Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1088 (2005) 224–233
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Routine application using single quadrupole liquid chromatography–mass spectrometry to pesticides analysis in citrus fruits

Carla Soler*, Jordi Mañes, Yolanda Picó

Laboratori de Bromatologia i Toxicologia, Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, València, Spain

Available online 22 April 2005

Abstract

A rapid and sensitive liquid chromatography–electrospray ionization–mass spectrometry method has been developed for the routine analysis of buprofezin, bupirimate, hexaflumuron, tebufenpyrad, fluvalinate and pyriproxyfen in citrus fruits. Extracts were obtained by matrix solid-phase dispersion (MSPD) using C_{18} as dispersant and dichloromethane-methanol (80:20, v/v) as eluent. Matrix effects were tested for all matrices by addition of standard to sample blank extracts (samples containing no detectable residues). Mean recoveries obtained at fortification levels between 0.01 and 5 mg kg^{-1} were 57–97% with relative standard deviations (RSDs) from 5 to 19%. The limits of quantification (LOQ) were in the range of 0.01–0.2 mg kg^{-1} and lower than maximum residue limits (MRLs) established by the Spanish legislation. The MSPD was compared with conventional ethyl acetate extraction, showing equivalent recoveries and precision. Although the sample is more concentrated (5-fold) by solid–liquid extraction (SLE) with ethyl acetate than by MSPD, LOQs obtained by both techniques, were almost equal, because MSPD reduces matrix effects, baseline noise, and interfering peaks from the matrix. The proposed method has been applied to the determination of selected pesticides in real samples. Liquid chromatography–tandem mass spectrometry (LC–MS–MS) with quadrupole ion trap (QIT) and triple quadrupole (TQ) have been used as confirmatory tool for positive samples according to a recent No. SANCO/10476/2003 European Union Guideline.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Food analysis; Citrus fruits; Pesticide residues

1. Introduction

Use of agrochemicals at various stages of cultivation and during post-harvest storage, plays an important role in food protection and quality preservation. However, widespread-used pesticides become a very important group of chemicals to be controlled because of their high toxicity to the human health and frequent presence of their residues in fruits and vegetables [1,2]. One of the most important aspects for minimizing the potential hazards to humans is the monitoring of pesticide residues in food. Maximum residue limits (MRLs) in fruits and vegetables have been set by the governmental agencies of each country [3,4] and the European Union (EU) [5] to guarantee consumer safety and to minimize their consumers' intake.

Analytical methodologies employed, owing to the strict regulation of MRLs, must be capable of residues measuring at trace levels [6,7] and of providing unambiguous evidence to confirm both, the identity and the quantity of any detected pesticide [8]. These routine methods should be simple, fast, and robust to minimize time spent per sample [9]. In the last decades, the on-line coupling of efficient liquid chromatography separation with mass spectrometry detector (LC–MS) has been used for the analysis of pesticide residues [10,11] and is rapidly becoming an accepted technique for regulatory monitoring purposes [12,13].

Advantages of the LC–MS are the reduction of sample preparation steps that provides a higher sample throughput and the high sensitivity and selectivity that enable the

* Corresponding author. Tel.: +34 963544958; fax: +34 963544954.
E-mail address: carla.soler@uv.es (C. Soler).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.03.106

analysis of target analytes at low concentrations. Main disadvantage of this technique is that using single quadrupole, an alternative technique is required to meet the European Union identification criteria established in the document No. SANCO/10476/2003 [14].

LC in combination with tandem mass spectrometry (LC–MS–MS) has provided the most powerful confirmatory tool for the pesticide residue analysis in food [15] because it discriminates more efficiently than LC–MS between the analyte and the matrix signal and it is especially relevant when the ultra-trace levels identification is needed [16]. Several multiresidue screening methods have been developed for routine application [17,18]. However, LC–MS–MS has the disadvantages for a routine analysis in the laboratories of being very expensive, requiring high-pure gas for collision-activated dissociation (CAD), having expensive replacements and being very delicate in its adjusting.

The common established extraction techniques are based on complex solvent extraction methods that for solid samples are also named solid–liquid extraction (SLE). These procedures have some drawbacks such as: they are time consuming, require high amount of sample and solvents, and lack sufficient specificity to avoid false positives [19]. That is the reason why they are replaced with faster, less expensive and easy handled protocols. Matrix solid-phase dispersion (MSPD) carries out simultaneously sample homogenization, extraction and clean-up [20] using a relative small sample size, low solvent volume and minimum amount of sample. In the last years, it has been increasingly applied for isolating pesticides from fruits and vegetables [19–21].

The aim of this work was to develop a rapid, specific and sensitive analytical method for the routine analysis of six widely used pesticides in citrus fruits at concentration levels lower than their respective MRLs. These pesticides have been scarcely studied previously. It involves a rapid and low time-consuming MSPD extraction that accomplished high sample throughput and routine determination of the sample using LC–MS with single quadrupole monitoring the main ion obtained for each analyte ($[M+H]^+$ or $[M+Na]^+$). The confirmation of positive samples was performed by LC–MS–MS using either triple quadrupole (TQ) or quadrupole ion trap (QIT) to meet the European Union requirements.

