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Multiple-stage mass spectrometric analysis of six pesticides in oranges by liquid chromatography–atmospheric pressure chemical ionization–ion trap mass spectrometry

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Abstract

Six pesticides were determined by liquid chromatography (LC) with positive ion (PI) atmospheric pressure chemical ionization quadrupole ion-trap tandem mass spectrometry (APCI–MS–MS). Ion fragmentation was studied by MS, MS^2 and MS^3. Fragmentation of the pesticides produced ions formed by various losses from the side-chains and through heterocyclic ring opening, but without any common fragmentation pathway. Multiple reaction monitoring (MRM) of MS, MS^2 and MS^3 was used to identify and quantify the pesticides. The samples were extracted with ethyl acetate and dried over anhydrous sodium sulfate. Comparison of the three MS modes showed that MS^3 is slightly less sensitive but much more selective. Recoveries from oranges were 72–94% at the limit of quantification (LOQ) level for all MS modes. The LOQs were 0.001–0.3 mg kg^{-1} quantifying by means of the MS^3 product ion. The method was used to analyse a number of orange samples. This is the first report on using MS^3 product ions for quantification of pesticide residues.

Keywords: Oranges; Food analysis; Quadrupole ion trap; Multiple stage mass spectrometry; Pesticides

1. Introduction

Modern agriculture has become dependent of pesticides use because they can increase farm profits by lowering labor and machinery costs [1,2]. However, pesticide residues are potentially toxics and are introduced purposefully into food, especially fruits and vegetables [1,3,4]. Residue limits for pesticides in food (called tolerances) are established by the governmental agencies in each country based on field trials and processing data [5–7]. However, to guarantee compliance of food with regulations, their monitoring for pesticide content is required [2,8]. Increased public awareness and the widespread use of pesticides have resulted in more frequent analysis by monitoring programs, which has created a growing interest in expanding the capability of current analytical methods and developing new ones to detect pesticide residues as proof of the safety of food [4].

Liquid chromatography–mass spectrometry (LC–MS) is attracting each time more attention because it meets the demands of sensitive and selective analyte detection in complex matrices, which are prerequisites in food analysis, according to recent national and international laws and regulations [5–7]. The detection of pesticides by mass spectrometry is now supplanting current spectrophotometric or fluorimetric detection, as demonstrates the many examples of application of LC–MS to the analysis of pesticide residues that can be found in recent chromatography and food literature [8–11]. When single stage mass spectrometry is used, there are still several analytical shortcomings to be overcome, derived from the lack of specific structure diagnostic ions and caused by known and unknown compounds that provide isobaric interferences or multiple-component spectrum definitely useless [8,10,12].

Tandem mass spectrometry gives the highest degree of certainty in analyte identification and, therefore, may be

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employed in accordance with recent guidelines to obtain data with relevant unambiguity [13,14]. Among the different mass analyzers that can perform tandem mass-spectrometry, ion trap answers this challenge with unsurpassed sensitivity down to the sub-picograms range [8,15–18]. Moreover, its multiple fragmentation stages provide data with excellent information content. These features allow highly reliable analyte identification even from mixtures or poorly separated pesticides and thus make an ion trap system MS one of the leading mass spectrometric techniques in multiresidue analysis. Most important drawback attributed to this mass analyzer is its limited dynamic range (i.e. it can not handle samples in which the ion abundances vary greatly and the range of ion traps is restricted $\sim 10^6$). The use of ion charge control (ICC) prevents this problem using automated scans to rapidly count the ions before they go into the trap, but the ICC can be a problem when trace elements, in particularly dirty matrices, are analyzed because the trap fills with both matrix ions (large number) and trace sample ions (very small number).

The performance of the ion trap for LC–MS determination of herbicides as phenylurea, triazines, carbamates, chlorinated phenoxyacetic acids, nitrophenols, and ammonium quaternary [19–24] in water has been reported in the literature since these compounds are readily soluble in water and their runoff into rivers and lakes posses several problems for the supply of clean drinking water. However, the presence of these herbicides in fruits for human consumption is scarce since they are not directly applied to them.

Although considered as one of the most powerful techniques for structure interpretation, LC–MS–MS has been seldom used in the analysis of pesticides residues in complex matrices such as fruits and vegetables. Recently, some papers dealing with the determination of chlormequat [25] or daminozide [26] in food samples have been published. However, they are single residue methods used for determining only one pesticide [25,27]. The use of ion trap to analyze multiple pesticide residues is restricted to a few applications dealing with some fungicides and carbamate insecticides using MS [28], as well as 48 pesticides in surface waters [24], five fungicides in fruits [29], and 17 pesticides in apples and apricots [30] by a second MS stage.

