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Comparison of liquid chromatography using triple quadrupole and quadrupole ion trap mass analyzers to determine pesticide residues in oranges

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Abstract

Liquid chromatography-triple quadrupole/mass spectrometry (LC-TQ/MS) and liquid chromatography-quadrupole ion trap/mass spectrometry (LC-QIT/MS) for determining bupirimate, hexaflumuron, tebufenpyrad, buprofezin, pyriproxyfen, and fluvalinate in fruits have been compared. The differences in the mass spectra obtained by triple and ion trap quadrupoles are discussed, showing how both of them provide interesting features. The evaluation of the two instruments was carried out by ethyl acetate extraction of oranges spiked with the studied pesticides at LOQ and 10 times the LOQ. Results obtained by LC-TQ/MS correlated well with those obtained by LC-QIT/MS. Recoveries were 70–94% by LC-TQ/MS and 72–92% by LC-QIT/MS with the R.S.D. from five replicate analysis 4–14% and 8–18%, respectively. Matrix effects were tested for both techniques by standard addition to blank extracts. Although the matrix effects are not originated in mass analyzer but in the LC/MS interface, they were, generally, more marked by LC-QIT-MS than by LC-TQ/MS. The limits of quantification (LOQs) were 0.005–0.2 mg kg^{-1} by both equipments—appropriate values for determining these pesticides in orange from the regulatory point of view. The results indicate that the TQ provides higher precision, better linearity, it is more robust, and when the purpose of the analysis is quantitative determination, preferable over the QIT. However, the application of both mass spectrometers to analyze orange samples conventionally treated showed that any can be used for qualitative and quantitative purposes.

Keywords: Food analysis; Oranges; Pesticide residues; Quadrupole ion trap; Triple quadrupole

1. Introduction

Most recent developed analytical methods for monitoring and screening pesticide residues in food should meet the EU requirements to ensure adequate sensitivity and selectivity [1]. For this reason, liquid chromatography/mass spectrometry (LC/MS) has become the most popular method to identify and quantify pesticides, and tandem mass spectrometry (MS/MS) is the recommended system because of its high sensitivity, selectivity, and analytical throughput [2–5].

Tandem MS multiplies the stages of mass analysis umpteenth times by preselecting an ion, and analyzing the induced fragments—for instance, by collision with an inert gas such as argon or helium. The most common tandem mass spectrometers for liquid chromatography (LC), triple quadrupole (TQ) and quadrupole ion trap (QIT), are becoming important tools in food analysis, especially in the area of pesticide residues determination in fruits and vegetables [3,6]. TQ combines two mass analyzers by means of a RF-only (quadrupolar or multipolar) collisions cell. The fragmentation is due to the collisions of DC-accelerated ions to a neutral gas, argon in most cases. In the QIT, ions are generated in an external source. A package of ions is trapped in the ion trap by means of low RF voltage on the ring electrode. A variety of procedures are available to scan, select, and perform multiple stage of MS/MS and so on.

The selectivity and easy use given by these new mass spectrometers over simple quadrupole ones make the methods development rapid, sensitive, selective, and reli-

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able. During these last years, a wide variety of analytical schemes have been proposed for many pesticides including ammonium quaternary herbicides, post-harvest fungicides, organophosphorus, and carbamates using both ion trap and triple quadrupole, without any synopsis of their strength and weakness for pesticide analysis [7–22]. There are, in fact, only a limited number of studies that compare both for chlormequat [23], and for triazine and phenylurea pesticides [24,25] because they are expensive and it is difficult dispose of both simultaneously. Several questions can be raised on the above discussion such as what instrument is preferable to determine pesticide residues in fruits and vegetables or how to exploit the possibilities of each one for a particular analysis.

The two mass analyzers have general advantages and disadvantages, widely reported in the literature that can be summarized [3,4,6,17,26–28]. Unique features of an ion trap are that it can perform MS^n, and has a greater sensitivity using scan mode. Main disadvantages are the difficult analyte quantification in complex matrices caused by QIT dynamic limited range and the low proportion of analyte ions compared with other unknown matrix component ions, and restriction of mass range of product ions—only ions with an *m*/*z* larger than ca. one third of the precursor-ion can be efficiently trapped [26,29]. The TQ presents the advantage of the screening strategies versatility because it can operate in full-scan, neutral loss, precursor-ion, and product-ion scan modes but its main drawback is the lack of sensitivity in the scan mode. Limits of detection only can be enhanced using selected reaction monitoring mode (SRM) [17,28].

There is no study that compares the best conditions for optimal sensitivity and selectivity using both mass analyzers to determine pesticides in fruits and vegetables, and that establishes the strong points of each instrument that can be profit to more sensitive, and selective quantification. The aim of this work is to compare the best conditions for bupirimate, hexaflumuron, tebufenpyrad, buprofezin, pyriproxyfen and fluvalinate using LC-TQ/MS and LC-QIT/MS and to discuss the advantages and disadvantages of both mass spectrometers.

2. Experimental

2.1. Chemicals, reagents and samples

Bupirimate, buprofezin, fluvalinate, hexaflumuron, pyriproxyfen, and tebufenpyrad were supplied by Riedel-de Haën (Seelze, Germany). Individual stock solutions were prepared dissolving 10 mg of each compound in 10 ml of methanol and stored in stained glass-stopper bottles at 4 °C; they were stable over a period of, at least, 3 months. Standard working mixtures, at different concentrations, were daily prepared by appropriate dilution of aliquots of the stock solution in methanol and into 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.

Method validation experiments were performed with biologically farmed oranges, obtained from an ecological farming cooperative (Valencia, Spain), which showed no pesticide concentrations. In addition, twelve conventionally farmed and treated orange samples of three different varieties (Naveline, New Hall, and Salustiane), 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.

