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Liquid chromatography–electrospray quadrupole ion-trap mass spectrometry of nine pesticides in fruits☆

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Abstract

A liquid chromatographic method, with electrospray ionization tandem mass spectrometry (LC–ESI-MS–MS), has been developed for determining acrinathrin, carbosulfan, cyproconazole, λ-cyhalothrin, kresoxim methyl, pyrifenox, pyriproxyfen, propanil, and tebufenpyrad in fruits. The ions prominent in ESI spectra were $[M + H]^+$ and $[M + Na]^+$. In the mass analyzer, collision-induced dissociation fragmentation involved common pathways, for example, product ions of $[M + H]^+$ resulted from the cleavage of the carbamic group or an oxygen bound. The utility of the method is demonstrated by the analysis of crude extracts obtained by matrix solid-phase dispersion (MSPD) using C_{18} as dispersant and dichloromethane-methanol as eluent, and by solid–liquid extraction (SLE) with ethyl acetate and anhydrous sodium sulfate. Mean recoveries ranged from 51.5 to 108%, with relative standard deviations <16%, were obtained for MSPD and from 59 to 101% with relative standard deviation <17% for SLE. However, for most compounds, limits of quantification are better by SLE (0.01–0.4 mg kg^{-1}) than by MSPD (0.05–2 mg kg^{-1}). During the validation process, the procedure was tested for matrix effects, blanks and stability of the system. Considerably matrix effects in the ESI ionization process were detected by comparing standard calibration, and matrix calibration. Because of this, detected residues were quantified from interpolation against calibration data obtained using matrix matched standards.

Keywords: Tandem mass spectrometry; Ion trap; Pesticides; Fruits; Matrix effects; Food analysis

1. Introduction

Nowadays, pesticides determination in food, especially in fruits and vegetables, is a priority objective to evaluate its quality and to avoid possible risk for the human health, because pesticide residues entered by way of the food chain are able to cause mainly cronical toxic effects [1]. They comprise a large group of substances with the only common characteristic of being effective against pest and constituting a challenge for the analyst since there is not a collective method to determine them [2,3].

For many years, gas chromatography (GC) was the technique of choice for determining pesticides because its favourable combination of very high selectivity and resolution, good accuracy and precision, wide dynamic concentration range and high sensitivity for thermostable and volatile molecules [2–5]. However, it is of limited value because most present-day extensively used pesticides are polar, low volatile and/or thermolabile compounds not directly determinable by GC [2,3,6,7]. Most of these polar pesticides can be efficiently separated by liquid chromatography (LC) [8] without a preceding laborious derivation step. Recent developments in the detection and separation facilities of LC have extended its applicability in pesticide residue analysis [9,10].

One of these developments was the introduction into the market of robust and easy-operating liquid chromatography–mass spectrometry (LC–MS) instruments capable to provide selective separation on-line with sensitive and selective mass

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detection [9–11]. Two major advances: the atmospheric interfaces [atmospheric chemical ionisation (APCI) and electrospray ionisation (ESI)], and tandem mass spectrometry [triple quadrupole (TQ) or quadrupole ion trap (QIT)] have opened new prospects in the field of pesticide residue determination in complex matrices as fruit [12–14]. Especially, QIT has found wide application in structure elucidation studies because main advantages of QIT are: (1) to obtain structural information of analytes that allows the study of unknown compounds; (2) to generate chromatograms and mass spectra that are less influenced by analytical background noise than those obtained by LC–MS; and (3) to increase the specificity of detection because there is no uncertainty on the origin of fragments in the product ion spectrum [15,16]. Its high sensitivity also provides the detection of levels of residues in agreement with EU requirements for pesticides analysis [17].

In quantitative analysis, one of the major problems is the enhancement or suppression of the analyte signal in the presence of the matrix components, which produces poor accuracy of results. Matrix signal suppression is believed to result from competition between matrix components and analyte ions [18], as a result, one of the possible approaches to eliminate matrix interferences is to reduce the amount of components that enter into the MS detector at the same time as the analyte, which can be reached by a more selective extraction procedure or more extensive sample clean-up [19]. Sample preparation is essential to develop an efficient method, practical and appropriate for determining pesticides and is still considered to be slow, labour-intensive, and even the restrictive step in laboratory processes [2,3,18]. As a consequence, extraction procedures need to be modified and simplified not only to shorten the working times but also to reduce the matrix components presents in the extracts. One of the most promising techniques to reduce the matrix interferences is matrix solid-phase dispersion (MSPD) that carry out extraction and clean-up in the same step with good recovery and reproducibility, saving the analysis time and organic solvent employed [20–22]. MSPD has been successfully applied to isolate carbamates [23], benzoylureas [24] and fungicides [25,26] from fruits and vegetables followed by LC–MS. However, its combination with tandem mass spectrometry only has been reported for some carbamates and organosphosphorus pesticides in fruit juices [27].