The present study focuses attention on carbendazim, thiabendazole and imazalil (fungicides), hexythiazox (acaricide), and methiocarb and imidacloprid (insecticides). They are structurally interesting pesticides, widely used in orange orchards and/or in post-harvest treatment. The use of APCI–MS^n in a quadrupole ion-trap mass spectrometer for structural characterization of the pesticides is explored. The objective is to interpret the fragmentation patterns obtained by multiple-stage MS–MS experiments, and to determine whether the fragmentations are structurally useful. To our knowledge, this is the first LC–MS^3 method that achieves the analysis of pesticides in fruit at levels below the maximum residue limits (MRLs).

2. Experimental

2.1. Chemicals and reagents

Carbendazim, hexythiazox, imazalil, imidacloprid, methiocarb and thiabendazole were supplied by Riedel-de Haën (Seelze, Germany). Individual stock solutions were prepared by dissolving 100 mg of each compound in 100 ml of methanol and stored in stained glass-stopper bottles at 4 °C. Standard working mixtures, at different concentrations, were prepared daily by appropriate dilution of aliquots of the stock solution in methanol and into "pooled" orange extracts.

HPLC-grade methanol and organic trace analysis grade ethyl acetate were purchased from Merck (Darmstadt, Germany). Anhydrous sodium sulfate (analytical grade) was bought from PanReac (Barcelona, Spain). Distilled water was deionized (< 18 cm MΩ resistivity) in a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA). All the solvents were passed through a 0.45 μm cellulose filter from Scharlau (Barcelona, Spain) before use.

2.2. Sample collection and preparation

Ten orange samples, collected from an agricultural cooperative, were tested. As far as possible, the samples were taken at various places distributed through the lot (size ~50 kg). They weighed ~2.5 kg and consisted of at least 10 individual fruits. The samples were analyzed unwashed and with the peel intact. They were cut into small pieces, and a 200 g portion was homogenized in a food chopper.

Organic solvent extraction was carried out by a common procedure as described elsewhere [31,32]. Briefly, 5 g of chopped orange was placed in 25 ml glass beaker and mixed thoroughly with 10 ml of ethyl acetate and 5 g of anhydrous sodium sulfate using a warring blender during 2 min. The homogenate was allowed to settle and the supernatant was passed through a filter paper into a 50 ml rotary-evaporation flask. The solid residue was again homogenized with 10 ml ethyl acetate, filtered through the anhydrous sodium sulfate and collected with the first extraction fraction. Five milliliter ethyl acetate was used twice to rinse the glass beaker and the rinsings were passed through the filter and collected. A rotary evaporator set at 40 °C and 250 mbar was used to evaporate the extract to dryness. The sample was reconstituted in 1 ml of methanol. A volume of 20 μl of the final extract was injected into the LC ion trap MS system.

2.3. Liquid chromatography–mass spectrometry

The liquid chromatography-ion trap-mass spectrometry (LC–IT–MS) system consisted of an Esquire3000 Ion Trap LC–MS^n system (Bruker Daltonik GmbH, Germany) and an Agilent 1100 Series LC system that includes a quaternary pump, an autosampler and a variable wavelength detector, a computer (HP PC) and a data acquisition/processing

Table 1
MS^n operation parameters

	Segment time definition (min)					
	Imidacloprid (0.0–9.5)	Carbendazim (9.5–10.2)	Thiabendazole (10.2–11.0)	Methiocarb (11.0–11.8)	Imazalil (11.8–13.2)	Hexythiazox (13.2–15)
Transition (MS^2)						
m/z	(256 → 209)	(192 → 160)	(202 → 175)	(226 → 169)	(297 → 255)	(353 → 228)
Width *m/z*	1.0	1.0	1.0	1.0	4.0	1.0
Cutoff mass	100	100	100	140	150	140
Amplitude (*V*)	1.2	2.0	2.0	2.0	2.0	2.0
Transition (MS^3)						
m/z	(209 → 175)	(160 → 132)	(175 → 131)	(169 → 121)	(255 → 159)	(228 → 168)
Width *m/z*	4.0	1.0	1.0	1.0	4.0	1.0
Cutoff mass	150	100	100	100	150	100
Amplitude (*V*)	3.0	1.6	1.6	2.0	2.0	1.2

Daltonic Esquire Control Software system 3.0. Separation was performed on a Phenomenex (Madrid, Spain) Luna C_{18} column (150 mm × 4.6 mm i.d., 5 μm) preceded by a Securityguard cartridge C_{18} (4 mm × 2 mm i.d.), using 20% of methanol (A) in water (B) maintained from 0 to 3 min, followed by a linear gradient to 90% A from 3–8 min. This composition was maintained from 8 to 15 min, and then returned to initial conditions in 5 min. Flow rate was 0.8 ml min^{-1} and 20 μl of standard solutions were injected.

