Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1073 (2005) 229–236
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Capillary electrophoresis for analyzing pesticides in fruits and vegetables using solid-phase extraction and stir-bar sorptive extraction

Ana Juan-García, Yolanda Picó, Guillermina Font*

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

Abstract

Two procedures based on solid-phase extraction (SPE) and stir-bar sorptive extraction (SBSE) in combination with micellar electrokinetic chromatography (MEKC)–diode array detection (DAD) were compared for the simultaneous extraction of acrinathrin, bitertanol, cyproconazole, fludioxonil, flutriafol, myclobutanil, pyriproxyfen, and tebuconazole in lettuce, tomato, grape, and strawberry. Selectivity and resolution of the MEKC procedure were studied changing the pH and the molarity of the buffer, the type and the concentration of surfactant, and the methanol content in the mobile phase. A buffer consisting of 6 mM sodium tetraborate decahydrate with 75 mM of cholic acid sodium solution (pH 9.2) gave the best results. Linearity, extraction efficiencies and limits of quantitation (LOQs) of both extraction methods were compared. The recoveries obtained by SPE ranged from 40 to 106% with relative standard deviations (R.S.D.s) from 10 to 19% whereas by the SBSE method, the recoveries were 12–47% and the R.S.D.s 3–17%. The LOQs were much better by SPE (0.2–0.5 mg kg^{-1} depending on the processed sample amount) than those obtained by SBSE (1 mg kg^{-1} for each compound). Advantages and disadvantages of both procedures are also discussed. As SPE is more robust, rapid, and sensitive than SBSE, its application in combination with MEKC is recommended because provided LOQs below the MRLs established, which is not always attained by SBSE.

Keywords: Food analysis; Solid-phase extraction; Stir-bar sorptive extraction; Pesticides; Micellar electrokinetic chromatography

1. Introduction

The social concern about the levels of pesticides in food and the constant trend observed in the current legislations to reduce the maximum residue levels (MRLs) allowed in a variety of fruits and vegetables is increasing the number of samples to be analyzed as well as the need for their accurate determination reducing the analysis' costs [1].

Sample preparation is often the most time-consuming and laborious part of the analytical process. An ideal procedure should be simple, inexpensive, efficient, able to extract the largest number possible of pesticides, and compatible with various determination techniques [2,3]. That is the reason why these methods strive towards the simplification and miniaturization. Solid-phase extraction (SPE) and stir-bar sorptive extraction (SBSE) are well-established preconcentration techniques in food analytical applications because they offer significant advantages such as economy in terms of time and solvent needs [4–12].

Side by side with the classical techniques for detecting pesticide residues—gas chromatography (GC) or liquid chromatography (LC)—an increasing number of methods involve the use of capillary electrophoresis (CE) [13–15]. CE joints separation of compounds with GC-like resolution, and the capability of LC to determine thermally labile or non-volatile compounds. CE shows great potential in the analysis of contaminants in food because of its features, such as higher separation efficiency, shorter analysis time, simplicity with regards to instrumentation, and very less consumption of expensive reagents and solvents [13,14]. In the beginning, CE presented the disadvantage of inappropriate sensitivity for contaminant analysis, as a result of the small sample volumes typically injected (ca. 1–10 nl), but it has already been solved by off- and on-column trace enrichment schemes [15–18]. This is often performed by SPE that can be used directly as

* Corresponding author. Tel.: +34 963544295; fax: +34 963544954.
E-mail address: guillermina.font@uv.es (G. Font).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.09.028

an extraction technique for liquid matrices, or as a clean-up method for solvents extracts [13,15]. SPE using C_{18} has already been applied for determining urea and fungicides in fruits and vegetables followed by CE [17,19–22]. However, the high matrix load required to achieve an appropriate pesticide concentration can cause the partial co-extraction of interfering substances, breakdown of the analytes, or clogging of the SPE material. SBSE, a relatively new extraction technique, uses as a magnetic rod encapsulate in a glass jacket and coated with polydimethylsiloxane (PDMS). Analytes are sorbed from aqueous samples based on partitioning between aqueous extract and PDMS. Because its unique characteristics, SBSE has the potential to overcome the problems associated with SPE of pesticides from fruits and vegetable extracts [8–12]. For example, SBSE, as an equilibrium technique, does not experience breakthrough and plugging, and can co-extract less water soluble co-extracts. To date, the application of SBSE to pesticide residues has focused mainly in GC determination [9–11]. Although there are recent reports on the application of SBSE to LC [8,12], no studies on the SBSE combined with CE determination have been reported.