2.2. Extraction procedure

Organic solvent extraction was carried out by a common procedure as described elsewhere [15,30]. 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 millilitres of ethyl acetate were 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 extract was reconstituted in 1 ml of methanol and filtered by a disposable syringe cellulose filter 0.22 μm from Análisis Vinicos (Tomelloso, Spain).

Spiked samples were prepared by adding 20 or 200 μl of working mixture to 5 g of chopped untreated fruit samples in a blender jar. The spiked sample was allowed to stand for 1 h before extraction to achieve the pesticide distribution in the fruits. Samples were extracted and analyzed in quintuplicate.

2.3. Chromatographic conditions

Analyses were carried out on a Agilent 1100 Series LC system (Agilent, Palo Alto, CA) that included a quaternary pump, an autosampler, and a variable wavelength detector, as well as on a Shimadzu system (consisted of a Shimadzu autoinjector SIL-AD, a Shimadzu high pressure pump LC-10 AD, a Shimadzu degasser GT-154, and a Shimadzu System Controller SLC-10 A.

Separation was always 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 75% of methanol (A) in water

(B) increased by a linear gradient to 90% A in 35 min, then returned to initial conditions in 10 min. The flow rate was 0.8 ml min^{-1}, the column effluent was directly introduced in the ESI interface without splitting, and 40 μl of standard solutions or extracts were injected.

2.4. Triple-quadrupole mass spectrometer conditions

A TQ mass spectrometer Quattro LC from Micromass (Manchester, UK), equipped with a pneumatically assisted electrospray probe, a Z-spray interface and a Mass Lynx NT software Ver. 3.5 was used for the MS/MS analyses coupled to the Shimadzu LC. Parameters were optimized by continuous infusion of a standard solution (10 μg ml^{-1}) via a syringe pump at a flow rate of 20 μl min^{-1}, which was mixed with the mobile phase at 0.8 ml min^{-1} by means of a T piece. Analysis was performed in both positive and negative ion modes (the positive or negative polarity of some voltages change according to the ionization mode). The ESI source values were capillary voltage, 3 kV; extractor, 2 V; RF lens, 2 V; source temperature, 120 °C; desolvation temperature, 350 °C; and desolvation and cone gas (nitrogen 99.99% purity) flows, 400 and 40 l h^{-1}, respectively. The analyzer settings were resolution, 15.0 (unit resolution) for the first and third quadrupoles; ion energy, 2; entrance and exit energies, 0; multiplier, 650; collision gas (argon, 99.995%) pressure 2.73×10^{-3} mbar; interchannel delay, 0.02 s; total scan time, 1.01 s. The MS was optimized for each collision/transition, and SRM chromatograms were recorded with the most favourable conditions for each analyte, by defining four time windows (0–7.5 min, 7.5–12.0 min, 12.0–17.0 min, and 17.0–35.0 min). A delay of 5 min was chosen to protect the source against contamination by salts and early elution compounds.

2.5. Quadrupole ion trap mass spectrometer conditions

The LC-QIT/MS system consisted of an Esquire3000 Ion Trap LC/MS(*n*) system (Bruker Daltonik GmbH, Germany), the Agilent HP1100 LC system, a computer (HP PC) and a data acquisition/processing Daltonic Esquire Control Software system 3.0.

The Esquire3000 was equipped with an ESI source, and operated in both positive and negative polarity. The mass spectrometer was tuned for each compound, optimizing the ionization source parameters, voltages on the lenses and trap conditions in the ExpertTune mode of the Daltonic Esquire Control software whilst infusing a standard solution in the same way that for the TQ at a flow rate of 4 μl min^{-1}. Operating conditions of the source were end Plate 450 V, capillary voltage, 4500 V; nebulizer pressure, 50 psi and drying gas flow 10 l min^{-1} at a temperature of 350 °C. The most adequate lens and block voltages were set by instituting the same four time segments that for the TQ as it is indicated in Table 1.

The mass spectrometer was run in full scan, and SRM modes. Negative and positive ions were detected using the standard scan at normal resolution (scan speed 10,300 *m*/*z*/s; peak with 0.6 FWHM/*m*/*z*). The trap parameters were set in ion charge control (ICC) using rolling averaging set at 2 with a target of 100,000, and maximum accumulation time of 50 ms at *m*/*z* range from 100 to 600 u. Fifteen individual scans were averaged. 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. The fragmentation steps for each compound were optimized visualizing the changes in the intensities of fragments ions, whereas the fragmentation cut-off and the fragmentation amplitude were manually varied.

3. Results and discussion

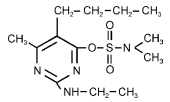
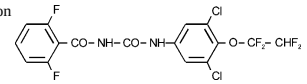
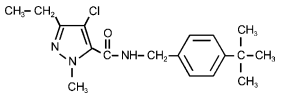
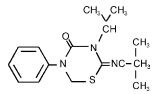
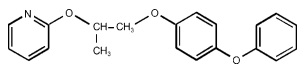
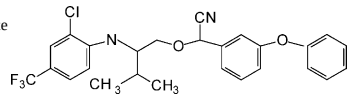
3.1. Comparison of mass spectra obtained by triple quadrupole and ion trap

The complete precursor-product fragmentation pathways observed for the studied pesticides as determined by

Table 1
Lens and block voltages

	Time windows (min)			
	0–10	10–14	14–22	22–35
Compound detected	Bupirimate	Hexaflumuron	Tebufenpyrad Buprofezin Pyriproxyfen	Fluvalinate
Polarity	Positive	Negative	Positive	Negative
Skimmer (V)	30	−54	85	−100
Capillary exit (V)	153	−100	100	−200
Octopole 1 (V)	4	−3.2	7	−7.5
Octopole 2 (V)	2.5	−3.2	4	−3
Trap driver (V)	63	107	90	100
Octopole reference (V)	50	152.5	50	100
Lens 1 (V)	−5	5	−7	1.5
Lens 2 (V)	−100	100	−100	100