The mass spectrometer was equipped with an APCI source, and operated in positive polarity. The conditions of the source were temperature, 450 °C; capillary voltage, 4000 V; the end plate offset was fixed at −500 V; corona current, 4000 nA; nebulizer pressure, 60 psi; and drying gas flow 4 l min^{-1} at a temperature of 350 °C. The Esquire 3000 was tuned for each compound, optimizing the voltages on the lenses in the ExpertTune mode of the Daltonic Esquire Control software whilst infusing a standard solution (10 μg ml^{-1}) by a syringe pump at a flow rate of 0.004 ml min^{-1}, which was mixed with the mobile phase at 0.8 ml min^{-1} by means of a T piece. The optimized tune parameters were set for each compound via time segments definition.

The mass spectrometer was operated in full scan and MRM modes. The trap parameters were detected in ion charge control mode using rolling averaging set at 2. Full scan mode was performed with a target of 70,000 and maximum accumulation time of 100 ms at *m/z* range from 50 to 400 u. MRM was carried out setting the target at 200 000 and maximum accumulation time at 200 ms for both, MS and MS^n experiments. Positive ions were detected at unit resolution (scan speed 10,300 u s^{-1}). Four scans were summarized for each spectrum, resulting in a spectral rate of 0.4 Hz. Collision induced dissociation (CID) was performed on the ion of interest by collisions with the helium background gas present in the trap for 40 ms. In these experiments, the protonated pesticide was subjected to CID to produce a first set of fragment ions, MS–MS or MS^2. Subsequently, one of the fragment ions from $[M + H]^+$ was isolated and fragmented to give the next set of fragment ions, MS^3. The fragmentation steps for each compound were optimized visualizing the changes in the intensities of fragments ions, whereas the fragmentation cutoff and the fragmentation amplitude were manually varied. Table 1 outline the values set for each of the studied pesticides.

3. Results and discussion

3.1. Quadrupole ion trap mass spectrometry

The only ion observed was the protonated molecule $[M + H]^+$ in full-scan MS in positive ion mode, except for hexythiazox (Fig. 1a), the mass spectrum of which gave also the fragment *m/z* 228 corresponding to the characteristic fragment $[M + H\text{-}C_6H_{11}N{=}C{=}O]^+$. The fragmentation of the protonated molecule exhibit major ions at *m/z* 228 and 271, and weak sings of the ions at *m/z* 168 and 194. The ion at *m/z* 271 is formed through the loss of cyclohexene. MS^3, of *m/z* 228, leads to the formation of *m/z* 168 via the loss of SCO unit.

The MS^2 spectrum of $[M + H]^+$ for carbendazim evidences an intense signal at *m/z* 160 that corresponds to $[M + H\text{-}CH_3OH]^+$. Further fragmentation of this ion was verified by MS^3, showing the intense signal at *m/z* 131 and an additional fragment at *m/z* 105, formed by the loss of carbon monoxide molecule and the combination of this loss with the opening of the ring and the loss of HCN molecule, respectively.

Imazalil (Fig. 1b) has chlorine atoms in its structure that yielded a characteristic pattern of isotopic doublet signals, consistent with the presence of two chlorine atoms in the molecule. The MS^2 analysis of imazalil with CID leads to four main fragment ions at *m/z* 255, 201,173 and 159 corresponding to the cleavage of the lateral chains and the opening of the pyrrolic ring, as it is indicate in Fig. 1b. The

