Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1098 (2005) 37–43
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Analysis of pesticides in fruits by pressurized liquid extraction and liquid chromatography–ion trap–triple stage mass spectrometry

Cristina Blasco *, Guillermina Font, Yolanda Picó

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

Received 22 December 2004; received in revised form 7 August 2005; accepted 10 August 2005
Available online 8 September 2005

Abstract

A multi-residue method using pressurized liquid extraction (PLE) and liquid chromatography–quadrupole ion trap–triple stage mass spectrometry (LC–IT–MS^3) has been developed for determining trace levels of pesticides in fruits. The selected pesticides can be distinguished in: benzimidazoles and azoles, organophosphorus, carbamates, neonicotinoids, and acaricides. PLE has been optimized to extract these pesticide residues from oranges and peaches by studying the effect of experimental variables on PLE efficiency. Samples were extracted at high temperature and pressure (75 °C and 1500 psi) using ethyl acetate as extraction solvent and acidic alumina as drying agent. The recoveries obtained by PLE ranged from 58% to 97% and the relative standard deviation (RSDs) from 5% to 19%. The limits of quantification (LOQs) of the compounds were from 0.025 to 0.25 mg kg^{-1}, which are well-below the maximum residue limits (MRLs) established by the European Union (EU) and the Spanish legislations.

Keywords: Food analysis; Pressurized liquid extraction; Pesticides; Fruits; Liquid chromatography; Triple stage mass spectrometry; Ion trap; Mass spectrometry

1. Introduction

Public concern, over pesticide residues in food, has been increased during the past 20–25 years. Consequently, legislations were approved in USA, European Union (EU), and other countries establishing new more restricted standards for pesticide residues in foods, which include the setting up of lower and lower maximum residue limits (MRLs) [1]. It is clear that a sound analytical methodology is indispensable for monitoring compliance with regulations.

There is continued interest in the development of alternative procedures of sample preparation, because of the need to reduce time, expenses, and hazardous wastes as well as in the automation of the already existing methods to increase sample throughput and reduce labor [1–4]. One of the most promising and recent sample preparation techniques is the pressurized liquid extraction (PLE; Dionex trade name ASE for accelerated solvent extraction), which offers the advantages of reducing solvent consumption and automating sample handling [4]. Its application in the pesticide residue field has, up to now, been limited to determine *N*-methylcarbamates [5] and few other pesticides in baby foods and adult diet samples [6] by gas chromatography (GC). PLE has a common angle with a widely used pesticide extraction procedure: matrix solid-phase dispersion (MSPD) because wet samples (such as fruits) must be previously mixed with a drying or dispersing agent [7–9].

The application of a non-selective extraction procedure with a wide-coverage of various classes of pesticides should be compensated by a specific determination technique, such as liquid chromatography-mass spectrometry, sensitive and selective enough to enable the unambiguous identification of the analyte [1–4]. Recently, pesticides have been widely determined using various LC–MS techniques, including single quadrupole [7,8], triple quadrupole [10–12] and quadrupole ion trap [9,13,14]. However, there is only one work that exploits MS^3 for determining six pesticide residues in oranges [15].

The aim of this study is to develop an analytical procedure that combines PLE and LC–IT–MS^3. The effect of several extraction parameters, such as solvent composition, temperature, pressure and static extraction time has been tested. The analytes (bitertanol, carbendazim, hexythiazox, imazalil, imidacloprid, methidathion, methiocarb, pyriproxyfen, thiabendazole and trichlorfon) were selected according to their use in fruit orchard and/or in post harvest treatments. Different peach, nec-

* Corresponding author. Tel.: +34 96 3544958; fax: +34 96 3564954.
E-mail address: cristina.blasco@uv.es (C. Blasco).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.08.037

tarine, orange and tangerine samples from local markets were analyzed using the developed method.

2. Experimental

2.1. Chemicals

Bitertanol, carbendazim, hexythiazox, imazalil, imidacloprid, methidathion, methiocarb, pyriproxyfen, thiabendazole and trichlorfon were supplied by Supelco (Madrid, Spain). The individual stock solutions were prepared by dissolving 100 mg of each compound in 100 ml of methanol, except for carbendazim, which was prepared in 10 M HCl instead of methanol. They were stored in glass-stopped bottles at 4 °C. Standard working solutions at various concentrations were daily prepared by appropriate dilution of aliquots of the stock solutions in methanol.

Methanol (gradient grade for liquid chromatography), ethyl acetate and dichloromethane (organic trace analysis) were purchased from Merck (Darmstadt, Germany). Deionized water (>18 MΩ cm resistivity) was obtained from 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.