Table 2
Precursor-product ion transition

Compound	TQ		QIT		
	Precursor-product ion fragmentation pathways		Precursor product-ion fragmentation pathways MS^2		Product-product ion fragmentation pathways MS^3
Bupirimate 	339 (40) $[M+Na]^+$	–	339 (20) $[M+Na]^+$		
	317 (100) $[M+H]^+ \rightarrow$	272 (5) $[-NH(CH_3)_2]$	317 (100) $[M+H]^+$	272 (20) $[-NH(CH_3)_2]$	
		237 (10) $[-SO_2CH_3]^+$		237 (100) $[-SO_2CH_3] \rightarrow$	210 (80) [–HCN]
		210 (30) $[-SO_2NH(CH_3)_2]^+$		210 (22)$[-SO_2NH(CH_3)_2]$	195 (20) $[-HCN\text{-}CH_4]$
		166 (100) $[C_4N_2HOHCH_3C_4H_9]^+$		195 (10) $[-SO_2NH(CH_3)_2\text{-}CH_4]$	181 (80) $[-HCN\text{-}H_2O]$
		150 (26)$[C_4N_2HCH_3C_4H_9]^+$		181 (10) $[-SO_2NH(CH_3)_2\text{-}H_2O]$	166 (100) $[-HCN\text{-}NC_2H_5]$
		108 (99) $[HSO_2NH(CH_3)_2]^+$		166 (10) $[-SO_2NH(CH_3)_2\ NC_2H_5]$	150 (90) $[-HCN\text{-}NC_2H_5\text{-}H_2O]$
				150 (10) $[-SO_2NH(CH_3)_2\ NC_2H_5H_2O]$	136 (30) $[-HCN\text{-}NC_2H_5\text{-}H_2O\text{-}CH_4]$
Hexaflumuron 	459 (100) $[M-H]^- \rightarrow$	439 (100) $[-FH]^-$	459 (80) $[M-H]^- \rightarrow$	439 (100) $[-HF] \rightarrow$	319 (100) $[-HF\text{-}C_2F_4]$
	439 (15) $[M-FH]^-$		439 (100) [–HF]		
Tebufenpyrad 	356 (100) $[M+Na]+$	–	356 (20) $[M+Na]^+$		
	334 (50) $[M+H]+ \rightarrow$	147 (100) $[CH_2C_6H_4C(CH)_3]^+$	334 (100) $[M+H]^+$	278 (20) $[-HC(CH_3)_3]$	
		117 (90) $[CH_3C_6H_4C_2H_3]^+$		200 (15) $[-C_6H_5C(CH_3)_3]$	
				171 (100) $[-NC_{11}H_{16}] \rightarrow$	156 (100) $[-CH_4]$
				147 (80) $[CH_2C_6H_4C(CH)_3]^+$	143 (12) $[-C_2H_4]$
				117 (20) $[CH_3C_6H_4C_2H_3]^+$	128 (40) $[-CH_4\text{-}C_2H_4]$
Buprofezin 	328 (10) $[M+Na]^+$		328 (15) $[M+Na]+$		115 (10) $[-C_2H_4\text{-}CO]$
		116 (100) $[-C_6H_5NCH_2\text{-}OCNCH(CH_3)_2]$	201 (75) $[-C_6H_5NCH_2]$		
	306 (100) $[M+H]^+ \rightarrow$	201 (35) $[-C_6H_5NCH_2]$	306 (100 $[M+H]^+ \rightarrow$	201 (100) $[-C_6H_5NCH_2] \rightarrow$	116 (100) $[-OCNCH(CH_3)_2]$
Pyriproxyfen 	344 (70) $[M+Na]^+$		344 (50) $[M+Na]^+$		
	322 (100) $[M+H]^+ \rightarrow$	227 (20) $[-C_5H_4NOH]$	322 (100) $[M+H]^+ \rightarrow$	227 (100) $[-C_5H_4NOH]$	
		96 (100) $[C_5NH_4OH+H]^+$			
Fluvalinate 	474 (100) $[M-HCN]^- \rightarrow$	446 (100) $[-CH_2CH_2]$	339 (100) $[M-(C_6H_5)_2O] \rightarrow$	162 (100) $[-C_6H_3CF_3Cl]$	