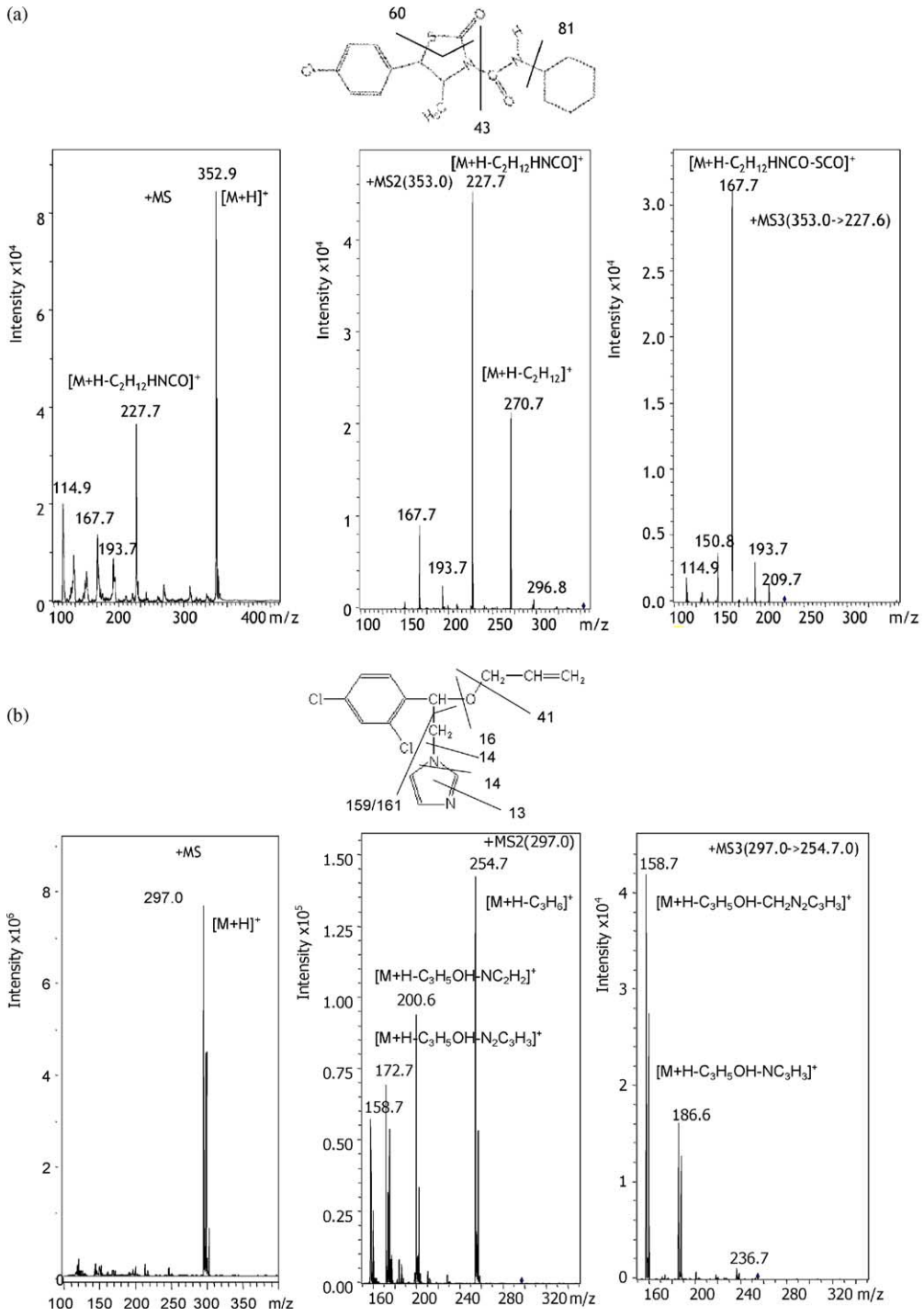

Fig. 1. Positive APCI mass spectrum obtained in methanol, the product ion mass spectra of the protonated molecules, the product ion mass spectrum (MS^3) of main positive ion derived from the protonated molecule and the proposed fragmentation pattern of (a) hexythiazox, and (b) imazalil.

spectrum derived from MS^3 of the ion at *m/z* 255 contains two peaks, at *m/z* 159 and 187. It was confirmed that all fragment ions retain the two chlorine atoms because they show the characteristic isotopic pattern.

A three-stage mass analysis of the protonated thiabendazole at *m/z* 202 illustrates a dominant fragmentation pathway: *m/z* 202 → *m/z* 175 → *m/z* 131. MS^2 of thiabendazole is characterized by the loss of HCN from the thiazolic ring. It results in an abundant product ion at *m/z* 175 and a further loss of CS, from the fragmentation of thiazolic ring, which leads to the little abundant ion of *m/z* 131. Further fragmentation of *m/z* 175 produces an abundant *m/z* 131 ion that confirms this fragmentation pathway.

The product-ion spectra of the protonated molecule of imidacloprid $[M + H]^+$ shows two specific ions, at *m/z* 209 (loss of HNO_2), and 175 (loss of HNO_2 and HCl).

Further fragmentation of the ion at *m/z* 209 yields the *m/z* 175 product ion.