The aim of this work is to develop a rapid, specific and sensitive method based in MSPD followed by LC–ESI–QIT-MS for determining nine pesticides and to compare it with traditional solvent extraction based on ethyl acetate use. These pesticides (acrinathrin, carbosulfan, cyproconazole, λ-cyhalothrin, kresoxim methyl, pyrifenox, pyriproxyfen, propanil, tebufenpyrad) were selected because they are widely used in the Valencia Community for oranges and strawberries. Individually, these compounds have been scarcely determined and there is no work reporting on their simultaneous analysis in any matrix.

2. Experimental

2.1. Materials and standards

Pesticides (acrinathrin, carbosulfan, cyproconazole, λ-cyhalothrin, kresoxim methyl, pyrifenox, pyriproxyfen, propanil, 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. All these stock solutions were stored no more than 3 months, except that of carbosulfan that was prepared each week to avoid compound degradation. Standard working mixtures at appropriated concentrations of each pesticide were prepared daily by dilution of aliquots of the stock solutions in methanol or in matrix extract.

HPLC-grade methanol was purchased from Merck (Darmstadt, Germany) and deionised water (<8 MΩ cm resistivity) was obtained from the Milli-Q SP Reagent Water system (Millipore, Bedford, MA, USA). All the solvents and solutions were filtered through a 0.45 μm cellulose filter from Scharlau (Barcelona, Spain) before use.

MFE C_{18} solid phase (particle diameter in the range of 45–55 μm and pore diameter of 60 Å) was acquired from Análisis Vínicos (Tomelloso, Spain).

2.2. Sample preparation

The samples analyzed—oranges and strawberries—were from organic farming without use of pesticides and obtained from a local market. The samples were taken in accordance with the guidelines of the European Union (EU) [22]; which means that, as far as possible, the sample would be taken at various places distributed throughout the lot (size ca. 50 kg). The samples, weighted 1 kg, consisted of 10 individual fruits. They were analyzed unwashed and with the peel intact. A representative portion of sample (200 g whole fruit) was chopped into small pieces and homogenized in a Bapitaurus food chopper (Taurus, Berlin, Germany).

2.2.1. Matrix solid-phase dispersion procedure

Portions of 0.5 g were weighed and placed into a glass mortar (50 ml) and were gently blended with 0.5 g of C_{18} for 5 min using a pestle, to obtain homogeneous mixture. For the preparation of fortified samples, 100 μl of the standard working solutions were added to 0.5 g of sample. Then, they were allowed to stand at room temperature for 3 h.

The homogeneous mixture was introduced into a 100 mm × 9 mm i.d. glass column, and eluted dropwise with 10 ml of a dichloromethane–methanol (50:50, v/v) mixture by applying a slight vacuum. The eluate was collected in a graduated conical tube (15 ml capacity) and concentrated, under a slight stream of nitrogen, to 0.5 ml.

2.2.2. Ethyl acetate extraction procedure

Fifty grams of chopped sample were placed in a 250 ml glass beaker and mixed thoroughly with 100 ml of ethyl ac-

Table 1
IT-MS^n conditions time scheduled

Parameters	Time windows			
	0–21 min	21–26.5 min	26.5–31 min	31–41 min
Nebulizer pressure (p.s.i.; 1 p.s.i. = 6894.76 Pa)		60		
Drying gas temperature (°C)		350		
Drying gas flow (ml min^{-1})		10		
Capillary (V)		−4500		
Skimmer (V)	24.1	40.1		22.13
Capillary exit (V)	127.37	172.1		118.03
Octopole 1 dc (V)	3.5	4.04		2.5
Octopole 2 dc (V)	1.5	2.30		1.74
Trap driver (arbitrary units)	39.02	46.8		40.25
Octopole reference (Vpp)	78.8	68.95		78.69
Lens 1 (V)	−3	−0.3		−5
Lens 2 (V)	−91.5	−100		−92.62
ICC		Yes		
Target (no. of ions)		10,000		
Max. accurate time (ms)		5		
Averages		8		

etate and 50 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 500 ml rotator-evaporation flask. The solid residue was again homogenized with 100 ml of ethyl acetate, filtered trough the anhydrous sodium sulfate and collected with the first extraction fraction. Twice, 25 ml ethyl acetate was used 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 <5 ml and then reconstitute it to 10 ml with ethyl acetate in a volumetric flask.