The present study compares SPE and SBSE as enrichment schemes for determining eight pesticides in strawberries, grapes, lettuces, and tomatoes by MEKC and diode array detection (DAD). As target analytes (see Fig. 1), four conazole fungicides (flutriafol, cyproconazole, tebuconazole, and myclobutanyl), one azole fungicide (bitertanol), one pyrrole fungicide (fludioxonil), one pyrethroid insecticide (acrinathrin), and one juvenile hormone mimic insecticide (pyriproxyfen) were selected based on their application in fruits and vegetables and on the legislation requirements of the EU [23].

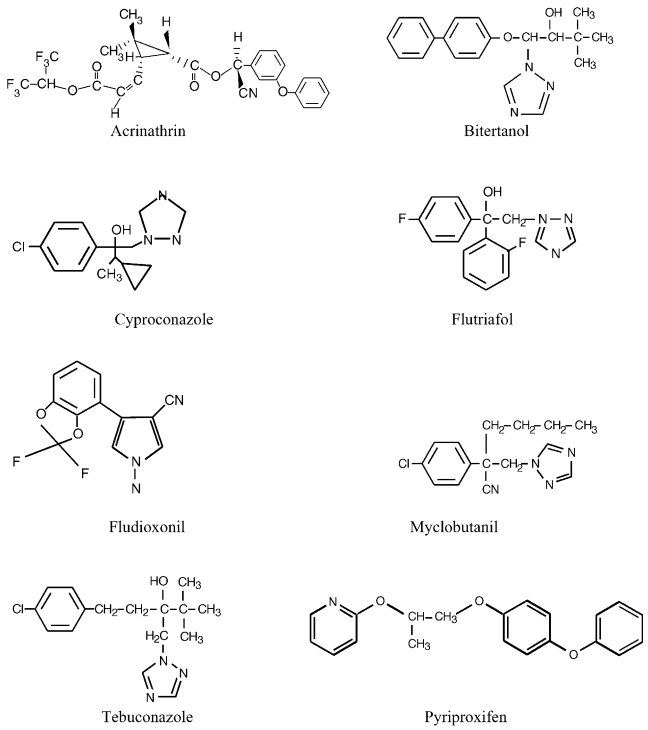

Fig. 1. Structures of the studied pesticides.

2. Experimental

2.1. Chemicals and reagents

Fludioxonil, cyproconazole, tebuconazole, bitertanol, acrinathrin, and flutriafol were purchased from Riedel-de-Haën (Seelze, Germany). Myclobutanil and pyriproxyfen were from Dr. Ehrenstorfer GmbH. Individual stock solutions were prepared at concentration of 1 mg ml^{-1} in methanol and stored in glass-stopper bottles at 4 °C. Working solutions, at different concentrations, were prepared by appropriate combination and dilution of the standard solutions with the running buffer. Mixtures were passed through a 0.45 μm cellulose filter from Scharlau (Barcelona, Spain).

HPLC-grade methanol and organic trace analysis grade dichloromethane were obtained from Merck (Darmstadt, Germany) and acetone from Mallinckrodt (ChromAR-HPLC, KY, USA). Deionized water (<18 MΩ cm resistivity) was used from the Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA).

Cholic acid sodium salt was purchased from Fluka (Buchs, Switzerland), sodium tetraborate decahydrate, and sodium dodecyl sulphate from Aldrich (Madrid, Spain), and sodium chloride, ortophosphoric acid, disodium hydrogenphosphate dihydrate, potassium dihydrogenophosphate, and trisodium phosphate dodecahydrate were of analytical grade from Scharlau (Barcelona, Spain).