Methiocarb is a pesticide representative of the class of carbamates. Its MS^2 spectrum presents only a product ion at *m/z* 169, derived from the neutral loss of the $CONCH_3$ group. MS studies of other carbamate pesticides have reported identical products ions [16,19,30]. In the further step (MS^3 of the ion at *m/z* 169) one product ion is formed at *m/z* 121 resulting from the loss of the group $HSCH_3$ located in the *p*-position at the carbamic group.

3.2. Method validation

3.2.1. Linearity and matrix effects study

Linearity was studied over a range of spiking levels from the LOQs obtained for MS^3 to 10 mg kg^{-1} by MS, MS^2 and MS^3. For all the compounds and all the MS modes, the calibration curves were linear in this range with regression coefficients >0.998.

However, in quantitative analysis one of the major problems is the suppression/enhancement of the analyte signal in presence of matrix components, which has been reported by many authors [23,25,28,33]. Response suppression caused by sample matrix components using the ES interface has been widely discussed in the literature [28,33]. However, the information about the effects of this class of interferences on APCI interface is more conflicting [28,30].

This interference can be established comparing the signal intensity obtained in a standard solution (methanol) with those obtained in matrix matched standards. Fig. 2 shows the differences in response of each analyte in pure solvent standard and in matrix matched standard at LOQ concentrations (Fig. 2a) and 10 times the LOQ concentration (Fig. 2b).

All pesticides, except hexythiazox, showed in orange matrix considerable differences in relation to the response obtained in a pure solvent standard using MS mode. For imidacloprid, carbendazim and thiabendazole, the detector response was enhanced by matrix component. This phenomenon can be attributed to the characteristics of the matrix. The studied pesticides are of basic nature and the matrix components of acidic character could promote the formation of $[M + H]^+$ ions of these analytes during the ionization process. It should be noted, that these analytes are eluted at low retention times and therefore coelution with such (polar) coextractants is probable. Methiocarb and imazalil showed response suppression in the presence of orange matrix. This decrease of ion intensities can be attributed to the gas-phase proton transfer.

The same course was noted using MS^2 and MS^3 modes. However, the intensity of the observed effect is lower than in MS mode. It is difficult to explain why MS^3 mode showed less matrix effect than MS^2, and this mode less one than MS mode. The matrix effects dictate the formation of $[M + H]^+$ current which should be directly proportional to the MS^2 and MS^3 ion current. However, one possibility is that the subsequent isolation and fragmentation steps help to minimize the

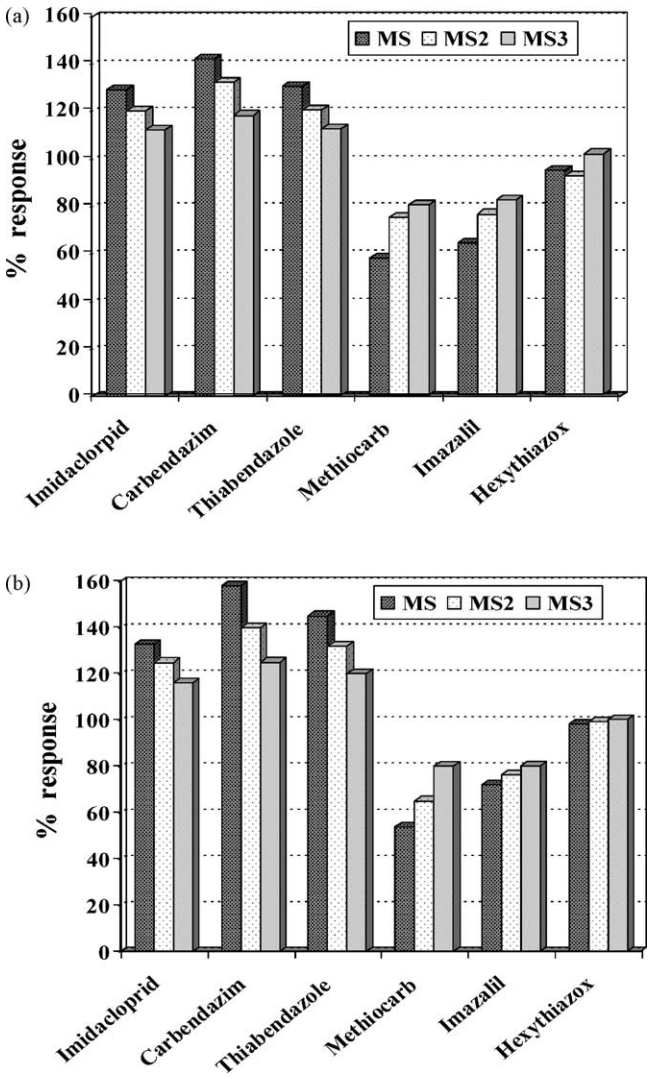

Fig. 2. Matrix effects in MS, MS^2 and MS^3 from orange extracts (a) sample at LOQ, and (b) sample at 10 LOQ, calculated as percentage of the response of each compound in matrix-matched standard compared with those obtained in pure solvent standard.