2.3. LC–multiple MS (MS^n) analysis

LC–QIT-MS was performed using an Esquire 3000 Ion Trap LC–MS^n system (Brucker Daltonik, Germany) and an Agilent 1100 Series LC System that includes a quaternary pump, an autosampler and a variable-wavelength detector. The mass spectrometer was equipped with an ESI source, and operated in positive polarity at a temperature of 325 °C at the conditions reported in Table 1. The Esquire 3000 was tuned for pesticides, optimizing the voltages on the lenses in the Expert Tune mode of Daltonic Esquire Control software whilst infusing a standard solution (10 μg ml^{-1}) by a syringe pump at a flow rate of 250 μl h^{-1}, which was mixed with the mobile phase at 0.6 ml min^{-1} by means of a T piece. The optimised tune parameters were setted for groups of compounds via time segments definition, as it is also summarized in Table 1.

The mass spectrometer was run in full scan and multiple reaction monitoring (MRM) modes. Ions were detected in ion charged control (ICC) mode. Full scan mode was performed with a target of 10,000 ions and maximum accumulation time of 5 ms at *m*/*z* range from 100 to 600 U. MRM was carried out setting the target at 10,000 ions and maximum accumulation time at 5 ms. Positive ions were detected at unit resolution (scan speed). Four scans were summarized for one spectrum, resulting in a spectral rate of 0.4 Hz. MS–MS was performed by selected product ion monitoring, isolation of the parent ion, and collision-induced dissociation (CID) with helium. Table 2 outlines the conditions optimized for product ions that were also regulated via time segment definition.

Table 2
Time scheduled MRM ions

Pesticides	Group	Time (min)	MRM ions			
			MS (*m*/*z*)	Cut off (*m*/*z*)	Amplitude (*m*/*z*)	MS–MS (*m*/*z*)
Propanil	1	0–21	217.6	100	1	161.6
Cyproconazole			291.8	100	1.5	124.8
Pyrifenox			294.8	250	2	262.7
Kresoxim methyl			336	150	1	245.7
Tebufenpyrad	2	21–26.5	356	100	1	146.7
Pyriproxyfen			321.8	100	2	226.6
λ-Cyhalothrin	3	26.5–31	473			
Acrinathrin			564			
Carbosulfan	4	31–40	381.1	100	1.5	159.8

The separation was achieved on an analytical column Luna C_{18} (250 mm × 4.6 mm i.d., 5 μm) preceded by a Securityguard cartridge C_{18} (4 mm × 2 mm i.d.), both from Phenomenex (Chesire, UK). The mobile phase was methanol–water at a flow-rate of 0.6 ml min^{-1}. The initial composition was 70% methanol from 0 to 5 min, followed by a linear gradient to 90% of methanol from 20 to 40 min. An aliquot of 20 μl of the final extract was injected into the LC apparatus.

3. Results and discussion

3.1. Mass spectrometry observations

Table 3 shows MS for studied pesticides in positive ionisation (PI) mode. The main ion observed in the mass spectrum was the protonated molecule $[M + H]^+$ for all compounds, except for acrinathrin, λ-cyhalothrin, kresoxim and tebufenpyrad, the mass spectra of which shows as main ion the adduct with sodium. Fig. 1 illustrated the total ion chromatogram and the extracted ion chromatogram obtained by LC–ion-trap MS.

A reliable way of obtaining structural information is to perform tandem MS experiments on specific ions of interest. Table 3 also shows the product ions obtained by MS–MS and MS^3 (when it is possible). The behavior of the pesticides can be divided into three models according to their capability to generate product ions.

Acrinathrin and λ-cyhalothrin constituted the first group that is typified because no fragment ion can be detected by MS–MS. Their common chemical characteristics are that both belong to the chemical family of synthetic pyrethroids and provide a mass spectrum that shows the formation of adduct with sodium.