MFE C_{18} solid phase (particle diameter in the range of 45–55 μm and pore diameter 60 Å) was acquired from Análisis Vínicos (Tomelloso, Spain). The solid-phase was placed into 100 mm × 9 mm i.d. glass column fitted with a coarse frit (No. 3). Prior to use, the column was activated by washing successively with methanol (10 ml) and deionized water (10 ml).

The stir bars (Twister) were from Gerstel (Mülheim, Germany) with a length of 10 μm and coated with a 1 mm PDMS layer. The stir bars were preconditioned by sonication 5 min into a vial containing 15 ml of methanol. The procedure was repeated three times.

2.2. Apparatus

All capillary electrophoresis separations were performed on a Beckman P/ACE System MDQ (Fullerton, CA, USA) equipped with a diode array detector and System Gold software for data acquisition. Uncoated fused-silica capillaries purchased from Beckman were used. The dimensions of the capillary were 57 cm × 75 μm i.d. The effective length of the capillary was 50 cm from the injection end of the capillary.

The electrolyte pH was measured by a pH meter (Model DM-21, Digined, Sao Paulo, Brazil).

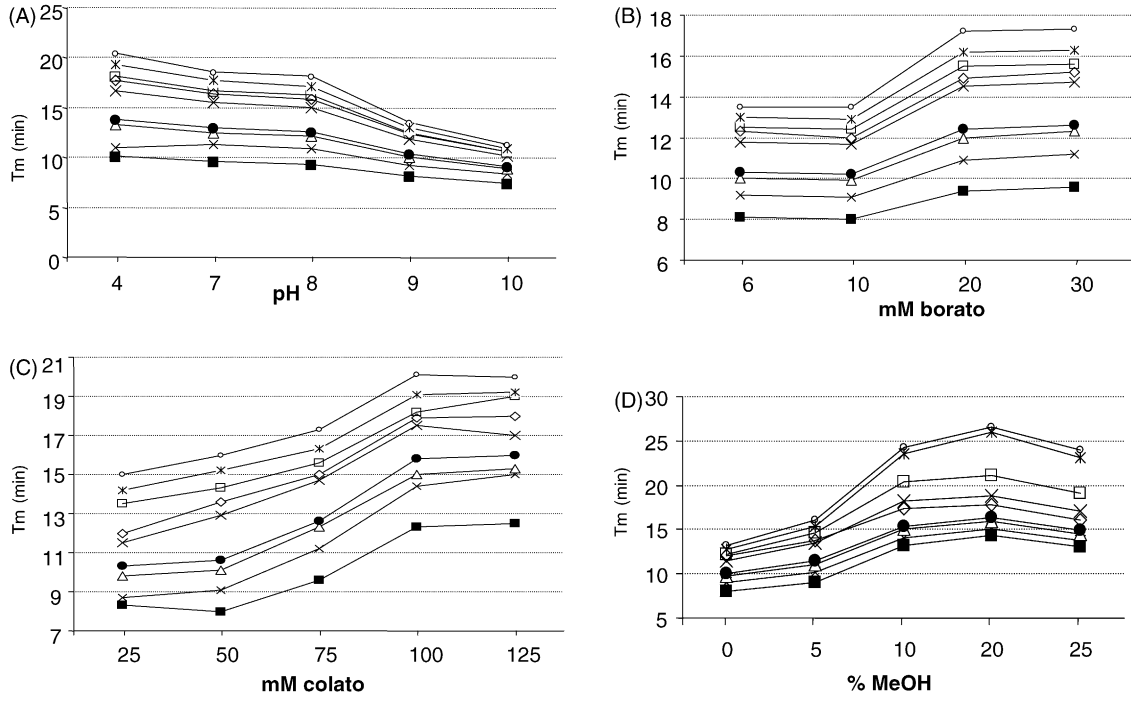

Fig. 2. Variations of the migration time of pesticides as a function of (A) pH, (B) electrolyte concentration, (C) sodium cholate concentration, and (D) percentage of methanol. Capillary: 57 cm × 50 μm i.d.; sample concentration: 10 μg ml^{-1}; detection wavelength 214 nm; other operating conditions: 20 kV, 25 °C. Curve identification: (■) flutriafol; (x) cyproconazole I; (Δ) cyproconazole II; (●) myclobutanil; (×) tebuconazole; (◊) acrinathrin; (□) bitertanol; (✱) fluodioxonyl; (◯) pyriproxyfen.

2.3. Preparation of running buffer and sample injection

Several parameters were studied during the optimization of the separation buffer, such as pH and concentration of the background electrolyte (BGE) ranging from 4 to 11 and from 3 to 30 mM, respectively with small increments. The presence of methanol as organic modifier, and the employment of different surfactants (cholic acid sodium salt and sodium dodecyl sulphate) at different concentrations were also tested. The buffer selected in MEKC was 6 mM sodium tetraborate decahydrate 75 mM cholic acid sodium salt prepared by diluting appropriate amounts in deionized water; complete dissolution was achieved by use of an ultrasonic bath.

