQ is the instrument most commonly used in this field because the lower LODs achievable permit compliance with the strict MRLs established by governing authorities. Both QqQ and QIT are frequently used in this field. The use of high performance mass analyzers such as TOF or QqTOF is also expected to increase in the next few years. This increased use will be for unknown metabolite identification, for non-target pesticide screening methods and for analyte confirmation in positive samples.

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