Going from one extreme to the other, it is the group formed by propanil and carbosulfan, which could be fragmented to the MS^3 stage at concentrations of relevance in food. The CID of the main fragments ions (MS^3) will often yield those formed by fragmentation of the lateral chains in the molecular structure or by opening of the heterocyclic rings.

The MS–MS spectrum of the protonated molecule of propanil evidences fragmentation of the side chain of the carbamic group resulting in a signal at *m/z* 161.6 that corresponds to $[M + H–CH_2CHCHO]^+$. Propanil has two chlorine atoms in its structure and the CID of the main structure ions (MS^3) showed a signal at *m/z* 126.8 formed by the loss of one chlorine atom from the previous fragment.

Carbosulfan is the most unstable of the analyzed compounds. The MS–MS spectrum presents the product ion at *m/z* 159.8. The ion at *m/z* 159.8 deriving from the neutral loss of the group $CONCH_3SN((CH_2)_3CH_3)_2$. In the further step ($MS^3$ of the ion at *m/z* 159.8), the product ion at *m/z* 117.8 is formed by the opening of the five-atom ring and cleavage of the propene group.

The last group of compounds includes cyproconazole, kresoxim methyl, pyrifenox, pyriproxyfen and tebufenpyrad that can be fragmented until the MS–MS spectrum using

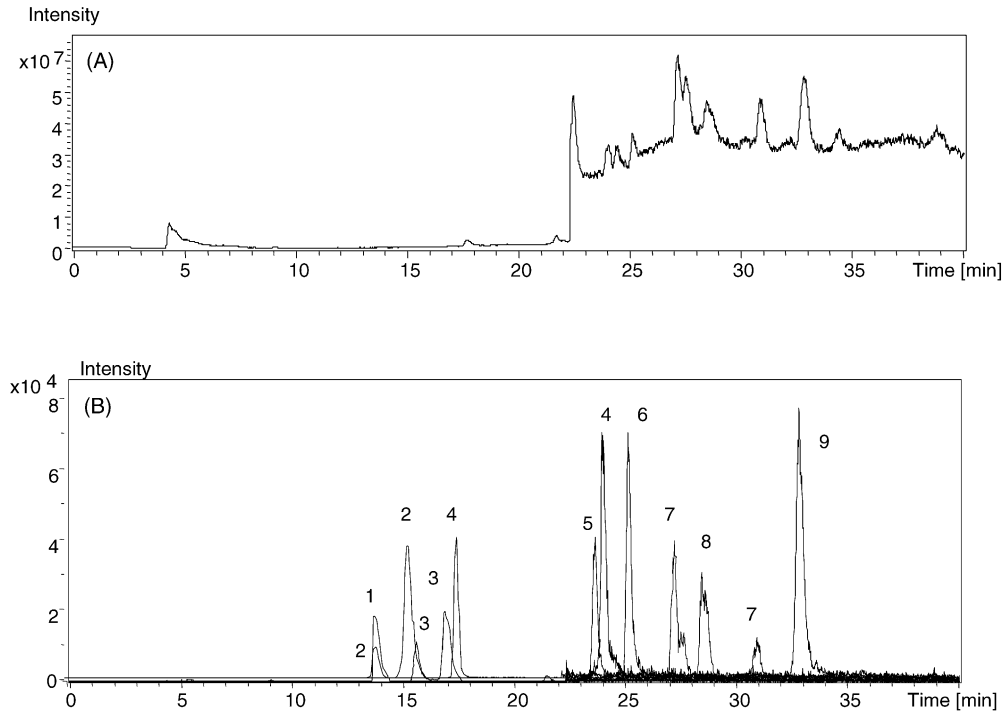
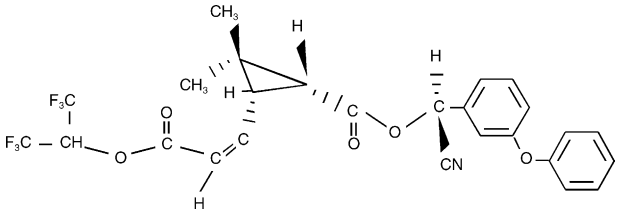
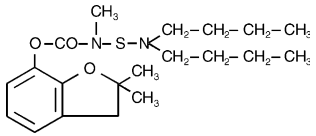
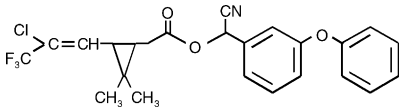
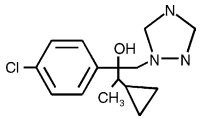
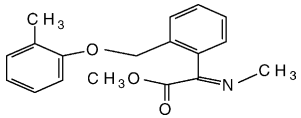
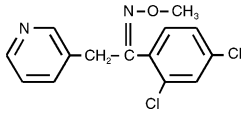
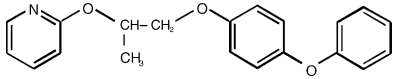
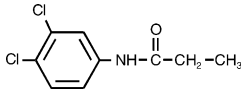
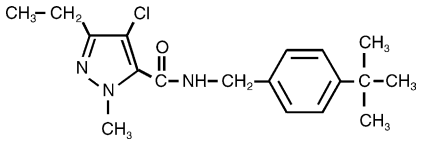