Neutral (pH of 6–8), acidic (pH of 4–5), and basic (pH 9–10) alumina were obtained from Merck (Darmstadt, Germany), silica and sodium sulfate anhydrous from Scharlau (Barcelona, Spain), Florisil® from Aldrich (Steinheim, Germany), and diatomaceous earth from Sigma (Steinheim, Germany).

2.2. LC–MS

The LC–IT–MS system consisted of an Esquire3000 Ion Trap LC/MS(n) system (Bruker Daltonik GmbH, Germany) and an Agilent 1100 Series LC equipment, which includes a quaternary pump, an autosampler and a variable wavelength detector, a computer (HP PC) and a data acquisition/processing Daltonic Esquire Control Software system 3.0.

Separation was performed on a Luna C_{18} column (150 mm × 4.6 mm I.D., particle size 5 μm) protected by a Security guard cartridge C_{18} (4 mm × 2 mm I.D.), both from Phenomenex (Madrid, Spain). The mobile phase was a methanol–water gradient at a flow-rate of 0.8 ml min^{-1}. During the first 5 min of the run the methanol content was kept isocratic at 40%, and then it was gradually increased to 80% in 3 min, kept for 10 min, increased to 90% in 2 min, and kept for 5 min. The injection volume was set to 20 μl. Operating conditions of the APCI interface in positive ion mode were vaporizer temperature, 350 °C; nebulizer gas (nitrogen) pressure of 60 psi (1 psi = 6894.76 Pa); drying gas (nitrogen) flow rate, 4 l min^{-1}; drying gas temperature, 350 °C; capillary voltage, 4000 V; and corona current, 4 μA.

The mass spectrometer was operated in full scan and multiple reaction monitoring (MRM) modes. The trap parameters were adjusted in ion charge control (ICC) mode using rolling averaging set at 2. Full scan mode was performed with a target of 10,000 and maximum accumulation time of 50 ms at *m*/*z* ranging from 50 to 400 U. MRM was carried out setting the target at 100,000 and maximum accumulation time at 5 ms for both, MS and MS^n experiments. Collision induced dissociation (CID) was performed on the ion of interest by collisions with the helium background gas for 40 ms. The cut-offs were between *m*/*z* 100 and 150, amplitudes between 1 and 2 V, and widths between 1.0 and 4.0.

2.3. Sample treatment

Samples analyzed, oranges, tangerines, peaches, and nectarines, were obtained from agricultural cooperatives. All samples were taken in accordance with the guidelines of the European Union (EU) Directive 79/700/CEE [16]; that is, as far as possible, to collect the sample at various places distributed throughout the lot (size ca. 50 kg). The sample, weighted at least 1 kg, and consisted of at least 10 individual fruits.

A representative portion of the sample (200 g of whole fruit) was chopped and homogenized. Portions of 2.5 g were blended with 20 g of the drying agent for 5 min in a mortar using a pestle. This mixture was introduced into a stainless steel extraction cell (22 ml capacity), which was positioned in the pressurized liquid extraction (PLE) system connected to a four-bottle solvent controller, both from Dionex (Synnyvale, CA, USA). Nitrogen at pressure of 10 bars was supplied to assist the pneumatic system and to purge the extraction cells. For the extraction, ethyl acetate (100% flush volume) was used at 75 °C and 1500 psi (1 psi = 6894.76 Pa) for 7 min static time, in two cycles, preheated 2 min and purge 60 s. The total volume of extract obtained under those conditions was 22 ml showing only very little variations, less than 0.5 ml, when analyzing different samples.

Each PLE extract was concentrated to ca. 1 ml in a Büchi R200 (Labortechnik, Flawil, Switzerland) rotary evaporator set at 40 °C and 250 mBar in 50 ml round-bottomed flasks. Then, the extract was transferred to a 15 ml conical tube and the round-bottomed flask was rinsed with twice 0.5 ml of methanol and evaporated to dryness using a multi-sample Turbovap LV Evaporator (Zymark, Hoptkinton, USA) provided with a nitrogen stream and a water bath at 50 °C. After solvent evaporation, it was reconstituted in 0.5 ml of methanol.