The pH of a borate buffer was adjusted to the desired pH value by mixing various proportions of 6 mM sodium tetraborate solution with the same concentration of boric acid sodium hydroxide.

At the beginning of each day, the capillary was conditioned with 0.1 M sodium hydroxide for 30 min. Before each injection, the capillary was pre-washed for 2 min with deionized water, 2 min with 0.1 M NaOH, 2 min with deionized water, and 2 min with running buffer applying an overpressure of 20 psi (1 psi = 6894.76 Pa). Sample injections were carried out in the hydrodynamic mode over 5 s of 0.5 psi. A voltage of 30 kV was applied to keep the total current less than 80 μA at 25 °C. The detection wavelength was set at 214 nm.

2.4. Extraction procedure

Strawberry, grape, lettuce, and tomato samples were taken at some local supermarkets. A representative portion of the sample was chopped in a food chopper and 100 g portions were stored in closed containers in a freezer.

A portion of sample (between 5 and 15 g) spiked or not with pesticides was placed in a 250 ml glass beaker and mixed and homogenized in an ultrasonic bath for 15 min, therefore 10 ml of water–acetone (50:50, v/v) was added and homogenized 15 min more. The resulting suspension was filtered through a Buchner funnel and the cake filter was washed twice with 20 ml of acetone. The extract was evaporated by rotavapour to eliminate the acetone.

2.4.1. Solid-phase extraction

The aqueous sample extract was passed through the C_{18} column at a flow rate about 2 ml min^{-1}. Retained pesticides were eluted with 10 ml of dichloromethane. The eluent was collected in a graduated conical tube (20 ml) and concentrated, under stream of nitrogen, to dryness. After that, it was redissolved with 0.5 ml of buffer solution.

2.4.2. Stir-bar sorptive extraction

The filtrate was placed into a 50 ml glass beaker and stirring with the stir bar, for 4 h at 900 rpm. After the extraction, the stir bar was removed from the aqueous sample with a magnetic stirring bar. The analytes were desorbed into 2 ml vial