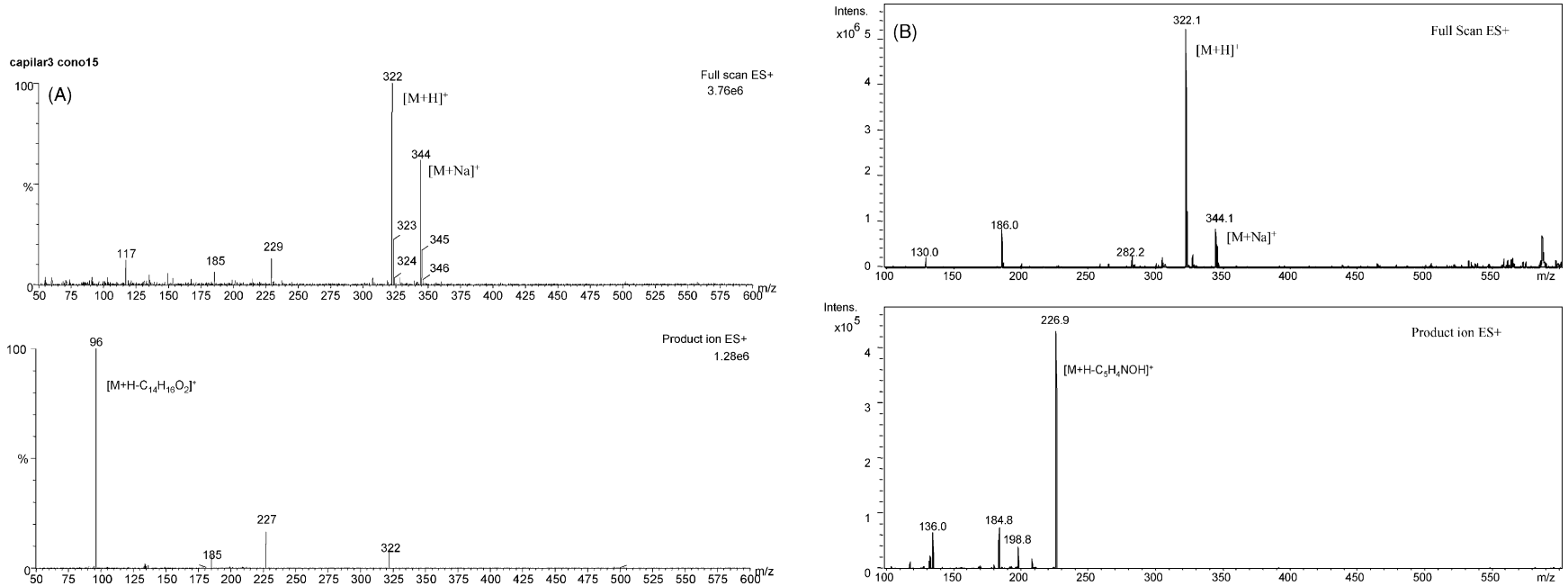


Fig. 1. Positive ion electrospray full scan mass spectrum and product ion spectrum of pyriproxyfen using *m*/*z* 322 as precursor obtained by (A) TQ and (B) QIT.

product ion and precursor ion scans are given in Table 2. The assignments for these transitions are also shown. Fragmentation is similar by QIT and TQ, except for fluvalinate. Although the fluvalinate MS is equal by both mass analyzers at high concentrations (main ion at *m/z* 474 corresponding to the neutral loss of HCN), at lower concentrations (those that are commonly present in fruits) QIT/MS is characterized by the fragment ion at *m/z* 339 corresponding to the neutral loss of phenol. In addition, a number of product ions observed for the pesticides in TQ differ from those observed in QIT product ions mass spectra. As it has been discussed in the literature [23–25], the reason is the differences between the two instruments, such as collisions with Ar or He, excitation by DC or *m/z*-selective RF waveforms, collision energy and that only ions with a *m/z* larger than ca. one-third of the precursor-ion *m/z* can be efficiently stored in the QIT for subsequent detection, the latter difference is especially important for pyriproxyfen, buprofezin and bupirimate, the most intense product ions of which were at *m/z* 96, 116 and 108, respectively, by TQ. They are lost by QIT. Fig. 1 illustrated the MS and MS/MS spectra for pyriproxyfen obtained by TQ and QIT.

A trend was observed for the formation of strong signals for sodium adducts using ESI interface, which did not provide product ions at any *m/z*. This is the reason why fluvalinate and hexaflumuron were determined in negative instead of the positive ionization mode. Using a QIT, the sodium adducts signal can be reduced in protonated molecule response interest by adjusting the lenses and block voltages as displays Fig. 2. The electrostatic lenses and split RF ion guide are responsible for focussing the ions from the source to the trap. The most probable theoretical background is that variations in the voltage modify the efficiency to focus in the source the different $[M+H]^+$ ions and $[M+Na]^+$. The relative abundance of the protonated molecules to the sodium adducts increase.

The unique feature of the ion trap is that it can perform multiple stages of MS^n. However, fluvalinate and pyriproxyfen only gave a MS/MS product ion that can not be further fragmented. Hexaflumuron, tebufenpyrad, and pyriproxyfen product ions lack the adequate sensitivity to achieve quantitative analysis by MS^3. Bupirimate is the only pesticide that provided MS^3 fragmentation with the adequate sensitivity. A study that uses MS^3 product ions for quantification of carbendazim, henythiazox, imazalil, imidacloprid, methiocarb,