3. Results and discussion

3.1. Evaluation of the PLE conditions

The influence of temperature, pressure, and static time on the recoveries of the pesticides in oranges is illustrated in Fig. 1. These parameters were also tested for peaches, providing similar results (data not shown). The extraction temperature (Fig. 1a) did not show a significant change on the recovery, indicating that there was no thermal degradation of pesticides, except for trichlorfon—an organophosphorus pesticide known for being thermodegrable, which disappear at 150 °C. The best results were obtained at 75 °C, with recoveries ranging from 40% to 108% and RSDs from 5% to 12%. An increase in color and in cloudy suspension as well as in the RSDs was visible noticed

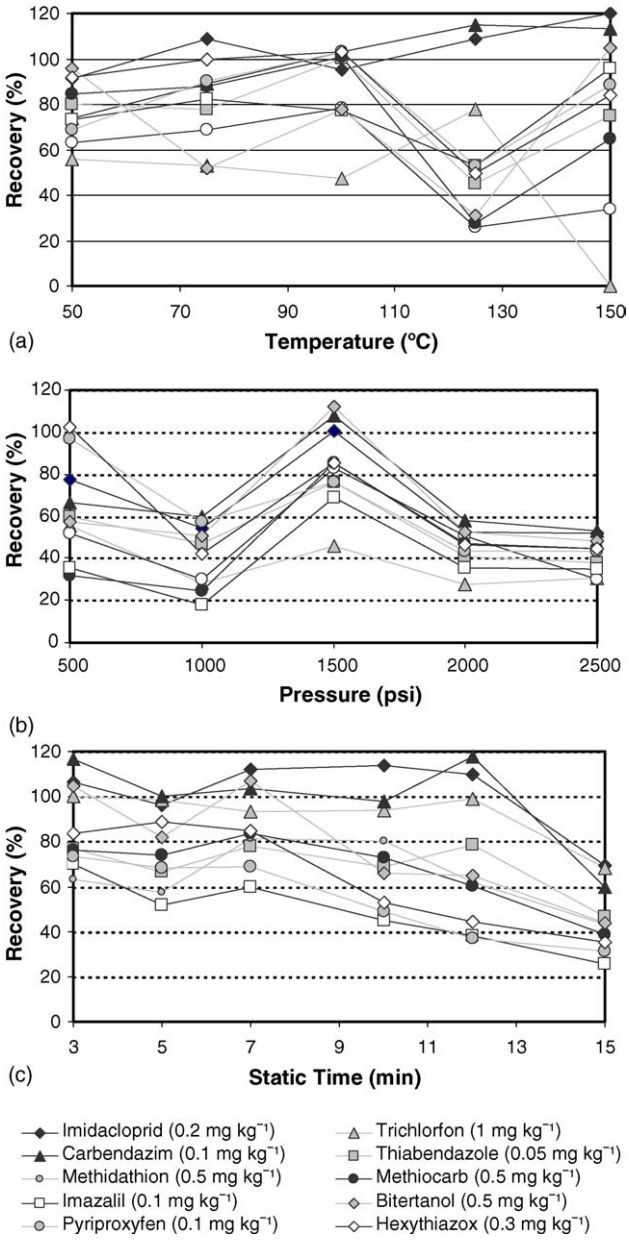

Fig. 1. Effect on the extraction efficiency of (a) temperature; (b) pressure; and (c) static time. Extraction conditions: ethyl acetate (flush 100%) in one static cycle; drying agent: anhydrous sodium sulfate.

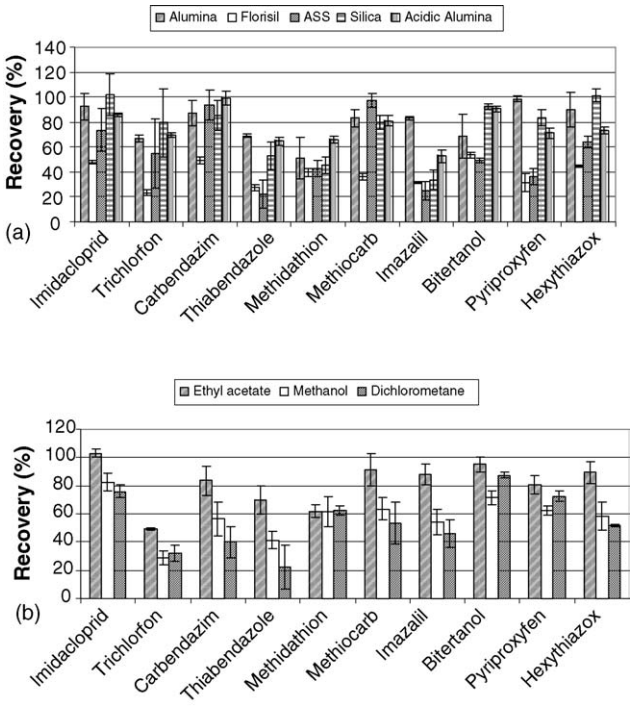

Fig. 2. Effect on the extraction efficiency of (a) different drying agents and (b) different extraction solvents. Other conditions and compounds concentration as in Fig. 1.

when the temperature increased from 90 to 150 °C because compounds of high molecular mass (carotenes, flavonoid, glucids, etc.) were co-extracted. Best recoveries were obtained at a pressure of 1500 psi (Fig. 1b). Operation at low pressure, close to 500 psi, the lowest possible with subsequent analysis, achieves also high recoveries. However, the system becomes unstable (overfilled collections vials), because of difficulties in maintaining the set pressure.