2. Experimental

2.1. Materials and standards

Pesticides (buprofezin, bupirimate, hexaflumuron, pyriproxifen, tebufenpyrad and fluvalinate) 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. Standard working mixtures for each pesticide at various concentrations were daily prepared by appropriate dilution of aliquots of the stock solutions in methanol or in matrix extract.

HPLC-grade methanol, ethyl acetate and dichloromethane (organic trace analysis) were purchased from Merck (Darmstadt, Germany). Deionized water (<18 mol cm^{-1} 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.

The solid phase for MSPD was C_{18} bonded silica (40–60 μm) from Analisis Vinicos (Tomelloso, Spain).

2.2. Sample preparation

Oranges, tangerines, grapefruits and lemons used as blank samples (samples with no detectable residues) and as spiked ones were from organic farming without use of pesticides and obtained from a local market. The developed procedure was also applied to the analysis of 80 samples that were taken, at random, out of those conventionally farmed. The samples were taken in accordance with the guidelines of the EU [5]; which means that, as far as possible, the sample was taken at various places distributed throughout the lot (size ca. 50 kg). The samples, weighting at least 1 kg, consisted of 10 individual fruits, were immediately stored in polyethylene bags for transporting to the laboratory. Samples were stored at 4 °C until the moment of extraction and analyses were carried out for the next 24 h to avoid problems of stability during the storage.

They were analyzed unwashed and unpeeled because Spanish legislation establishes the MRLs in mg kg^{-1} of whole sample. A representative portion of sample (200 g whole fruit) was chopped into small pieces and homogenized in a Bapitaurus food chopper (Taurus, Berlin, Germany). Two subsamples (30–40 g) of these representative portions were stored at −20 °C because it was necessary to repeat the analysis. No degradation of the pesticides, when they are present, was detected under these conditions.

2.2.1. Matrix solid-phase dispersion procedure

Portions of 0.5 g of chopped sample were weighed, placed into a glass mortar (50 ml) and gently blended with 0.5 g of C_{18} bonded silica for 5 min using a pestle, to obtain homogeneous mixture.

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 (80:20, v/v) mixture by applying a slight vacuum. The eluated was collected in a graduated conical tube (15 ml capacity) and concentrated, under stream of nitrogen, to 0.5 ml. An aliquot of 5 μl of the final extract was injected into the LC apparatus.

2.2.2. Solid–liquid extraction procedure employing ethyl acetate

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

and 50 g of anhydrous sodium sulfate using a Warring blender for 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 through the anhydrous sodium sulfate and collected with the first extraction fraction. Twice 25 ml ethyl acetate were used to rinse the glass beaker and the rinsings were passed through the filter and collected. The extract was evaporated to less than 5 ml using rotary evaporator, set at 40 °C and 250 mbar. Then, it was passed to a graduate conical tube (15 ml) and evaporated to dryness under a stream of nitrogen. The sample was reconstituted in 10 ml of methanol. A volume of 5 μl of the final extract was injected into the LC–MS system.

2.3. Routine LC–MS analysis using a single quadrupole

The separation was achieved on an analytical column Luna C_{18} (150 mm × 4.6 mm I.D., 5 μm) preceded by a securityguard cartridge C_{18} (4 mm × 2 mm I.D.), both from Phenomenex (Cheshire, UK). The mobile phase was methanol-water at a flow-rate of 0.6 ml min^{-1}. The solvent composition was 70% methanol at 0 min, and linearly increased to 90% methanol at 35 min. The separation conditions were the same for the three LC–MS equipment used: the single quadrupole, TQ and QIT.

LC was performed using a Hewlett-Packard (Palo Alto, CA, USA) HP-1100 Series LC/MSD system consisted of an autosampler, a binary solvent pump, and a mass spectrometry detector (MSD), equipped with an electrospray ionization (ESI) interface in positive ionization (PI) mode. Optimization of the LC–MS conditions was carried out by varying them in flow injection analysis (FIA) of the analytes (20 μl of 10 mg ml^{-1} individual standard solutions). The optimized parameters of the interface were: vaporizer temperature, 325 °C; nebulizer gas (nitrogen) pressure, 60 psi (1 psi = 6894.758 Pa); drying gas (nitrogen) flow rate, 10 ml min^{-1}; and temperature, 350 °C; capillary voltage, 4500 V; fragmentator, 80 V; gain, 3.

Full-scan LC–MS chromatograms were obtained by scanning from *m*/*z* 100 to 600. Time scheduled conditions for monitoring pesticides are reported in Table 1.

2.4. LC–MS^n confirmatory analysis

In positive samples, two confirmatory analyses were conducted as is indicated in the document No. SANCO/10476/2003 European Union Guidelines.

2.4.1. 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 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.

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 0.6 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 quadruples; 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. Transition selected and conditions used are summarized in Table 2.

2.4.2. 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 mass spectrometer was run in full scan, and multiple reaction monitoring (MRM) modes. Negative and positive ions were detected using the standard scan at normal resolution (scan speed 10,300 *m*/*z* s^{-1}; peak width 0.6 full width at half maximum (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. The fragments and fragmentation conditions are summarized in Table 2.