Fig. 1. Chromatographic separation of the pesticides studied using LC–QIT-MS: (A) total ion chromatogram and (B) extracted ion chromatogram. Peak identification: (1) propanil; (2) cyproconazole; (3) pyrifenox; (4) kresoxim methyl; (5) tebufenpyrad; (6) pyriproxyfen; (7) λ-cyhalothrin; (8) acrinathrin; (9) carbosulfan.

Table 3
Structure and molecular and fragment ions obtained by LC–ESI-MS^n

Pesticides	M_w	Structure	*m*/*z* and tentative ions		
Name			MS	MS–MS	MS^3
Acrinathrin	541.1		564 $[M + Na]^+$		
Carbosulfan	380.0		381.1 $[M + H]^+$	159.8 $[M + H{-}COSN_2C_9H_{21}]^+$	117.9 $[M + H{-}COSN_2C_9H_{21}{-}C(CH_3)_2]^+$
λ-Cyhalothrin	449.9		473 $[M + Na]^+$		
Cyproconazole	291.5		291.8 $[M + H]^+$	124.8 $[M + H{-}ON_3C_8H_{13}]^+$	
Kresoxim methyl	313		336 $[M + Na]^+$	245.7 $[M + Na{-}CH_3C_6H_4]^+$	
Pyrifenox	294		294.8 $[M + H]^+$	262.7 $[M + H{-}CH_3OH]^+$	
Pyriproxyfen	321.3		321.8 $[M + H]^+$	226.6 $[M + H{-}C_5H_4NOH]^+$	
Propanil	218.1		217.6 $[M + H]^+$	161.6 $[M + H{-}CH_2{-}CH{-}COH]^+$	126.8 $[M + H{-}CH_2{-}CH{-}COH{-}Cl]^+$
Tebufenpyrad	333		356 $[M + Na]^+$	170.6 $[M - NC_{11}H_{16}]^+$	

concentrations relevant in food. In the CID (MS–MS) of the protonated molecule of pyrifenox, the ion spectra corresponds to the cleavage of the oxime bond that results in the product ion *m*/*z* 262.7 $[M + H–CH_3OH]^+$ formed by the neutral loss of methanol.

The MS–MS analysis of kresoxim methyl and pyriproxyfen lead to a fragment ion corresponding to the cleavage of the ether bond. Kresoxim methyl presents only a product ion spectrum at *m*/*z* 245.7 derived from the neutral loss of methylbenzene, and pyriproxyfen presents also a product spectrum ion at *m*/*z* 226.6 corresponding to the neutral loss of 2-hydroxypyridine.

Tebufenpyrad belongs to the class of carbamates. The MS–MS spectrum presents an ion at *m*/*z* 170.6 formed by the cleavage of the carbamic group and the neutral loss of *p*-aminomethylisobutylbenzene.

The MS–MS spectrum of cyproconazole gave a product ion at *m*/*z* 124.8 deriving from cleavage of the lateral chains in the carbon contiguous to the phenyl group, and the location of the positive charge in the carbon atom stabilizing by resonant structures.

3.2. Study of the extraction procedure

Fig. 2 displays the LC–ESI-MS–MS chromatogram obtained after MSPD extraction of an orange sample spiked with the selected fungicides at the limit of quantification (LOQ) levels and is typical of the data obtained using this determination procedure. As it is shown in Table 2, the time schedule for data acquisition includes one set of 4, two sets of 2 and one set of 1 MRM channels. Chromatograms obtained after solid–liquid extraction (SLE) were similar. These chromatograms demonstrated how the enhanced selectivity afforded by MS–MS detection attained discrimination between the studied pesticides that were not separated under the LC conditions.