Extraction efficiency remained constant for all the static times tested (Fig. 1c), which can be explained by the high solubility of the studied pesticides in ethyl acetate and/or the weak analyte–matrix interactions, as have been reported previously [5,6]. Since the length of the static cycle did not influence the extraction efficiency, the extraction time was set at to 7 min to assure a rapid extraction as well as a constant recovery without observable variations.

The percentage of flush (from 50% to 150%) and the number of extraction cycles (from 1 to 5) were checked. The highest recoveries were reached at 100% flush, keeping constant for the bigger percentages. Extraction efficiency was constant from one to three extraction cycles, whereas starting from the fourth cycle a remarkable decrease was noted. A justification of this behavior is that the more cycles were used, the greater amounts of interfering substances were extracted.

Alumina, floristic, silica, and anhydrous sodium sulfate were tested, as drying materials, for PLE in oranges and peaches. In addition, alumina was tested in the three pH ranges available (basic, neutral, and acidic). Recoveries were very similar for all the compounds, except for trichlorfon, the recovery of which decreases from 75% using acidic silica to 32% using basic alumina. A probable explanation is that Trichlorfon is quickly degraded in slightly basic aqueous solutions. Fig. 2a shows the recoveries and RSDs obtained from oranges using these sorbents, excepting basic alumina. As it can be seen, alumina and silica provided almost the same recoveries for all the compounds, except for imazalil, which is better recovered from alumina. However, RSDs obtained using alumina were lower than those obtained using silica, especially for the most polar compounds (imidaclorid, trichlorfon, carbendazim, and thiabendazole). Neutral and acidic alumina provided very similar recoveries; however, slightly better recoveries were observed working acidic alumina, particularly for trichlorfon. Florisil gave lower recoveries for all the compounds, and anhydrous sodium sulfate gave also low recoveries for thiabendazole, imazalil, bitertanol,

pyriproxyfen and hexythiazox. In addition, this last sorbent provides the dirtiest extracts with a cloudy and strong color.

Ethyl acetate, methanol, and dichloromethane were tested as extraction solvent as Fig. 2b reports. An increase in the extraction efficiency was observed from dichloromethane to ethyl acetate, except for bitertanol, trichlorfon, pyriproxyfen, and methiocarb. Dirtiest extracts were obtained with methanol because it also extracts other food components with higher efficiency as flavonoids, carotenes, and sugars than dichloromethane or ethyl acetate.

The optimum procedure was to disperse the sample with acidic alumina and to extract it with ethyl acetate (100% flush) at 75 °C and 1500 psi for 7 min in two cycles.

3.2. Liquid chromatography–triple stage mass spectrometry

Table 1 shows the selected transitions for the studied pesticides. The triple-stage mass spectrum of six of them – imidacloprid, carbendazim, thiabendazole, methiocarb, and hexythiazox – as well as their ability to quantify pesticide residues in citrus fruits had already been studied in a previous work [15]. The second-stage mass spectra of bitertanol [12] and pyriproxyfen [9] have also been studied. However, they have never been determined by triple-stage mass spectrometry. Bitertanol, trichlorfon and pyriproxyfen can be determined, at the required detection limits, using triple stage mass spectrometry. On the contrary, methidathion can only be determined by a second-stage mass spectrometry because it gave the product ion at *m/z* 145, which fragmentation is very unstable and in the limits of the instrumental capabilities of the ion-trap.

Quantification of the extracts was based on a six points matrix matched standard curves covering the range from LOQ to 100 times the LOQ. Matrix-matched standard curves were prepared by extracting blank orange and peach at 75 °C for 7 min, using ethyl acetate, thus assuring a perfect match between samples analyzed and standard curves. The need to use matrix-matched standard curves was demonstrated by evaluating calibration curves based on standard diluted in methanol or in matrix extracts. The results are showed in Table 2. These calibration curves presented good linearity (*r* value of all curves were >0.991), but different slopes. Carbendazim, imidacloprid, and thiabendazole are the compounds less affected by the matrix, showing suppression in the response <15% in matrix matched standards compared to those prepared in methanol. For hexythiazox, methiocarb, and pyriproxyfen response suppression <25% was noted, and for imazalil, methiocarb, and trichlorfon response suppression was <50%, whereas for bitertanol an increase on the response of 50% was observed. This results are in agreement with those previously reported [9,12,15].