3. Results and discussion

3.1. LC–MS analysis and quality parameters

Both atmospheric pressure interfaces (API), atmospheric pressure chemical ionization (APCI) and ESI, were studied. Using ESI source, all the studied pesticides can be determined in positive ionization mode (PI), whereas in negative one, only hexaflumuron and fluvalinate gave response. Using

Table 1
Structure and molecular and fragment ions obtained by LC–ESI–MS

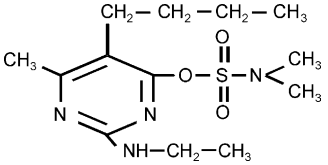
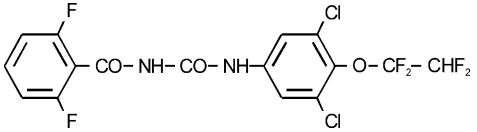
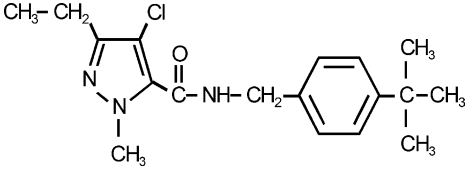
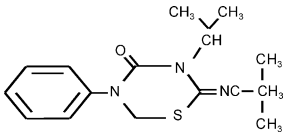
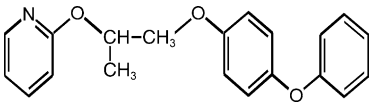
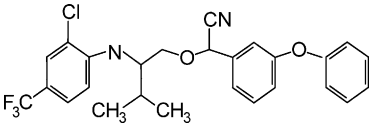
Compound	Structure	Time (min)	SIM ion (*m*/*z*)	Main ions (*m*/*z*) (relative abundance)	Confirmatory (*m*/*z*) ions	Optimal fragmentor to see the confirmatory ion (V)
Bupirimate (316)		7.99	317	317 $[M + H]^+$ (90) 339 $[M + Na]^+$ (100)	237 $[M - SO_2CH_3]^+$	100
Hexaflumuron (460)		12.89	483	483 $[M + Na]^+$ (100)		
Tebufenpyrad (333)		15.44	356	356 $[M + Na]^+$ (100) 334 $[M + H]^+$ (65)	147 $[CH_2C_6H_4C(CH)_3]^+$	160
Buprofezin (305)		16.4	306	306 $[M + H]^+$ (100) 328 $[M + Na]^+$ (10)	201 $[M - C_6H_5NCH_2]^+$	120
Pyriproxyfen (321)		17.52	322	322 $[M + H]^+$ (100) 344 $[M + Na]^+$ (60)	227 $[M + H\text{-}C_5H_4NOH]^+$	100
Fluvalinate (502)		29.9	525	525 $[M + Na]^+$ (100)		

Table 2
Transitions and conditions used by TQ and QIT

Compound	TQ				QIT			
	Transitions	Cone (V)	Collision (eV)	Dwell (s)	Transitions	Cutoff	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 → 146 334 → 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

APCI in PI mode, bupirimate, buprofezin, tebufenpyrad and pyriproxyfen provided signal, but not hexaflumuron and fluvalinate. ESI source provided greater sensitivity than APCI.

According to the reported ESI theory [23,25], this interface present substantial advantages because the sample can be directly ionized in the liquid phase at quasi-ambient temperature (the interface is at ambient temperature and only the drying gas blown into it for evaporating and droplet shrinkage is a 350 °C), minimizing the degradation of thermolabile compounds. The soft ionization assumes that the detected gas-phase ions are a true representation of the ions in the sample [22–25].

The main ions obtained and their tentative assignations are shown in Table 1. The ions monitored in SIM were separated in four windows to detect the analytes with sufficient instrumental sensitivity. The mass spectra showed the molecular sodium adducts $[M + Na]^+$ for hexaflumuron and fluvalinate and both, the protonated molecules $[M + H]^+$ and the sodium adducts $[M + Na]^+$ for bupirimate, pyriproxyfen, tebufenpyrad and buprofezin. Formation of sodium adducts has extensively been reported in ESI for the pesticides with functional groups that can donate a lone pair of electrons [11,16]. The source of sodium is debatable because it can come from the glassware that contacts with the sample, from the methanol used in the mobile phase (it is almost ubiquitous as impurity), from the metal tubing, etc. [17].

The ions monitored in selected ion monitoring (SIM) (Table 1) were the base-peaks of the mass spectrum for each pesticide. For bupirimate, buprofezin and pyriproxyfen were the protonated molecule, and for fluvalinate, tebufenpyrad and hexaflumuron, the sodium adduct.

Although ESI is the most soft-ionization technique of API sources, ions can be fragmented to produce characteristic fragments as it is reflected in Table 1. This fragmentation is performed in the single quadrupole by increasing the potential between the entrance capillary and the first skimmer (fragmentor) in the ion-focusing region of the instrument [26]. Bupirimate, tebufenpyrad, buprofezin and pyriproxyfen at high fragmentor voltages generate one fragment ion (at *m*/*z* 237, 147, 201 and 227, respectively) that can be used as diagnostic ion. Fluvalinate and hexaflumuron, which only form the sodium adduct, did not fragment.