The analysis of a blank extract in the MRM mode did not show interferences with other compounds in the MRM. The organic oranges and strawberries analyzed showed also no pesticide concentrations after MSPD or SLE.

The most important problem in the LC–MS using ESI interface is the matrix effect, which has been widely reported in the literature [11,13,19]. The matrix effect depends on the properties of the analyte itself and the presence of other ionizable material. It was established comparing the signal intensity obtained in a standard solution (methanol) with those obtained in matrix matched standards. Table 4 illustrates the differences in response of each analyte in pure solvent standard and in matrix matched standard at LOQ concentrations, and 10 times the LOQ concentrations, by MSPD and SLE, respectively.

All pesticides showed in orange matrix considerable suppression in relation to the response obtained in a pure solvent standard, except pyriproxyfen, the signal of which was greater in the presence of matrix components. The enhancement on the pyriproxyfen response can be attributed to the

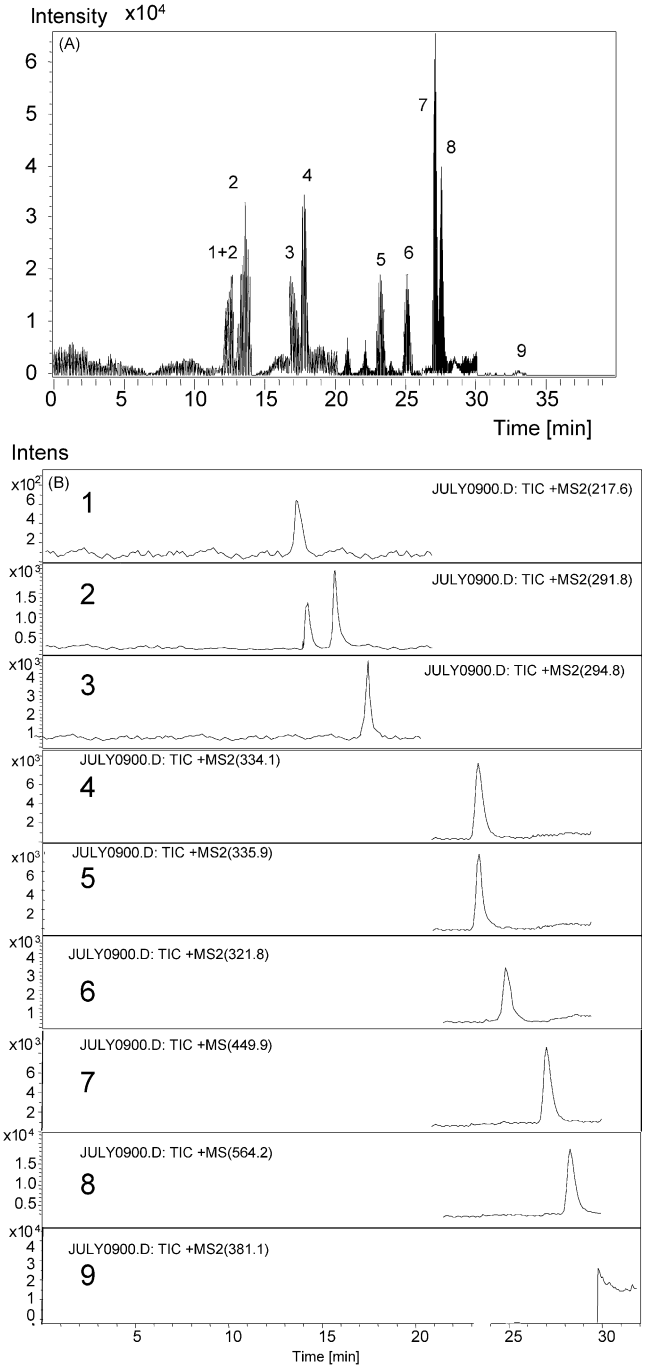


Fig. 2. LC–QIT-MS–MS obtained after MSPD of orange spiked with the studied pesticides at the LOQ level: (A) MRM total ion chromatogram; (B) extracted ion chromatograms. Peak identification as in Fig. 1.

characteristics of the matrix. The pyriproxyfen is 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. The decrease of ion intensities can be attributed to the gas-phase proton transfer, as it has been already described [11]. The results for strawberries do not show any noticeable difference from those obtained for oranges.