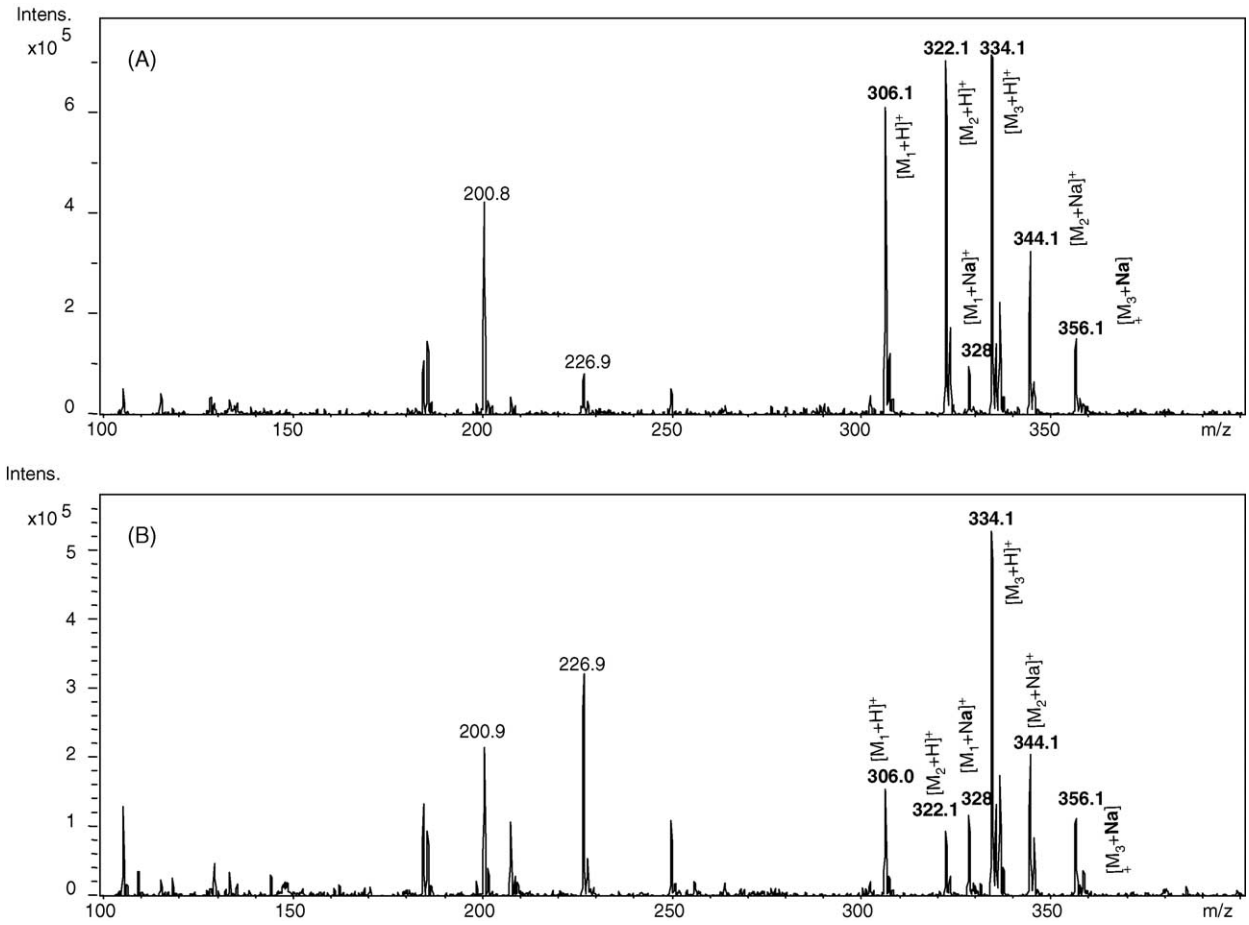


Fig. 2. Positive ion electrospray full scan mass spectra of M_1 = buprofezin, M_2 = pyriproxyfen, and M_3 = tebufenpyrad. Highlight the differences in the intensity of the protonated molecule and the sodium adduct varying lenses and block voltages: (A) skimmer = 85 V, capillary exit = 100 V, trap driver = 90 V and (B) skimmer = 100 V, capillary exit = 200 V, trap driver = 125 V.

and thiabendazole residues in oranges [30] demonstrated that, in some cases, it is possible to characterize pesticides in a QIT by MS^3. The LOQ obtained for the analytes were between 0.001 and 0.3 mg kg^{-1}. The unquestionable advantage is the improvement of the method selectivity. Main disadvantage is that most pesticides do not provide a MS^3 fragment with enough sensitivity to obtain LOQs below the established MRLs. The selectivity even with one transition is quite adequate. However, selectivity and specificity are parameters that are always good to increase. The guidelines of the EU [31], recommended that, if possible, the ions selected for medium/high resolution MS/MS should be characteristic of the analyte, not common to many organic compounds. Although, the selected ions are typical of the studied pesticides, there are always other organic compounds that can be fragmented to them. In ion trap mass spectrometers, the MS/MS may be carried out repetitively on a sequence of product ions (MS^n), which is not usually practical with low-level residues. However, when good sensitivity can be achieved is very interesting, selective and specific.

The TQ provides inadequate sensitivity using product scan-mode. Because of the structural diversity of these pesticides, the constant neutral loss is not useful for the current type of application. The wide range of scanning modes is restricted to the SRM mode. On the contrary, QIT provides an adequate sensitivity using product-scan mode. The transitions of interest are easily extracted from the product scan chromatogram to further quantify.

The selected ion transitions used for the quantitative LC-ESI/MS/MS method are indicated in Table 3. These ions were selected according to highest sensitivity where possible.

Qualitative identification criteria in the target compounds was based on the LC retention time of the analyte compared to that of a standard (±2%), the specific transition selected, which were characteristic of the analyte, not common to many organic compounds, and ration of different product ions (when it is possible) within the 10% of the ratios obtained for the standard. According to EU guidelines [31] MS/MS provides sufficient evidence of the identity and quantity.

3.2. Comparison of method validation

The performance of the method was evaluated according to EU guidelines [1]. The LOQ was established as the lowest analyte concentration that provides acceptable recoveries (>70%) and precision (<20%). The percentage of recovery, the repeatability (within-day precision) and the reproducibility (day-to-day precision) were determined at two spiked levels (LOQ and 10 times the LOQ). The recoveries were determined using standards prepared in orange extract to compensate the matrix effects (even though, in the case of TQ, were not necessary as it was demonstrated latter). Recovery obtained does not depend on the type of MS system but the sample pre-treatment procedure. However, coeluting, undetected matrix components may reduce or enhance the ion intensity of the analytes and affect the reproducibility and accuracy of the assay [32].

The results are presented in Table 4. Good recoveries, repeatabilities and reproducibilities were obtained for all pesticides using the two mass analyzers. Recoveries were 70–94%, with a reproducibility ranged from 8 to 19% by LC-TQ/MS and 72–92% with a reproducibility ranged from 12 to 19% by LC-QIT/MS. Although recovery was similar by both mass analyzers and precision was within the range of the EU guidelines, it should be noted the best precision obtained using TQ.