The MS analysis was carried out by MS^3 according to the transitions reported in Table 1 (except for trichlorfon that

Table 1
Time schedule, precursor/product ions, and their relative abundance (*A*, %) at different MS stages

Time window (min)	Analyte	MS		MS^2		MS^3	
		m/z (*A*, %)	Assignment	*m/z* (*A*, %)	Neutral loss	*m/z* (*A*, %)	Neutral loss
0–9.2	Imidacloprid	256 (100)	$[M+H]^+ \rightarrow$	209 (100) 175 (75)	$[-NO_2] \rightarrow$ $[-NO_2,-Cl]$	175 (100)	[−Cl]
	Trichlofon	256 (100) 228 (50)	$[M]^+ \rightarrow$ $[M-C_2H_4]^+$	221 (100)	$[-HCl] \rightarrow$	145 (50) 127 (100)	– $[C_2Cl_2]$
	Carbendazim	192 (100)	$[M+H]^+ \rightarrow$	160 (100)	$[-CH_3OH] \rightarrow$	160 (6) 132 (100) 105 (25)	 [−CO] [−CO,−HCN]
	Thiabendazole	202 (100)	$[M+H]^+ \rightarrow$	175 (100) 131 (20)	$[-HCN] \rightarrow$ [−HCN,−SC]	131 (100)	[−SC]
9.2–20	Methidathion	303 (100) 145 (80)	$[M+H]^+ \rightarrow$ $[M-PS_2(OCH_3)_2]$	145 (100)	$[-PS_2(OCH_3)_2]$		
	Methiocarb	226 (100)	$[M+H]^+ \rightarrow$	169 (100)	$[-CONCH_3] \rightarrow$	121 (100)	$[HSCH_3]$
	Imazalil	297 (100)	$[M+H]^+ \rightarrow$	257 (100) 201 (75) 173 (50) 159 (45)	$[-C_3H_6] \rightarrow$ $[-C_3H_6,C_3N_2H_4]$ $[-C_3H_6,C_3N_2H_4,O]$ $[-C_3H_6,C_3N_2H_4,CO]$	187 (50) 159 (100)	$[-C_3N_2H_4]$ $[-C_3N_2H_4,CO]$
	Bitertanol	338 (100)	$[M+H]^+ \rightarrow$	269 (100) 251 (20)	$[-C_2H_3N_3] \rightarrow$ $[-C_2H_3N_3, -H_2O]$	251	$[-H_2O]$
20–25	Pyriproxyfen	322 (100)	$[M+H]^+ \rightarrow$	227 (100) 199 (10) 185 (20)	$[-C_5NH_4OH] \rightarrow$ $[-C_5NH_4OH,-C_2H_4]$ $[-C_5NH_4OH,-C_3H_6]$	199 (30) 185 (100) 134 (20)	$[-C2H_4]$ $[-C_3H_6]$ $[-C_6H_5OH]$
	Hexythiazox	353 (100) 228 (40)	$[M+H]^+ \rightarrow$ $[M+H-C_6H_{12}NCO]^+$	271 (50) 228 (100) 168 (20)	$[-C_6H_{12}]$ $[-C_6H_{12}, NCO] \rightarrow$ $[-C_6H_{12}, NCO,-SCO]$	168	[−SCO]

Table 2
Matrix calibration of blank oranges in comparison with standard calibration[a]

	Concentration range (mg kg^{-1})	r (standards in methanol)	r (matrix matched standards)	Slope matrix/slope standard
Imidacloprid	0.01–1	0.9966	0.9997	0.96
Trichlorfon	0.25–25	0.9992	0.9909	0.87
Carbendazim	0.02–2	0.9991	0.9998	0.66
Thiabendazole	0.02–2	0.9999	0.9975	0.99
Methidathion	0.04–4	0.9938	0.9989	0.84
Methiocarb	0.04–4	0.9999	0.9976	0.68
Imazalil	0.02–2	0.9991	0.9999	0.50
Bitertanol	0.04–4	0.9961	0.9924	1.58
Pyriproxyfen	0.02–2	0.9995	0.9988	0.75
Hexythiazox	0.09–9	0.9971	0.9934	0.82

[a] The data are obtained by six level calibration in triplicate.

was determined at MS^2). For identification purposes, the ratio between the different fragment ions (when there are) in the product ion full scan mass spectrum was measured. Quantification was carried out by conventional external standard procedure using matrix matched standards.