The quality control procedures established by the EU [14] indicate that the minimum requirement for confirmation of identity is data from two ions of *m*/*z* >200; or three ions of *m*/*z* >100. If these requisites cannot be met, additional supporting evidence should be provided.

Confirmatory analysis was carried out by MS–MS using either QIT or TQ. In this case, fluvalinate and hexaflumuron were determined by NI mode, as it was studied in a previous work [27], because using these mass spectrometers, the ionization mode can be alternated in the same run and the deprotonated molecule can be fragmented for identification purposes. Taking into account the transitions and conditions reported in Table 2, which were optimized in a previous work [27] the EU criteria is met by TQ for the six studied pesticides and by QIT for five of them, excluding only fluvalinate.

The limits of detection (LODs), limits of quantification (LOQ), linearity, run-to-run and day-to-day precision were obtained for standards of the studied pesticides in methanol using SIM mode to validate LC–MS procedure. The LODs based on a signal-to-noise ratio of 3 ranged from 35 pg for bupirimate to 500 pg for fluvalinate. With an injection volume of 5 μl, this corresponds to LODs from 0.005 to 0.1 μg ml^{-1} (detailed in Table 3). The LOQs, based on a signal-to-noise ratio of 10, ranged from 0.02 to 0.4 μg ml^{-1}, as are also listed in Table 3.

The calibration graph was plotted (five points) for standards solutions between 0.3 and 30 mg kg^{-1}, the response function was found to be linear with a coefficient of determination $r = 0.998$. The relative standard deviations (RSDs) for an injection of 0.3 mg kg^{-1} ranged from 3.3 to 4.6% for run-to-run precision, and from 4.2 to 8.3% for day-to-day precision.

Table 3
Instrumental LODs and spiked levels used to validate the procedure

Compound	LMR Spain legislation (mg kg^{-1})				Spiking level I (LOQ) (mg kg^{-1})	Spiking level II (10 LOQ) (mg kg^{-1})	LOD (μg ml^{-1})
	Oranges	Tangerines	Grapefruits	Lemons			
Bupirimate	0.05	0.05	0.05	0.05	0.02	0.2	0.005
Hexaflumuron	0.5	0.5	0.5	0.5	0.2	2	0.05
Tebufenpyrad	0.5	0.5	0.5	0.5	0.2	2	0.05
Buprofezin	0.2	0.2	0.2	0.2	0.08	0.8	0.02
Pyriproxyfen	0.5	0.5	0.5	0.5	0.2	2	0.05
Fluvalinate	1	1	1	1	0.4	4	0.1

3.2. Matrix effect

One drawback, especially when using electrospray ionization, is the presence of matrix components that can affect the ionization of the target analytes. The mechanism and the origin of the matrix effect results from competition between matrix and analyte ions in the sprayed solution for access to droplet [28]. Depending on the environment in which ionization and ion-evaporation take place, this competition may effectively decrease (ion suppression) or increase (ion enhancement) the efficiency of analyte ion formation [29].

The matrix effect was evaluated by comparison of the response of pesticides standards prepared in orange, tangerine, grapefruit, and lemon extracts with standards in methanol, at LOQ and 10 times LOQ concentrations. Matrix matched standards for MSPD and ethyl acetate methods were prepared extracting samples that contain no detectable residues according to the procedures described in Sections 2.2.1 and 2.2.2, respectively. The final extracts were evaporate to dryness and then, redissolved in standards prepared in methanol at appropriate concentration. Fig. 1 illustrates the differences in response observed at LOQ level by MSPD and SLE.