Figs. 3 and 4 show a chromatogram of the extraction and separation of the six pesticides spiked at LOQ levels as they are defined by the EU [1] (see Table 4) in orange and an extract blank orange. The results, at this concentration level, are fully satisfactory and confirmation of the target compounds can be easily achieved. The reported LOQ can be a little different of those expected from the S/N but it should be taken into account that recovery and R.S.D. are also taken into account. From these chromatograms, the limit of detections (LOD, defined as S/N = 3) were estimated to be in the range of 0.5–20 μg kg^{-1} by either of the two mass analyzers, which are in agreement with those reported in the literature [7,18,22,30]. No peaks were detected in

Table 3
Transitions and conditions used for quantification by TQ and QIT

Compound	TQ				QIT			
	Transitions	Cone (V)	Collision (eV)	Dwell (s)	Transitions	Cut-off	Amplitude	Width
Bupirimate	317 → 166 317 → 108	30	25	1.2	317 → 237	100	1.5	1
Hexaflumuron	459 → 439	20	10	0.5	459 → 439	100	1.2	1
Tebufenpyrad	334 → 146 334 → 171	30	30	1.2	334 → 171	100	1.5	1
Buprofezin	306 → 201 306 → 116	12	20	1.2	306 → 201	100	1.0	1
Piryproxyfen	322 → 96 322 → 227	15	15	1.2	322 → 227	100	2.0	1
Fluvalinate	474 → 446	20	12	0.3	339 → 163	100	1.0	2.0

Table 4
Recoveries, repeatability and reproducibility at the LOQ and 10 times LOQ levels

Compound	Concentration (mg kg^{-1})	TQ			QIT		
		Recovery (%)	Repeatability (R.S.D. %, $n=5$)	Reproducibility (R.S.D. %, $n=5$)	Recovery (%)	Repeatability (R.S.D. %, $n=5$)	Reproducibility (R.S.D. %, $n=5$)
Bupirimate	0.005	80	16	19	82	18	19
	0.05	86	6	12	89	9	16
Hexaflumuron	0.05	74	5	16	72	8	18
	0.5	77	4	10	75	10	14
Tebufenpyrad	0.05	80	6	18	76	12	19
	0.5	83	7	11	81	11	15
Buprofezin	0.02	87	7	10	79	10	14
	0.2	82	5	9	83	8	12
Piryproxyfen	0.1	93	8	11	89	12	15
	1.0	94	6	8	92	13	17
Fluvalinate	0.2	79	9	13	69	11	16
	2.0	70	8	10	88	17	19

unfortified biologically farmed oranges for any of the studied pesticides.

Determination of matrix effects was carried out by analyzing in duplicate six standards of different concentrations ranging from LOQ to 1000 times LOQ in methanol and in orange extract, and comparing the slopes of the calibration curves. For each pesticide the slope of the calibration curve obtained for the standards in methanol and orange extract