3.3. Method validation

The method was validated for oranges/tangerines and peaches/nectarines, according to EU guidelines [17,18]. Table 3 shows the recovery, precision and quantification limits (LOQs) obtained. In oranges, the lowest average recovery (60%) was obtained for methidathion, whereas the highest was 98% for imidacloprid) with RSDs from 5% (methidathion) to 19% (trichlorfon). Slightly low recoveries were observed for imidacloprid, imazalil, and methiocarb in peaches and nectarines, the average recoveries ranged from 48% (imazalil) to 98% (carbendazim) and the RSDs were between 5% (imazalil) and 19% (bitertanol). LOQs were well-below the MRLs documented by different national and international governmental statements that ensures a reliable determination.

Typical chromatograms for tangerine samples (non-spiked and spiked at the LOQ levels) are shown in Fig. 3a and b. The chromatographic resolution and the peak performance were satisfactory for the studied pesticides in the spiked samples. The sample that no contains any of the studied pesticides show the lack of interfering peaks that can give a false positive sample.

3.4. Fruit sample extractions: PLE versus conventional ethyl acetate extraction

Results were compared with those obtained using the conventional solvent extraction (SE) with ethyl acetate and anhydrous sodium sulfate. Fig. 4 shows the recoveries and the RSDs of both methods, obtained from oranges at the concentrations used in the optimization experiments. Recoveries obtained using PLE methods ranged from 48% to 98%, whereas those obtained by SE were in the range of 32% to 98%. PLE gives better recoveries for all pesticides in both matrices, except for trichlorfon. The RSDs showed no differences with any of the two methods, even through PLE is automated programmed. Table 4 summarizes several parameters indicative of the analytical performance of both procedures. As a consequence of the better accuracy provided by PLE for imidacloprid, imazalil, bitertanol and pyriproxyfen, the LOQ obtained for these compounds by PLE is almost half than that obtained by SE.

Table 3
Recovery and repeatability of the method ($n = 5$)

	Spiking level[a] (mg kg^{-1})	Oranges		Peaches		Lowest MRLs (mg kg^{-1})
		Recovery (%)	RSDs (%)	Recovery (%)	RSDs (%)	
Imidacloprid	0.01, 0.1	98, 97	17, 12	63, 74	16, 10	0.05[b]
Trichlorfon	0.25, 2.5	75, 78	19, 14	67, 72	17, 13	1.00[b,c]
Carbendazim	0.02, 0.2	85, 87	12, 8	95, 98	11, 9	0[d]–1.00[b,c]
Thiabendazole	0.02, 0.2	77, 82	12, 10	76, 78	18, 15	0.05[b,c]
Methidathion	0.04, 0.4	60, 67	5, 5	65, 69	18, 15	0.05[d]
Methiocarb	0.04, 0.4	90, 94	11, 9	65, 69	18, 9	0.05[b,e]
Imazalil	0.02, 0.2	89, 91	12, 10	48, 54	15, 9	0.02[b,c]
Bitertanol	0.04, 0.4	88, 89	15, 10	82, 87	18, 11	0.05[b,c]
Pyriproxyfen	0.02, 0.2	89, 91	15, 13	79, 81	19, 16	0.05[b]
Hexythiazox	0.09, 0,9	79, 82	17, 14	77, 85	17, 13	0.5[b,e]

[a] Spiking levels corresponding to the LOQ and 10 times the LOQ according to EU guidelines [17,18]; LOQ was the lowest concentration that provides acceptable recovery (>70%) and reproducibility (<20%) [17].
[b] MRL established by Spanish Legislation [19].
[c] MRL established by EU [20].
[d] MRL established by USA [21].
[e] MRL recommended by Codex Alimentarius [22].