effect. This result could also be attributed to space–charge losses since the trap target levels, 200,000 counts and 200 ms, are high. The ICC conditions were carefully optimized by varying the trap target levels and maximum accumulations times for standard in methanol and also matrix matched standard. Low values resulted in lowest sensitivity while higher values (up to 500 ms) provided changes in the signal shape indicating that ion trap was overloading. The matrix effects observed were almost equal at different values.

Different authors reported useful approaches to compensate or eliminated matrix effects as more selective extraction and/or cleanup procedure, modification of the mobile phase composition, or special a calibration techniques (matrix matched standards, isotopically labelled internal standards, post-column addition or eco-peak analysis) [28]. The first strategy is time consuming and not always successful, and the second one could results in the suppression of the

Table 2
Method quantification limits (LOQ) and recoveries by LC–MS, LC–MS^2 and LC–MS^3 for the analysis of the target pesticides in orange samples[a]

Pesticide	LC–MS		LC–MS^2		LC–MS^3	
	LOQ ($mg\,kg^{-1}$)	Recovery, (percentage R.S.D., n=5)	LOQ ($mg\,kg^{-1}$)	Recovery, (percentage R.S.D., n=5)	LOQ ($mg\,kg^{-1}$)	Recovery, (percentage R.S.D., n=5)
Imidacloprid	0.0005	83 (19)	0.001	85 (15)	0.001	80 (10)
Carbendazim	0.01	72 (14)	0.02	76 (12)	0.02	79 (9)
Thiabendazole	0.01	76 (10)	0.02	75 (6)	0.02	75 (6)
Methiocarb	0.01	82 (18)	0.02	80 (14)	0.04	84 (8)
Imazalil	0.005	75 (17)	0.01	72 (16)	0.01	77 (12)
Hexythiazox	0.06	94 (14)	0.2	92 (11)	0.3	92 (7)

[a] Results were calculated using matrix matched standards.

standard by the buffers. Among the different calibration methods, the calibration with matrix-matches standards was chosen because of its simplicity, and economy.

3.2.2. Accuracy and precision

Table 2 summarizes recoveries, and repeatabilities of the three MS procedures described. Recoveries were 72–94%, almost equal for the three MS stages, and similar to those previously reported for LC–MS (SIM) [31,32]. The relative standard deviation ($n = 5$) for MS determination was below 19%, for MS^2 below 16% and for MS^3 below 12%, showing that MS^3 is most precise procedure followed by MS^2. Data also showed the good accuracy achieved by matrix matched standard calibration.

3.2.3. Limits of detection and quantification

The LOQs obtained using LC–MS, LC–MS^2 and LC–MS^3 in MRM mode are shown in Table 2. They were calculated as the lowest level for which acceptable recoveries (>70%) and repeatabilities (<20%) are obtained [5]. Although fragments selected in MS^2 and MS^3 were the highest intensity ones, the fragmentation of a precursor ion do not provided only one fragment with 100% efficacy. This is the reason why working with standards prepared in methanol, each MS stage reduces sensitivity 10 times. In contrast, analyzing orange extracts, MS mode achieved LOQs twice lower than those obtained by MS^2 and MS^3 that were of the same order of magnitude. The explanation is that the high selectivity of MS^2 and MS^3 achieves a reduction of the background, improving signal-to-noise ratio, which is a very important feature when complex samples are analyzed.

LOQs by MS^3 were similar to those reported in the literature by MS^2 that were 0.1–3 $\mu g\,mg^{-1}$ for benzimidazoles, azoles, and carbamate pesticides in apples and apricots using ion trap [30] and 0.5–2 $\mu g\,kg^{-1}$ for a wide polarity range of pesticides in carrots and potatoes using triple quadrupole [34]. From the literature [21], LOQs achievable with the triple quadrupole are better or equal to those obtained with the ion trap. The unquestionable advantage of using MS^3 is that the method selectivity is improved maintained sensitivity. Main disadvantage is that most pesticides do not provide a MS^3 fragment with enough sensitivity to obtain LOQs below the established MRLs, making the proposed procedure hardly extended to other pesticides.