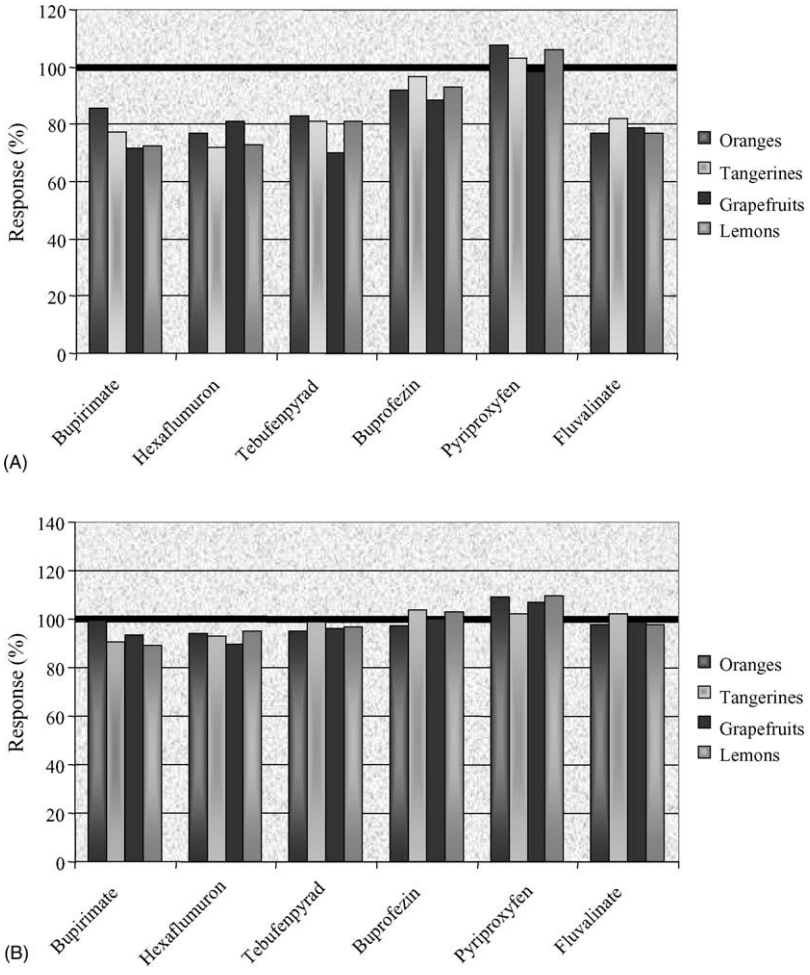


Fig. 1. Matrix effects by LC–ESI–MS after (A) SLE with ethyl acetate and (B) MSPD extraction procedure at LOQ concentrations.

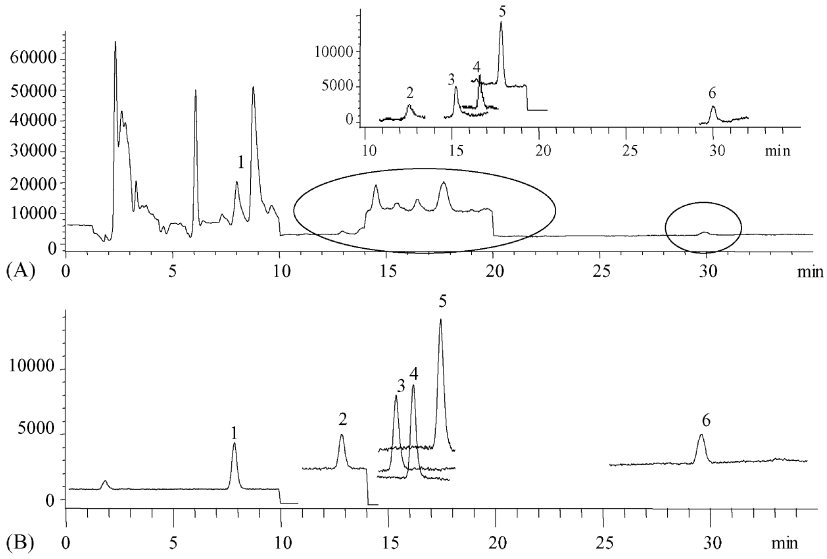


Fig. 2. Chromatograms of spiked orange at LOQ concentration (see Table 3) by (A) ethyl acetate and (B) MSPD. Peak identification: (1) bupirimate, (2) hexaflumuron, (3) tebufenpyrad, (4) buprofezin, (5) pyriproxyfen and (6) fluvalinate.

All pesticides showed in matrix obtained by SLE a considerable suppression in relation to the response obtained in pure solvent standard. This suppression is less highlighted for buprofezin but is higher than 20% for hexaflumuron, tebufenpyrad, bupirimate and fluvalinate. Pyriproxyfen presents an enhancement in its response (15%). On the contrary, the response of pesticides obtained by MSPD shows an enhancement or suppression <10%. The use of matrix matched standards to compensate the signal suppression is required for the SLE but not for MSPD.

3.3. Method validation

The accuracy (as recoveries), precision (as repeatability) and LOQs were established to validate the procedure.

LOQs, according to the EU guidelines were defined as lowest concentration that provided acceptable recoveries and RSDs (<19%) [30]. LOQs correspond to the lower calibration level reported in Table 3 as it is empirically verified by analyzing samples spiked with the pesticides at these concentration levels. Fig. 2 shows chromatograms obtained from a spiked orange sample at LOQ concentrations by MSPD and SLE with ethyl acetate. MSPD provided higher quality clean-up than ethyl acetate extraction as it is reflected in the fact that the chromatogram obtained with MSPD has less matrix peaks which can interfere with the analyte signal than that obtained by ethyl acetate.

Although the sample is more concentrated (5-fold) by ethyl acetate extraction than by MSPD, LOQs obtained by both techniques were almost equal because MSPD reduces matrix effects, baseline noise and interfering peaks from the matrix. The recovery and the relative standard deviations obtained from spiked samples at two fortification levels, LOQ and 10 LOQ, by MSPD are shown in Table 4. The recoveries of pesticides meet the EU criteria (>70%), except for hexaflumuron (∼60%). They seem to be independent of matrix and the spiked level. A good repeatability ($n = 5$) with RSDs ranging from 5 to 16% at LOQ level and from 8 to 19% at 10 times LOQ level was observed.