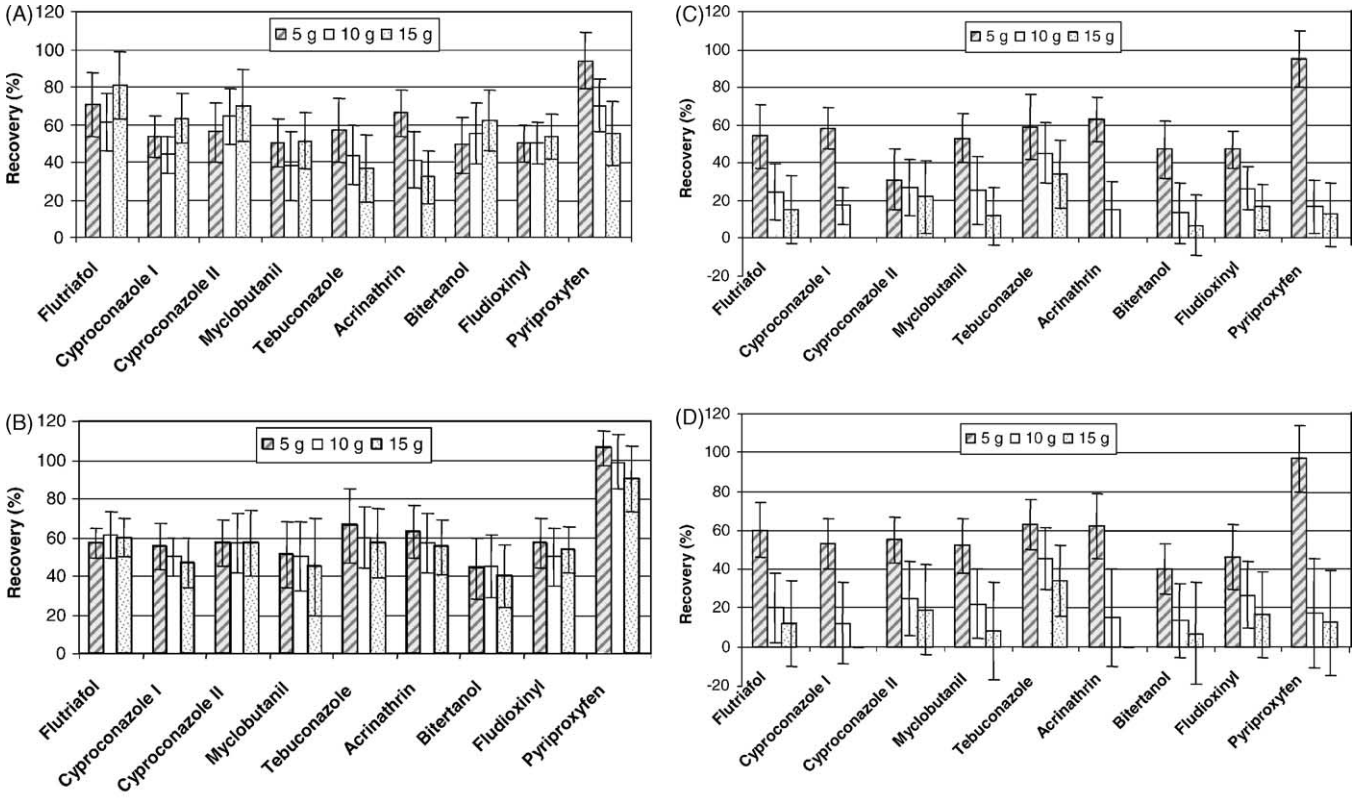

Fig. 3. Effect of sample amount on pesticide recoveries and R.S.D.s by SPE in (A) lettuce, (B) tomato, (C) strawberry, and (D) grape.

filled with 1 ml of methanol, concentrated to dryness under a stream of nitrogen, and redissolved with 0.5 ml of buffer.

3. Results and discussion

3.1. Optimization of the electrophoretic conditions

The optimization of the electrophoretic conditions is shown in Fig. 2. The pH of the separation buffer was varied between 4 and 10. All buffers were 6 mM borate and 75 mM sodium cholate. The migration times decreased with pH (Fig. 2A). The lower analysis time with appropriate resolution between analytes was at pH 10. Although the analysis time was higher, the pH 9.2 was selected as optimal because life-time of the capillary since higher pH degraded the silica inner wall of the capillary too quickly.

The influence of sodium tetraborate molarity on the pesticide separation was examined in the range 6–30 mM using a buffer 75 mM sodium cholate at pH 9.2 (Fig. 2B). For all the analytes, the migration times were almost the same until 10 mM, then increased until 20 mM maintaining again until 30 mM. Because of these results a sodium tetraborate concentration of 6 mM was selected.