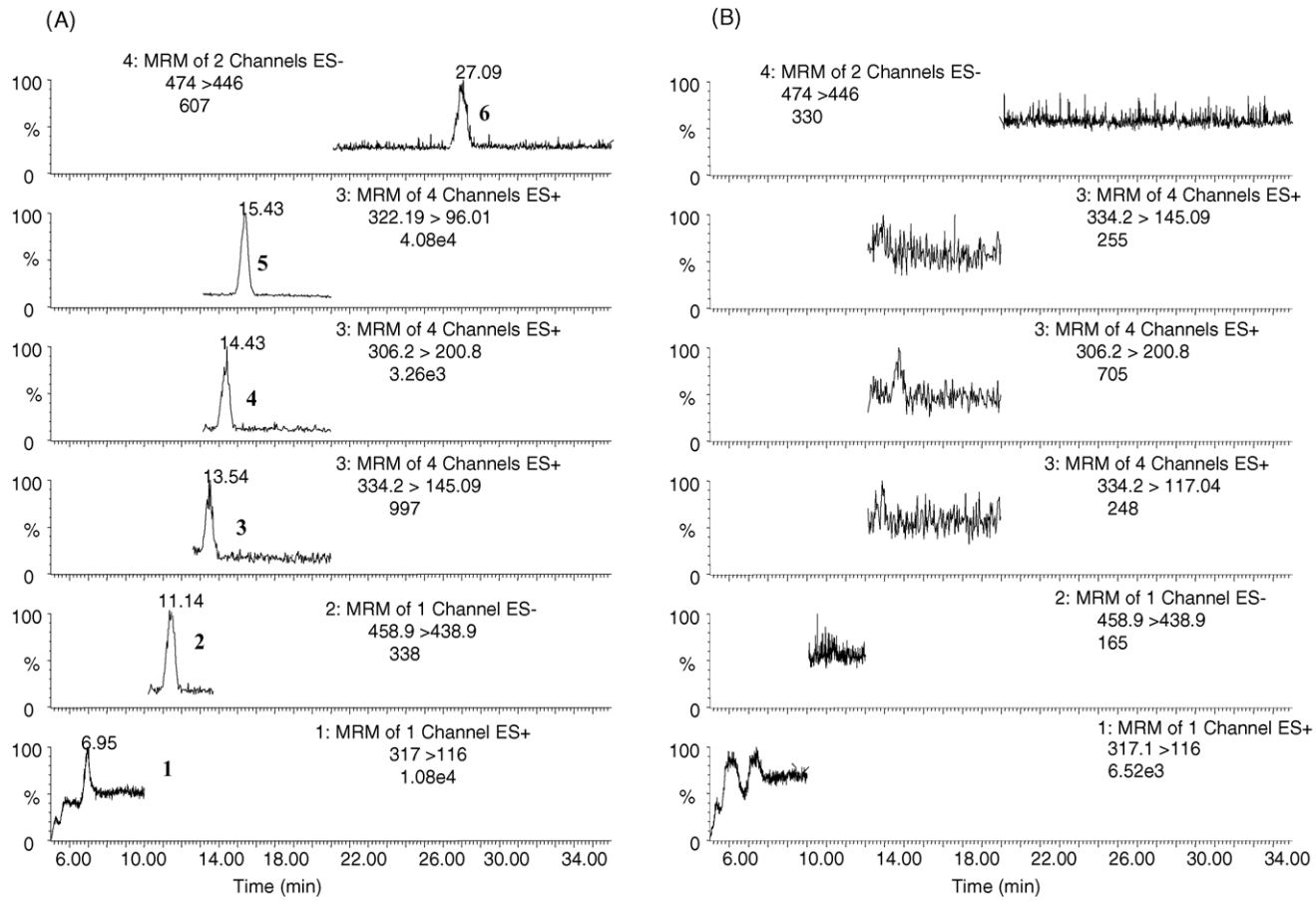


Fig. 3. LC/MS/MS chromatograms (SRM mode, for *m/z* transitions, see Table 3) using a TQ of (A) orange extract spiked at the LOQ level, and (B) orange extract without the pesticides. Peak identification: (1) bupirimate, (2) hexaflumuron, (3) tebufenpyrad, (4) buprofezin, (5) pyriproxyfen and (6) fluvalinate.

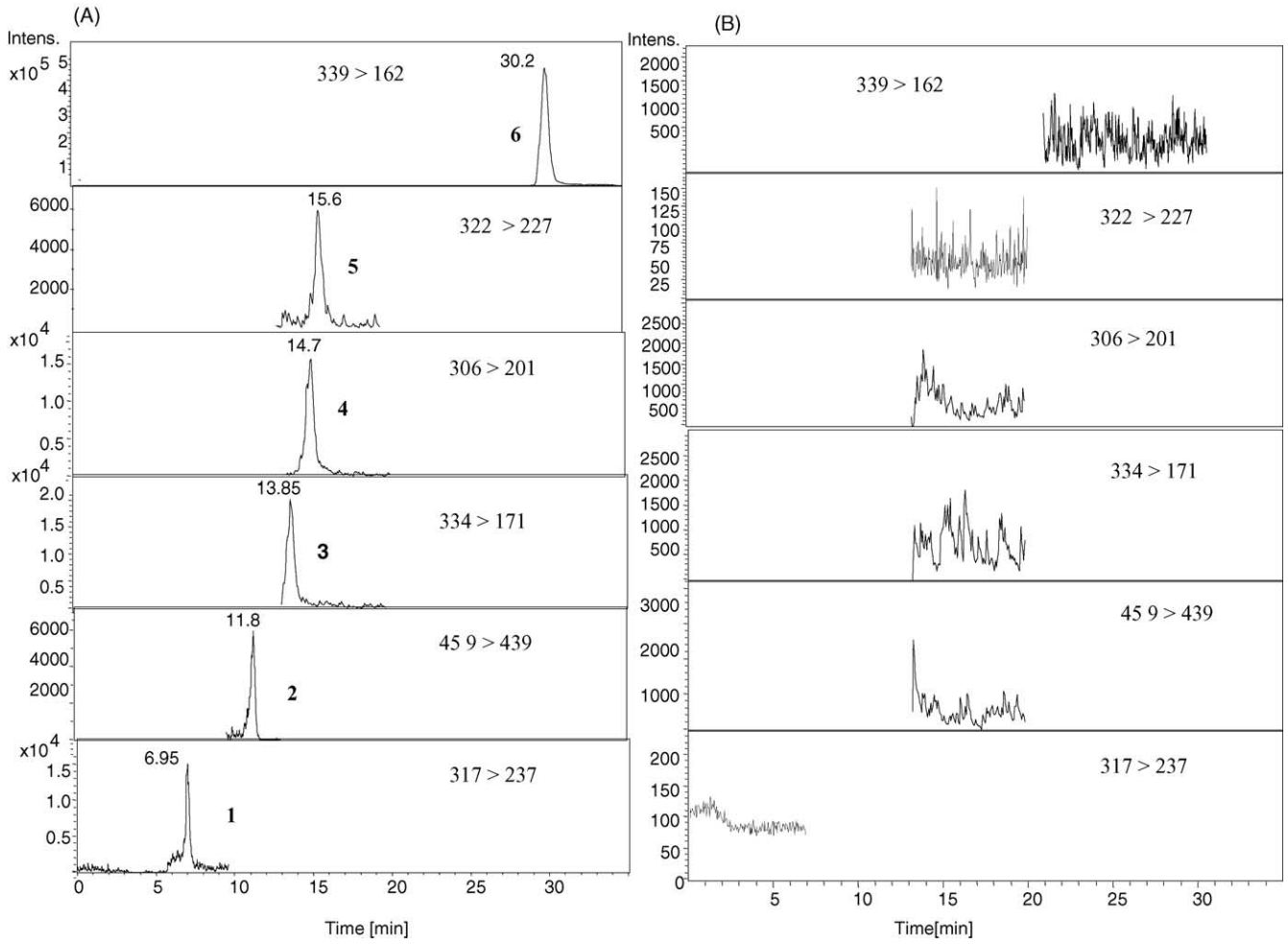


Fig. 4. LC/MS/MS chromatograms (SRM mode, for *m*/*z* transitions, see Table 3) using a QIT of (A) orange extract spiked at the LOQ level, and (B) orange extract without the pesticides. Peak identification as in Fig. 3.