Table 4
Recovery and repeatability of the method by MSPD extraction

Sample	Concentration	Bupirimate Recovery, % (RSD,%)	Hexaflumuron Recovery, % (RSD,%)	Tebufenpyrad Recovery, % (RSD,%)	Buprofezin Recovery, % (RSD,%)	Pyriproxyfen Recovery, % (RSD,%)	Fluvalinate Recovery, % (RSD,%)
Orange	Spiking level I	97 (16)	59 (8)	75 (9)	87 (15)	74 (14)	79 (12)
	Spiking level II	84 (11)	65 (9)	73 (10)	88 (19)	81 (16)	75 (9)
Tangerine	Spiking level I	84 (9)	57 (8)	80 (10)	82 (12)	69 (8)	81 (15)
	Spiking level II	92 (10)	61 (10)	76 (9)	87 (11)	76 (11)	78 (10)
Grapefruit	Spiking level I	86 (9)	58 (7)	69 (5)	81 (13)	68 (8)	71 (8)
	Spiking level II	91 (8)	63 (10)	72 (11)	78 (9)	51 (9)	75 (10)
Lemon	Spiking level I	85 (14)	59 (8)	68 (8)	85 (10)	73 (13)	72 (9)
	Spiking level II	87 (19)	64 (9)	75 (10)	79 (9)	75 (9)	71 (8)

Spiking level I: LOQ concentrations; spiking level II: 10 LOQ concentration.

The developed MSPD method was compared to an established ethyl acetate extraction procedure. Table 5 lists the recoveries and RSDs obtained for SLE with ethyl acetate followed by LC–MS. The recoveries ranged from 68 to 92% and the RSDs ranged from 4 to 13%. The results are almost equal than those obtained by MSPD. The most marked difference is observed for hexaflumuron, which is better extracted using SLE with ethyl acetate.

Of the two method studied for isolating pesticides, MSPD was preferred for determining pesticides in real samples because it offers simplicity and less consumption of solvent as advantages when it is compared with a classical SLE method.

3.4. Application in real samples

The procedure was applied to the analysis of 80 samples from conventional farming, taken from different local markets. With each batch of 10 samples, a five-point calibration curve was prepared for analyte concentrations between the LOQs and 10 LOQs by injections before and after those of the sample extracts. In addition, 2 quality control (QC) samples