Table 4
Percentage of response of each analyte from spiked extracts obtained by MSPD and SLE compared with those obtained for methanol standards

Compounds	MSPD		SLE	
	LOQ	10 LOQ	LOQ	10 LOQ
Propanil	81	78	60	48
Cyproconazole	88	60	50	40
Pyrifenox	102	96	68	76
Kresoxim methyl	60	50	98	75
Tebufenpyrad	66	76	46	62
Pyriproxyfen	130	138	138	128
λ-Cyhalothrin	50	54	60	63
Acrinathrin	55	58	80	78
Carbosulfan	15	10	17	15

Almost no difference in matrix effect was recorder for ethyl acetate extraction and MSPD (see Table 4). MSPD has been reported as a technique that avoids matrix interferences in the LC–MS determination of benzoylurea, carbamates and fungicides [23–25]. Rather surprisingly, the results obtained for the pesticide selected in this study showed that matrix effects are very similar to those obtained by ethyl acetate extraction.

The use of matrix-matched calibration standards was done to compensate for signal suppression/enhancement of the studied pesticides in matrix solution compared to their response in pure solvent. Calibration curves, obtained from extracted ion chromatogram peak area measurements from matrix-matched standards, were calculated for all the studied pesticides. These curves displayed good linearity over the selected concentration range with linear regression correlation coefficients better than 0.99.

Table 5 shows detailed recovery data for the studied pesticides in oranges free of pesticides, spiked at LOQ and 10 times the LOQ by MSPD. The LOQ was defined as the lowest level for which acceptable recoveries and repeatabilities (<20%) are obtained [22]. Mean recoveries were between 51.5 and 108%, with the exception of carbosulfan that was <15%. The reason for the low recovery of carbosulfan is its instability—it quickly degradates to carbofuran and 3-hydroxycarbofuran. The relative standard deviations (R.S.D.s) ranged from 4 to 15%, except for carbosulfan that was >25%, which was also indicative of the irregular recoveries obtained for it throughout this study. The results obtained in samples spiked at LOQ are very similar to those obtained with samples spiked at 10 times LOQ.

The sensitivity is one of the most important parameters in pesticides residues determination. The LOQs obtained by MSPD are between 0.05 and 2 mg kg^{-1} pyriproxyfen being the most sensitive and λ-cyhalothrin the less.

SLE with ethyl acetate and anhydrous sodium sulfate was also evaluated with respect to accuracy, precision and limits of quantification, as is shown in Table 6. Mean recoveries ranged from 59 to 101% and R.S.D.s from 8 to 16%, with the exception of carbosulfan, that was recovered <15% with R.S.D.s >25%. The results obtained in samples spiked at LOQ were also very similar to those obtained with samples spiked at 10 times LOQ.

Table 5
Concentration and recovery of the studied compounds in oranges obtained by MSPD

Compounds	Concentration (mg kg^{-1})	Recovery (%) ($x \pm$ R.S.D., $n = 5$)	Concentration (mg kg^{-1})	Recovery (%) ($x \pm$ R.S.D., $n = 5$)
Propanil	0.2	65.2 ± 5	2	62.6 ± 8
Cyproconazole	0.3	51.5 ± 6	3	51.6 ± 10
Pyrifenox	0.1	102.5 ± 6	1	103.8 ± 9
Kresoxim methyl	0.2	108.1 ± 7	2	92.2 ± 11
Tebufenpyrad	0.1	55.7 ± 4	1	60.8 ± 8
Pyriproxyfen	0.05	95.6 ± 8	0.5	90.2 ± 13
λ-Cyhalothrin	2	72.1 ± 10	20	73.4 ± 15
Acrinathrin	0.3	86.3 ± 6	3	85.1 ± 9
Carbosulfan	0.2	14.1 ± 25	2	10.6 ± 31

Table 6
Concentration and recovery of the studied compounds in oranges obtained by SLE with ethyl acetate