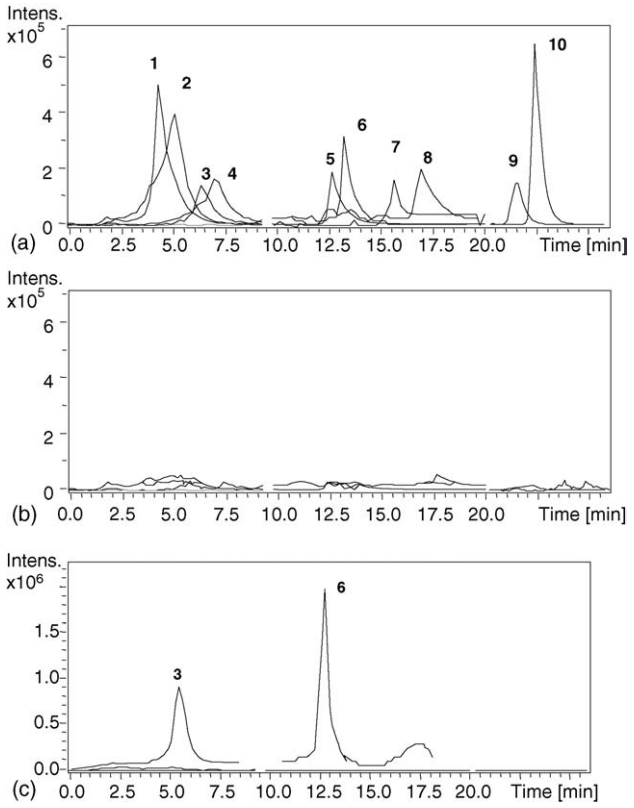

Fig. 3. LC–IT–MS chromatograms obtained after PLE for (a) spiked orange; (b) unspiked orange; and (c) sample no. Peak identification: (1) imidacloprid, (2) trichlorfon, (3) carbendazim, (4) thiabendazole, (5) methidathion, (6) methiocarb, (7) imazalil, (8) bitertanol, (9) pyriproxyfen; and (10) hexythiazox.

The linearity of the calibration curves, constructed from the analysis of spiked samples was good in both procedures, with correlation coefficients always greater than 0.99. Matrix interference studies conducted by both procedures showed that, for both, important enhancement or suppression of the response is observed for the majority of compounds.

Table 5 shows the different pesticide residues detected, as well as their concentration levels (concentrations were corrected for the recoveries). Residues of pesticides were found in 18 of 40 sets of samples analyzed. Carbendazim and imidacloprid were frequently present. Although both procedures gave comparable results, the proposed methodology gives increased possibilities

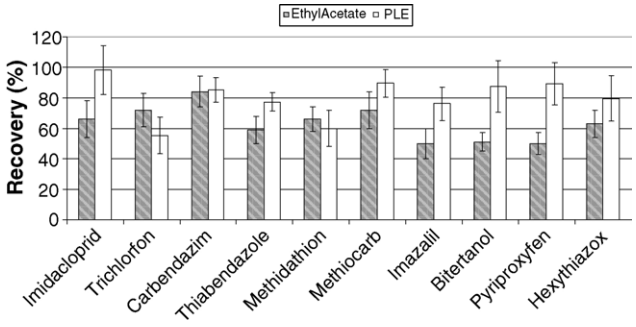

Fig. 4. Comparison of PLE and conventional SE with ethyl acetate results of pesticide recovery in spiked oranges.

Table 4
Performance comparison

	PLE	Ethyl acetate
Spiking concentration (mg kg^{-1})	LOQ–10 LOQ	LOQ–10 LOQ
Recovery (n = 5, %)	48–98	32–98 Worst for most compounds
Repeatibility (RSD, %; n = 5)	<19	<19
Linearity (r^2)	>0.994	>0.992
Matrix interferences	Suppression (15–50%) for most compounds, enhancement for bitertanol	Suppression (15–50%) for most compounds, enhancement for bitertanol
Sensitivity (LOQ, mg kg^{-1})	0.01–0.25	0.01–0.25 Worst LOQ for imidacloprid, imazalil, bitertanol, pyriproxifen

of automation with no extra cleanup step needed, leading to substantial time savings as compared with classical methodologies. Fig. 3c shows an LC–IT–MS^3 chromatogram for an orange in which carbendazim and methidathion were found.

Table 5
Pesticide concentrations in oranges, tangerines, peaches and nectarines obtained from agricultural cooperative

Samples	Pesticides	Content, n = 3, mg kg^{-1}(RSD, %)	
		PLE	Ethyl acetae
Oranges			
1	Carbendazim	0.02 (12)	0.02 (16)
	Hexythiaxoz	0.09 (15)	0.07(9)
4	Carbendazim	0.03 (8)	0.01 (7)
7	Carbendazim	0.04 (12)	0.05 (8)
10	Carbendazim	0.03 (5)	0.05 (11)
11	Carbendazim	0.04 (18)	0.05 (11)
13	Carbendazim	0.10 (3)	0.16 (14)
15	Carbendazim	0.06 (12)	0.05 (5)
16	Carbendazim	0.03 (15)	0.02 (8)
	Methidathion	0.16 (6)	0.12 (8)
18	Carbendazim	0.09 (9)	0.14 (17)
19	Carbendazim	0.16 (4)	0.11 (14)
	Imazalil	0.55 (13)	0.95 (5)
	Carbendazim	0.05 (8)	0.02 (2)
Peaches			
1	Carbendazim	0.55 (12)	0.45 (15)
3	Carbendazim	0.09 (14)	0.12 (18)
	Imidacloprid	0.02 (19)	0.03 (13)
4	Carbendazim	0.69 (7)	0.76 (7)
	Imidacloprid	0.02 (8)	0.04 (8)
7	Carbendazim	0.17 (6)	0.23 (19)
12	Thiabendazole	0.03 (18)	0.02 (8)
14	Carbendazim	0.36 (12)	0.44 (1)
16	Carbendazim	0.57 (9)	0.34 (4)
	Imidacloprid	0.17 (8)	0.14 (12)