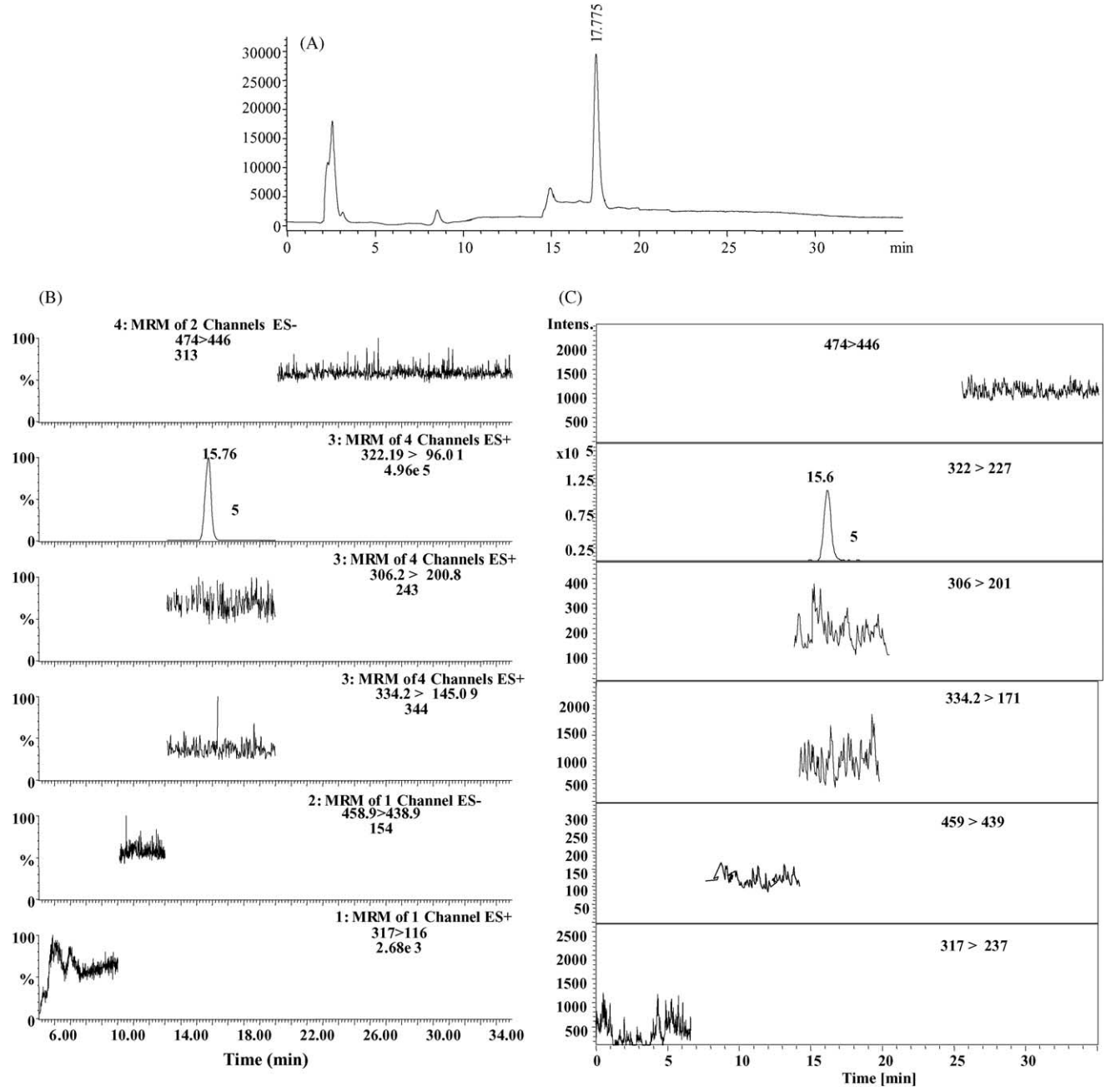


Fig. 3. Chromatogram of tangerine sample no. 8 that contains pyriproxyfen at 0.6 mg kg^{-1} (see Table 6) after MSPD obtained by (A) single quadrupole, (B) TQ and (C) QIT. Peak identification as in Fig. 2.

Table 5
Recovery and repeatability of the method by SLE extraction with ethyl acetate

Sample	Concentration	Bupirimate Recovery (%) (RSD)	Hexaflumuron Recovery (%) +RSD	Tebufenpyrad Recovery (%) +RSD	Buprofezin Recovery (%) +RSD	Pyriproxyfen Recovery (%) +RSD	Fluvalinate Recovery (%) +RSD
Orange	Spiking level I	79 (16)	74 (9)	78 (8)	85 (10)	90 (18)	70 (10)
	Spiking level II	82 (10)	77 (10)	83 (9)	82 (9)	91 (9)	77 (8)
Tangerine	Spiking level I	81 (12)	75 (13)	80 (10)	92 (12)	88 (10)	72 (6)
	Spiking level II	89 (17)	72 (10)	82 (11)	89 (13)	81 (11)	75 (11)
Grapefruit	Spiking level I	77 (14)	73 (8)	84 (12)	86 (9)	89 (12)	76 (9)
	Spiking level II	79 (15)	69 (11)	81 (17)	83 (11)	83 (10)	78 (10)
Lemon	Spiking level I	76 (11)	70 (9)	85 (14)	90 (15)	82 (15)	68 (8)
	Spiking level II	75 (9)	82 (12)	81 (13)	84 (12)	78 (9)	71 (14)

Spiking level I: LOQ concentrations; spiking level II: 10 LOQ concentration.

were injected in every batch of samples. The QC samples were blank lemon sample fortified at LOQ level and 10 times the LOQ level. All the samples were injected in duplicate.

The results for samples that contain pesticides residues are summarized in Table 6. The concentration levels were lower than the MRLs established for all pesticides, except pyriproxyfen, that exceed in one positive sample, reaching the value of 0.6 mg kg^{-1}.

Pyriproxyfen was found and quantified in seven different samples because this pesticide is a widespread-used insecticide in the citrus crops in the Valencian Community. However, hexaflumuron was not detected in any commercial samples analyzed, because it is more applied to another type of crops such as apples and pears.

Table 6
Pesticides detected in real samples

No. sample	Compound	Concentration (mg kg^{-1})	Confirmation	
		MSPD	TQ	QIT
Oranges				
1	Pyriproxyfen	0.2	+	+
2	Tebufenpyrad	0.1	+	+
	Fluvalinate	0.4	+	+
3	Pyriproxyfen	0.2	+	+
4	Buprofezin	0.1	+	+
Tangerines				
5	Pyriproxyfen	0.2	+	+
6	Fluvalinate	0.3	+	+
7	Tebufenpyrad	0.4	+	+
	Fluvalinate	0.5	+	+
8	Pyriproxyfen	0.6	+	+
Grapefruits				
9	Pyriproxyfen	0.2	+	+
	Bupirimate	0.1	+	+
10	Fluvalinate	0.3	+	+
11	Pyriproxyfen	0.3	+	+
Lemons				
12	Bupirimate	0.2	+	+
13	Buprofezin	0.1	+	+
14	Pyriproxyfen	0.1	+	+
15	Tebufenpyrad	0.2	+	+

Fig. 3 shows one chromatogram corresponding to the tangerine sample (sample no. 8) that contained pyriproxyfen at concentration level of 0.6 mg kg^{-1}. This figure shows the chromatogram obtained by LC–MS using single quadrupole and the confirmatory chromatograms obtained LC–MS/MS with TQ and QIT.

The identity of the detected residues was confirmed by LC–MS–MS using TQ and QIT. As it is deduced from Table 6 and Fig. 3, no false positives were detected by conventional LC–MS with single quadrupole. So, although LC–MS needs a confirmatory tool as it is regulated in the European legislation, this technique provide an efficient and fast method identifying and quantifying pesticides in citrus samples.

4. Conclusions

MSPD followed by LC–ESI–MS has been validated for determining bupirimate, buprofezin, hexaflumuron, fluvalinate, pyriproxyfen and tebufenpyrad in citrus fruits. The appropriate selectivity and sensitivity accomplish identification and quantification of low levels of the determined pesticides. Further evidence of the identity can be obtained by MS–MS using either TQ or QIT.