LOQs were well-below MRLs established or recommended internationally [5–7] that ensures a reliable determination by the three MS modes. The USA legislation set limits of 1.0 $mg\,kg^{-1}$ for hexythiazox, 0.7 $mg\,kg^{-1}$ for imidacloprid and 10 $mg\,kg^{-1}$ for imazalil and thiabendazole. The EU has established limits for carbendazim, imazalil and thiabendazole (5 $mg\,kg^{-1}$). Finally, the FAO/WHO proposed tolerances of 0.5 $mg\,kg^{-1}$ for hexythiazox, 5 $mg\,kg^{-1}$ for imazalil, 0.05 $mg\,kg^{-1}$ for methiocarb and 10 $mg\,kg^{-1}$ for thiabendazole.

Examples of typical chromatograms in the MRM mode are showed in Fig. 3. The chromatographic resolution and peak performance were satisfactory for the studied pesticides in spiked samples.

3.2.4. Feasability of the method

All the experiments performed with the ion trap took about three months and the system was continuously switched with 6–8 analyses per day. Hence over a total of 1000 samples were analyzed. The weakness attributed to this mass analyzer on the limited dynamic range, when particularly dirty matrices, are analyzed has not been observed using the proposed procedure. Orange is a difficult matrix but the results obtained showed that quantification of pesticide residues is reliable.

APCI intensities varied throughout the day, its response was controlled injecting standards before and after the sample extracts. The number of samples injected between the two calibration batches was adjusted in that way that the difference in the response was lower than 15%. About ten samples were analyzed between two standards in each sequence to monitor the detector response. As soon as the difference in the response between two calibration batches becomes unacceptable, the sequence was interrupted and the corona discharge needle in the APCI cleaned.

3.2.5. Application

The viability of LC–MS and LC–MS^n to determine the selected pesticides in oranges was evaluated by analyzing ten samples from an agricultural co-operative located near Valencia city (Valencia, Spain). Table 3 summarizes the

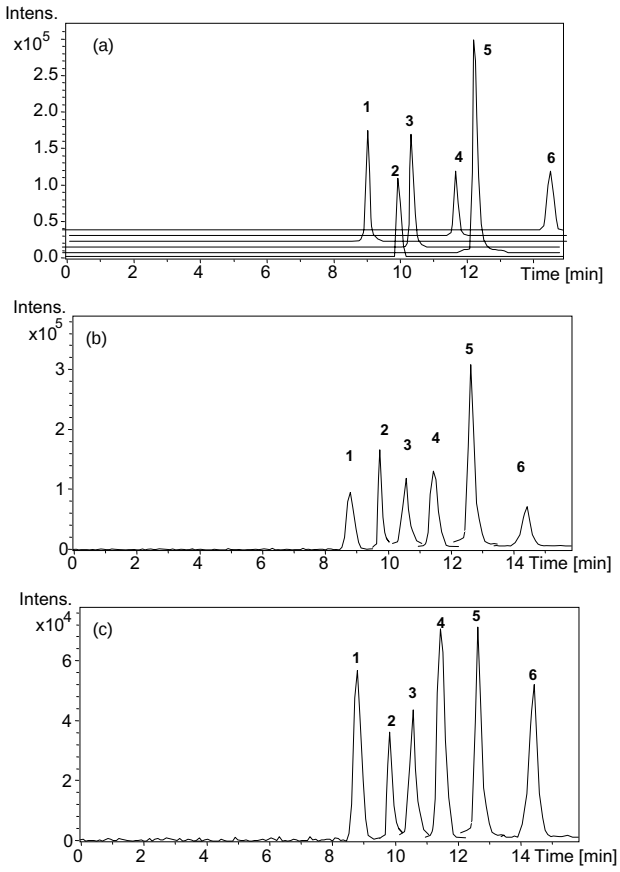

Fig. 3. Extracted ion chromatograms of spiked orange at MS^3 LOQ levels obtained by (a) LC–MS (b) LC–MS^2 and (c) LC–MS^3. Peak identification: 1. Imidacloprid, 2. Carbendazim, 3. Thiabendazole, 4. Methiocarb, 5. Imazalil, and 6. Hexythiazox.