Sodium dodecyl sulphate and sodium cholate were tested as surfactants. Peak resolution was much better using sodium cholate, which was selected for further experiments, this last surfactant achieved the differentiation of two isomers of cyproconazole, even through no chiral buffer is used. Different behavior was observed when the concentration of cholate was varied in the range 25–125 mM. Although the migration order of pesticide compounds with cholate concentration remains the same, the resolution varies considerably as cholate concentration increased from 25 to 75 mM. In addition, it can be see in Fig. 2C that the migration time increased, when cholate concentration varied from 75 to 125 mM. This behavior can be explained by both the increase of the ionic strength of the separation buffer and the greater interaction between analytes and micelles caused by the use of highest cholate concentrations. Therefore, the best separation was obtained with intermediate 75 mM cholate concentration in the buffer.

In order to find the best compromise between resolution and analysis speed, the effect of methanol concentration as organic modified was investigated, varying from 0 to 25% (see Fig. 2D). It was observed that increasing the percentage of methanol, the analysis time was increased and does not lead to real improvements in the resolution of the analytes. So, the use of organic modifier was avoided.

Table 1 outlines some analytical parameters of the proposed method. The complete separation of the eight studied pesticides was obtained with resolution values >1. The repeatability and reproducibility of migration times were fairly high; the R.S.D. values did not exceed 2.0 and 3.0%, respectively. The repeatability and reproducibility of the peak area were worst; the R.S.D. values for different analytes ranged

Table 1
Analytical parameters of MEKC separation (R.S.D., %, $n = 5$)

Pesticides	Resolution[a]	Average t_m (min)	Run-to-run R.S.D. (%)	Day-to-day R.S.D. (%)	Average area	Run-to-run R.S.D. (%)	Day-to-day R.S.D. (%)
Flutriafol	0	7.89	1.9	2.3	9,272	2.3	3.8
Cyproconazole I	5.3	9.01	1.6	2.1	3,382	3.4	2.9
Cyproconazole II	4.0	9.82	2.0	2.3	3,597	2.2	3.5
Myclobutanil	1.6	10.14	2.0	2.5	6,351	5.0	5.1
Tebuconazole	6.5	11.67	1.6	2.6	7,016	2.1	2.8
Acrinathrin	1.7	12.08	1.7	2.6	2,174	4.3	4.7
Bitertanol	1.1	12.37	1.5	2.6	20,857	4.6	5.0
Fludioxinil	1.7	12.92	1.6	2.9	25,626	3.3	4.3
Pyriproxifen	1.6	13.46	1.6	3.0	14,478	5.3	5.8

[a] Resolution was calculated using the equation: $Rs = 2(t_1 - t_2)/(w_1 + w_2)$, where t_1 and t_2 are the migration times and w_1 and w_2 are the widths of the peak at base line.

from 2.1–5.3 and 2.9–5.8%, respectively. The limits of detection (LODs), estimated as three times signal-to-noise ratio were 0.1 μg ml^{-1} for bitertanol, fludioxonyl, and pyriproxyfen, and 0.5 μg ml^{-1} for the rest.

3.2. SPE procedure

Fruit and vegetable samples are matrices that do not allow direct SPE of pesticides. They must be extracted with polar solvents to have the pesticides in an aqueous extract. Acetone was selected as extraction solvent because it is easy of evaporating and avoids losses of pesticides.

The influence of the amount of sample was also examined to obtain the smallest LOQs. Different sample sizes were tested from 5 to 15 g. Fig. 3 shows the effect of the sample amount on pesticide recoveries for the four matrices tested. Two different behaviors were observed. The amount of lettuce and tomato samples could be increase to 15 g without important variations in recovery values and with R.S.D.s within the guidelines of the EU (<20%). On the contrary, the amount of grape and strawberry samples presented important influence on the recoveries because of the high viscosity of the extract. The maximum amount of these matrices that could be processed was 5 g to can recover all the analytes with R.S.D.s within the guidelines of the EU (see Fig. 3C and D). When the sample amount was increased the recoveries for most compounds diminished and R.S.D.s increased to unacceptable values.