The application of MSPD to complex matrices can reduce the matrix effects. Moreover, other advantages of this extraction procedure are, the reduction in amount of sample needed, and of required organic solvent that increases sample throughput, and that the results obtained compare-well with more established procedures, making MSPD an attractive alternative for the more conventional extraction techniques such as ethyl acetate extraction.

The applicability of the method to routine analysis was tested in real samples with good performance. Most strict confirmatory methods showed that the proposed method does not provide false positive.

Acknowledgements

This work was financially supported by the Spanish Ministry of Science and Technology and the European Regional

Development Funds (ERDF) (Project AGL2003-01407) and to the Generalitat Valenciana (GRUP 03/164). C.S. thanks the Conselleria d'Educació i Ciéncia for a research training grant.

References

[1] M. Eddleston, L. Karalliedde, N. Buckley, R. Fernando, G. Hutchinson, G. Isbister, F. Konradsen, D. Murray, J.C. Piola, N. Senanayake, Lancet 360 (2002) 1163.
[2] C. Saieva, C. Aprea, R. Tumino, G. Masala, S. Salvan, G. Frasca, M.C. Giurdanella, I. Zanna, A. Decarli, G. Sciarra, D. Palli, Sci. Total Environ. 332 (2004) 71.
[3] Codex Alimentarius Commission. Codex Maximum Residue Limits for pesticides, http://apps.fao.org/faostat/collections?version=ext&hasbulk=0&subset=FoodQuality, FAO/WHO, Rome 2005.
[4] Spanish Secretary of Tourism and Trade, http://www.mcx.es/plaguicidas/espanol.asp, Ministerio de Industria, Turismo y Comercio, Spain, 2005.
[5] European Union on-line, Plant Health. Pesticide Residue, http://europa.eu.int/comm/food/plant/protection/pesticides/index_en.htm, European Union, Brussels, 2005.
[6] M.J. Taylor, K. Hunter, K.B. Hunter, D. Lindsay, S. Le Bouhellec, J. Chromatogr. A 982 (2002) 225.
[7] L. Mondello, G. Dugo, P. Dugo, Recent Appl. LC–MS Nov (2002) 2.
[8] A. Sannino, L. Bolzoni, M. Bandini, J. Chromatogr. A 1036 (2004) 161.
[9] J.L. Martinez-Vidal, F.I. Arrebola, M. Mateu-Sanchez, Rapid Commun. Mass Spectrom. 16 (2002) 1106.
[10] C. Blasco, G. Font, Y. Picó, J. Chromatogr. A 1028 (2004) 267.
[11] Y. Picó, G. Font, J.C. Moltó, J. Mañes, J. Chromatogr. A 882 (2000) 153.
[12] T. Reemtsma, Trends Anal. Chem. 20 (2001) 500.
[13] E. Lacassie, M.F. Dreyfuss, J.L. Daguet, M. Vignaud, P. Marquet, G. Lachatre, J. Chromatogr. A 830 (1999) 135.
[14] Document No. SANCO/10476/2003, Quality Control Procedures for Pesticides Residue Analysis.
[15] O.J. Pozo, J.M. Marin, J.V. Sancho, F. Hernandez, J. Chromatogr. A 992 (2003) 133.
[16] C. Soler, J. Mañes, Y. Picó, J. Chromatogr. A 1048 (2004) 41.
[17] A. Agüera, S. López, A.R. Fernández-Alba, M. Contreras, J. Crespo, L. Piedra, J. Chromatogr. A 1045 (2004) 125.
[18] C.L. Hetherton, M.D. Sykes, R.J. Fusell, D.M. Goodall, Rapid Commun. Mass Spectrom. 18 (2004) 2443.
[19] M. Fernández, Y. Picó, J. Mañes, J. Chromatogr. A 871 (2000) 43.
[20] S.A. Barker, J. Chromatogr. A 880 (2000) 63.
[21] C. Blasco, Y. Picó, J. Mañes, G. Font, J. Chromatogr. A 947 (2002) 227.
[22] J. Klein, L. Alder, J. AOAC Int. 86 (2003) 1015.
[23] P. Sandra, G. Vanhoenacker, F. Lyen, L. Li, M. Schelfaut, LC–GC Eur. 12 (2001) 8.
[24] J.M.F. Nogueira, T. Sandra, P. Sandra, Anal. Chim. Acta 505 (2003) 209.
[25] E. Rosenberg, J. Chromatogr. A 1000 (2003) 841.
[26] Y. Picó, C. Blasco, G. Font, Mass Spectrom. Rev. 23 (2004) 1.
[27] C. Soler, J. Mañes, Y. Picó, J. Chromatogr. A 1067 (2005) 115.
[28] B.K. Choi, D.M. Hercules, A.I. Gusev, J. Chromatogr. A 907 (2001) 337.
[29] S.A. Barker, J. Chromatogr. A 885 (2000) 115.
[30] Document No. SANCO/825/00, Residues analytical methods — for post-registration control and monitoring, 17 March 2004.