Table 5
Matrix calibration of a biologically farmed orange in comparison with standard calibration[a]

Compound	TQ				QIT			
	Slope	*y*-intercept	*r*	Slope matrix/ slope standard	Slope	*y*-intercept	*r*	Slope matrix/ slope standard
Bupirimate	102208 (M)[b] 102266 (S)[c]	−71 (M) −70 (S)	0.999 (M) 0.999 (S)	0.999	28679700 (M) 30189158 (S)	−278734 (M) −232374 (S)	0.993 0.994	0.95
Hexaflumuron	539 (M) 539 (M)	−41 (M) −41	0.999 (M) 0.999 (S)	1.0	404115 (M) 449017 (S)	50065 (M) 45687 (S)	0.992 0.995	0.90
Tebufenpyrad	1201 (M) 1199 (S)	84 (M) 82 (S)	0.999 (M) 0.999 (S)	1.002	3325103 (M) 4156379 (S)	−80885 (M)	0.994 0.997	0.8
Buprofezin	11538 (M) 11538 (S)	698 (M) 697 (S)	0.999 (M) 0.998 (S)	1.0	11907253 (M) 11919172 (S)	−442253 (M) −400232 (S)	0.990 0.996	0.999
Piryproxyfen	65474 (M) 65477 (S)	3192 (M) 3191 (S)	0.999 (M) 0.999 (S)	0.999	1124026 (M)	−36922 (M)	0.991 0.993	1.25
Fluvalinate	133 (M) 157 (S)	−16 (M) −17 (S)	1.000 (M) 0.998 (S)	0.846	1489107 (M) 1789792 (S)	163588 (M) 159996 (S)	0.941 0.980	0.832

[a] The data are obtained by six level calibration in triplicate.
[b] (M) = matrix-calibration.
[c] (S) = standard-calibration.

Table 6
Concentration of studied pesticides in oranges

Sample	Compound	Concentration mg kg^{-1} (R.D.S. %, $n = 3$)	
		TQ	QIT
1	Pyriproxyfen	3.24 (9)	5.01 (12)
2	Pyriproxyfen	0.03 (16)	0.01 (22)
	Fluvalinate	0.16 (12)	0.25 (16)
3	Tebufenpyrad	0.12 (14)	0.11 (14)
	Pyriproxyfen	0.08 (20)	0.10 (24)
4	Pyriproxyfen	0.05 (17)	0.03 (22)
	Fluvalinate	0.85 (8)	1.02 (12)
5	Tebufenpyrad	0.25 (12)	0.24 (14)
6	Hexaflumuron	0.31 (12)	0.35 (14)
	Tebufenpyrad	0.09 (16)	0.08 (19)
7	Pyriproxyfen	0.64 (10)	0.59 (14)

are included in Table 5. The standard calibration functions of the LC-TQ/MS/MS system were linear for three orders of magnitude, $r > 0.998$. In addition, matrix effects were considered not significant, except for fluvalinate that presents a decrease in the response ca. 15% in orange extracts. Quantitative analyses can be done by external standard method using the standards prepared in methanol.

On the contrary LC-QIT/MS/MS showed poorer linearity over the calibration range. For bupirimate, buprofezin, pyriproxyfen and tebufenpyrad the calibration could be interpolated by linear regression, which fitted very well at higher concentrations but deviate at the lower ones. The calibration curve of hexaflumuron is characterized by an increase in the response at the lower values and a decrease at the higher ones. Fluvalinate provided no linear response at all. The above describe poor linearity of the LC-QIT/MS/MS system has already been discussed in the literature [18], which recommend to solve the problem, 2-point calibration, using standards surrounding the sample according to the EU guidelines [1]. Matrix effects observed with the QIT were also more marked than with the QT. Most of the compound showed some suppression in the response in orange extracts that is almost negligible for bupirimate (5%) and hexaflumuron (10%) and ca. 20% for tebufenpyrad and fluvalinate. Only pyriproxyfen presented a enhancement in the response and buprofezin showed no matrix effect. Therefore, to avoid biased results, matrix-matched standards should always be used for calibration of unknown samples. Although the matrix effects are originated in the LC/MS interface, not in the mass analyzer, this could be explained because the different design of the interface in both equipments.

3.3. Application to fruit analysis

Optimized methods for MS/MS analysis by QIT and TQ were applied to 12 conventionally farmed orange samples. Seven samples contain one or more of the studied pesticides. Method precision was checked by analyzing the samples in triplicate. The mean values obtained, as well as, the associated relative standard deviations (R.S.D.), are presented in Table 6. It is interesting to note the good accuracy obtained by both mass analyzers to quantify tebufenpyrad and hexaflumuron. Satisfactory results were obtained for the studied pesticides by TQ with R.S.D. below 19%. For QIT, higher R.S.D. were found, with values from 14 to 24%. The most detected pesticide at highest levels was pyriproxyfen that was present five samples in the range of 0.03–3.24 mg kg^{-1} by TQ and of 0.01–5.01 mg kg^{-1} by QIT.

4. Conclusion

Some of the generic advantages reported for each tandem mass spectrometer are not applicable when trace analysis is performed, because, in this case, the restricting parameter is sensitivity. The use of MS^n (with $n = 3$ or higher) with the QIT or of the screening strategies other than SRM of the TQ provided detection limits higher than those required to determine pesticides at levels lower than MRLs.

Each instrument has its good and bad points. TQ provides higher precision, better linearity, less matrix interferences, and it is more robust than the QIT to determine pesticide residues in complex matrices, such as oranges. Although TQ can only monitor a number of selected specific transitions, it would be possible to acquire simultaneously at least two transitions for each pesticide, which is sufficient for this type of application with respect to selectivity/confirmation of peak identity. Limitations of QIT occur in dynamic range, accurate mass measurement, and quantitative precision. As a compensation for, it is possible use product-ion scan with an excellent sensitivity. The results presented in this report indicated that QIT is a possible alternative option to determine the selected compounds in orange samples.

At a summary, both, TQ and QIT enable sensitive and selective analysis of bupirimate, buprofezin, fluvalinate, hexaflumuron, pyriproxyfen, and tebufenpyrad in oranges at μg kg^{-1}, when the adequate measures to prevent errors or lack of precision are taken by the analyst.

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