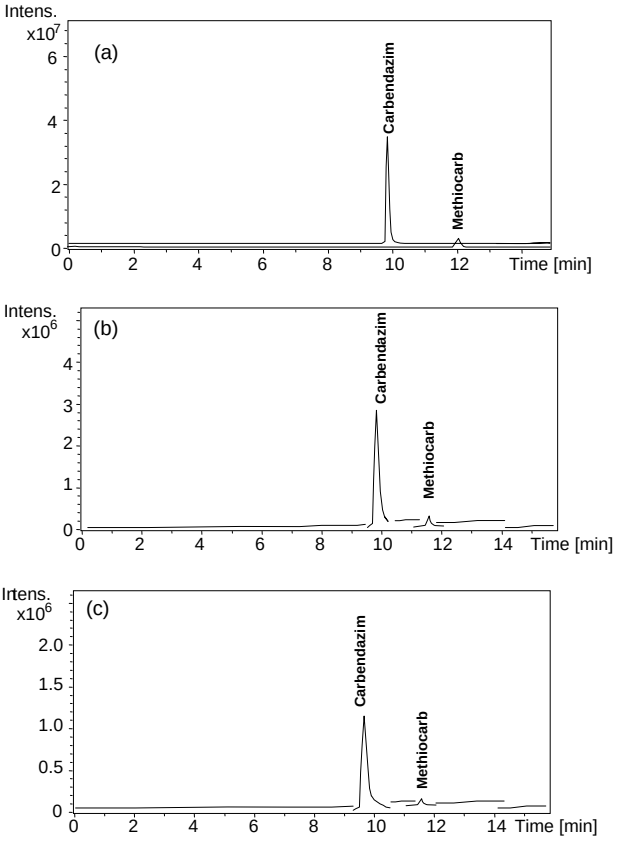

Fig. 4. Extracted ion chromatograms of field treated sample 2 obtained by (a) LC–MS (b) LC–MS^2, and (c) LC–MS^3.

results of the samples containing pesticides, and reveals the presence of several of the studied pesticides in oranges from human consumption, at concentration usually in the $\mu g\,kg^{-1}$ range. There is a sample that has been not included in the Table because no pesticide was detected. It is interesting to note the good agreement between the results obtained by MS, MS^2, and MS^3, except for methiocarb in sample 1. Differences in repeatability between the three MS methods can be clearly observed in this Table. This study also demonstrates that carbendazim, imazalil, imidacloprid and

Table 3
Concentration of the studied pesticides in oranges

Sample	Compound	Concentration $mg\,kg^{-1}$ (percentage R.S.D., $n = 3$)[a]		
		MS	MS^2	MS^3
1	Carbendazim	0.42 (10)	0.45 (9)	0.44 (6)
	Methiocarb	0.06 (16)	0.05 (12)	0.05 (10)
2	Carbendazim	0.29 (9)	0.30 (9)	0.28 (6)
	Methiocarb	0.05 (17)	0.04 (13)	0.04 (9)
3	Carbendazim	0.38 (11)	0.38 (10)	0.38 (6)
	Imazalil	0.42 (8)	0.44 (9)	0.45 (8)
5	Imidaclorprid	0.19 (16)	0.20 (14)	0.18 (10)
	Carbendazim	0.28 (15)	0.28 (12)	0.28 (8)
7	Carbendazim	0.50 (9)	0.52 (13)	0.48 (6)
	Imazalil	0.40 (12)	0.42 (10)	0.41 (8)
9	Carbendazim	0.58 (9)	0.59 (7)	0.60 (5)

[a] Results were calculated using matrix matched standards.

methiocarb are the most omnipresent of the selected compounds. The pesticide concentrations found in oranges were always lower than the limits established by the EU or USA legislations.

As an example, the chromatograms obtained for the orange sample 2 by MS, MS^2 and MS^3 are presented in Fig. 4. Carbendazim, and methiocarb were detected by the three MS methods.

4. Conclusions

This work demonstrates that using APCI–MS, APCI–MS^2 and APCI–MS^3 is possible to characterize pesticides in a quadrupole ion-trap. MS^3 study showed characteristic fragmentation pattern for each compound that provided sufficient structural information and permitted the election of the specific transition for their identification. In this example, MS^3 spectra were used for the first time to quantify a mixture of pesticides.

This method is sensitive and selective for the target analytes in oranges. The limits of quantification of the analytes were between 0.001 and 0.3 mg kg^{-1} for a 5 g sample size, and false positives were not observed. Sample preparation was held to a minimum, consisting of an initial ethyl acetate extraction step. The tandem mass spectrometry with an ion trap provides a reliable and robust tool that can be used for routine analysis of pesticides in orange samples.

The MS^3 provided better results in terms of selectivity and repeatability than MS^2 and MS modes. However, the general results demonstrated that the three MS modes can be successfully applied to determine pesticides in oranges.

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