4. Conclusions

The efficiency of PLE is comparable to conventional techniques to extract pesticide residues from fruits. The required solvent volume is smaller, and this extraction procedure is less time consuming and the handling of the sample is reduced.

APCI–MS^2 and APCI–MS^3 can be combined in the same chromatographic run to characterized pesticides in a QIT. The studied multiple-stage spectrum showed a characteristic fragmentation pattern for each compound, which provides sufficient information and achieves the unequivocal identification of the compounds.

PLE coupled with LC–IT–MS^3 enables rapid and accurate determination of pesticides in fruit samples with LOQs in the range of 1–50 $\mu g\,kg^{-1}$, which are below the MRLs established by the EU. The low LOQs allow application of the presented method for monitoring priority list of LC-amenable pesticides in fruits.

Acknowledgements

This work has been financially supported by the Conselleria d'Empresa, Universitat i Ciencia (project No. GVA05/109).

References

[1] F.E. Ahmed, Trends Anal. Chem. 20 (2001) 649.
[2] Y. Picó, C. Blasco, G. Font, Mass Spectrom. Rev. 23 (2004) 45.
[3] Y. Picó, G. Font, J.C. Moltó, J. Mañes, J. Chromatogr. A 882 (2000) 153.
[4] P.L. Buldini, L. Ricci, J.L. Sharma, J. Chromatogr. A 971 (1) (2002) 234.
[5] M. Okihashi, H. Obana, S. Hori, Analyst 123 (1998) 711.
[6] J.C. Chuang, K. Hart, J.S. Chang, L.E. Boman, J.M. van Emon, A.W. Reed, Anal. Chim. Acta 444 (2001) 87.
[7] C. Blasco, Y. Picó, J. Mañes, G. Font, J. Chromatogr. A 947 (2002) 227.
[8] X. Pous, M.J. Ruiz, Y. Picó, G. Font, Fresenius' J. Anal. Chem. 371 (2001) 182.
[9] C. Soler, J. Mañes, Y. Picó, J. Chromatog. A 1048 (2004) 41.
[10] M.J. Taylor, K. Hunter, K.B. Hunter, D. Lindsay, S.L. Bouhellec, J. Chromatogr. A 982 (2002) 225.
[11] T. Goto, Y. Ito, H. Oka, I. Saito, H. Masumaoto, H. Nakazawa, Anal. Chim. Acta 487 (2003) 2001.
[12] C. Soler, J. Mañes, Y. Picó, J. Chromatog. A 1067 (2005) 115.
[13] C. Blasco, J. Mañes, G. Font, Y. Picó, Anal. Chem. 75 (2003) 3274.
[14] J. Zrostlýkova, J. Hajslova, T. Kovalczuk, R. Stephan, J. Poustka, J. AOAC 359 Int. 86 (2003) 612.
[15] C. Blasco, G. Font, Y. Picó, J. Chromatog. A 1043 (2004) 231.
[16] EC Comision Directive 79/700/EEC (24 July 1979) establishing community methods of sampling for the official control of pesticide residues in and on fruit and vegetables, Official Journal L207, 15/08/1979, Brussels, Belgium, p. 0026.
[17] European Commission, Directorate General Health and Consumer Protection, Residue analytical methods for post-registration control and monitoring, Sanco/825/00 (rev. 7) 17 March 2004.
[18] European Commission, General Health and Consumer Protection, Quality control procedures for pesticide residues analysis, Sanco/10476/2003.
[19] http://www.mcx.es/plaguicidas/plaresul.asp, Ministerio de Economía, Secretaría General de Inspección, Certificación y Asistencia Técnica del Comercio Exterior, Madrid, Spain, 2003.
[20] EU MRLs shorted by pesticides updated 04/11/2004 disposable at http://europ.eu.int European Union, Brussels, 2005.
[21] http://www.gpoaccess.gov/cfr/index.html Code of Federal Regulations, USA, 2004.
[22] http://apps.fao.org/default.jsp Codex Alimentarius Commision, FAO/OMS, Rome, Italy, 2004.