Compound	Concentration (mg kg^{-1})	Recovery (%) ($x \pm$ R.S.D., $n = 5$)	Concentration (mg kg^{-1})	Recovery (%) $x \pm$ R.S.D., $n = 5$)
Propanil	0.04	69.3 ± 16	0.4	63.1 ± 12
Cyproconazole	0.06	101.3 ± 12	0.6	97.6 ± 8
Pyrifenox	0.02	59.2 ± 14	0.2	68.4 ± 10
Kresoxim methyl	0.04	62.3 ± 11	0.4	65.4 ± 9
Tebufenpyrad	0.02	99.1 ± 12	0.2	98.1 ± 14
Pyriproxyfen	0.01	86.4 ± 15	0.1	81.5 ± 12
λ-Cyhalothrin	0.4	71.1 ± 10	10	76.4 ± 12
Acrinathrin	0.06	95.9 ± 8	1	92.3 ± 10
Carbosulfan	0.04	13.8 ± 29	0.5	12.2 ± 25

Table 7
Method performance comparisons

	MSPD	SLE (ethyl acetate)
Spiking concentrations (mg kg^{-1})	0.05–20	0.01–10
Accuracy (recovery, %)	51.5–108	59–101.3
Repeatability (R.S.D., %)	<16	<17
Linearity (r)	>0.997	>0.992
Sensitivity (LOQ)	0.05–2	0.01–0.4
Applicability	All the studied pesticides except carbosulfan	All the studied pesticides except carbosulfan

Table 8
Maximum residues limits (MRLs) and limits of quantification (LOQs) of studied compounds

Pesticide	MRL in oranges (mg kg^{-1})				LOQ (mg kg^{-1})	
	Spain	EU	Codex Alimentarius	USA	MSPD	SLE
Acrinathrin	0.1				0.3	0.06
Carbosulfan	0.01	0.05			0.2	0.04
λ-Cyhalothrin	0.05	0.1	0.2	0.01	2	0.4
Cyproconazole	0.05			0.1	0.3	0.06
Kresoxim methyl	0.5	0.05			0.2	0.04
Propanil	0.5				0.2	0.04
Pyrifenox	0.1				0.1	0.02
Pyriproxyfen	0.02			0.3	0.05	0.01
Tebufenpyrad	0.05				0.1	0.02

The LOQs obtained by SLE were between 0.01 and 0.4 mg kg^{-1}, which are better than those obtained using MSPD because of the higher concentration factor attained with the ethyl acetate extraction (five against one).

Table 7 summarizes the parameters indicatives of the analytical performance of both procedures. MSPD and SLE provide similar accuracy, repeatability and extract all the studied pesticides, except carbosulfan which is transformed in its metabolites. Main advantage of MSPD compared with SLE is the avoidance of long concentration procedures and the significant reduction of the organic solvent required. On the other hand, the main disadvantage of the procedure is the inferior LOQs obtained.

Table 8 indicates the MLR of studied pesticides in oranges established by EU, USA and Spanish legislations and by the Codex Alimentarius guidelines and compares those with the LOQ obtained applying both extraction procedures. Using MSPD the only compounds that present LOQs higher than maximum residue limit (MRL) established by Spanish legislation were tebufenpyrad, pyriproxyfen and acrinathrin. LOQ of pyrifenox reached the MRL established in Spain and LOQ of pyriproxyfen achieved the MRL established by the USA legislation.

LOQs obtained by SLE are better than those obtained by MSPD, only LOQs of carbosulfan and λ-cyhalothrin were higher than MRLs established by the Spanish legislation, and LOQ of λ-cyhalothrin was over the MRL established by the EU.

4. Conclusion

LC–QIT-MS–MS is a suitable technique for determining pesticides in real samples. It provides an unequivocal identification of compounds and its selectivity and specificity is appropriated for pesticides determination. Although suppression of the analyte signal is a phenomenon frequently observed using ESI interfaces, calibration with matrix-matched standards can provide accurate results avoiding incorrect quantification.

MSPD and SLE in combination with LC–QIT-MS–MS enable selective and sensitive analysis of acrinathrin, cyproconazole, λ-cyhalothrin, kresoxim methyl, pyrifenox, pyriproxyfen, propanil, and tebufenpyrad in oranges and strawberries. However, carbosulfan cannot be determined in real samples because it is quickly degraded to carbofuran and 3-hydroxycarbofuran. Both techniques are simple and did not require any purification step. Linearity, accuracy and precision obtained by MSPD and SLE are similar but SLE has demonstrated to be more sensitive than MSPD, which is a requirement to meet the MRLs established by the most commonly applied legislations.

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