In this study, LOQs were determined according to the guidelines of EU [23], as the lowest concentration for which acceptable recoveries (>50%) and repeatabilities (<20%) are obtained. Table 2 presents LOQs lower than 0.5 mg kg^{-1}, satisfying the EU [23], Codex Alimentarius [24], US Food and Drug Administration [25], and Spanish [26] MRLs. For lettuce and tomato samples, LOQs can be diminished until 0.2 mg kg^{-1} working with 15 g of samples instead of 5 g. Considering these results, the method is adequate to determine the studied pesticides in grapes, lettuce, strawberries, and tomatoes. Table 2 shows also the recoveries and precision obtained from spiked samples at LOQ levels. Average recoveries were between 40 and 106% and R.S.D.s ranged from 10 to 19%. Recoveries were similar in any of the matrix studied. Higher concentrations were also tested (data not shown) providing results in the same interval.

The good performance of the electropherograms obtained from SPE extracts using 15 g of sample is illustrated in Fig. 4. Unspiked samples do not show peaks from the matrix that can interfered with the studied compounds. The lettuce sample presents pyriproxyfen (Fig. 4B) as it was confirmed by liquid chromatography–mass spectrometry (LC–MS) using a HP 1100 system equipped with a APCI interface, using the standard source conditions, in positive ionization (PI) mode. The analysis was carried out in selected ion monitoring mode (SIM) selecting three characteristic ions of pyriproxyfen at *m/z* 322, 227, and 185 and using a fragmentor voltage of 100 V, according to a method previously reported

Table 2
Accuracy and precision at LOQ (amount of sample processed 5 g) SPE and MRLs established by the Spanish legislation [26]

Pesticide	Concentration (mg kg^{-1})	Recovery, % (R.S.D., %, $n = 5$)				*MRL*
		Lettuce	Tomato	Grape	Strawberry	
Flutriafol	0.5	62 (17)	60 (14)	57 (8)	54 (17)	0.5–2
Cyproconazole I	0.5	58 (11)	53 (13)	55 (12)	58 (14)	0.05–1
Cyproconazole II	0.5	60 (16)	55 (12)	57 (12)	31 (18)	0.05–1
Myclobutanil	0.5	56 (13)	52 (14)	51 (17)	53 (15)	0.02–1
Tebuconazole	0.5	60 (17)	63 (13)	66 (14)	59 (15)	1–5
Acrinathrin	0.5	66 (12)	62 (17)	63 (14)	63 (19)	0.2–1
Bitertanol	0.5	49 (15)	40 (13)	44 (16)	47 (13)	2–3
Fludioxinyl	0.5	50 (10)	46 (17)	57 (13)	47 (19)	1–2
Pyriproxyfen	0.5	94 (15)	97 (17)	106 (9)	95 (18)	0.05–1

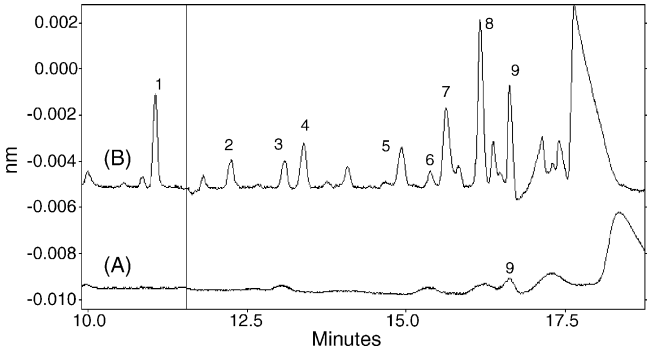

Fig. 4. Electropherograms of SPE extracts from 15 g sample of (A) lettuce that contains pyriproxyfen at 0.2 mg kg^{-1} sample, and (B) lettuce sample spiked with the pesticides at 0.5 mg kg^{-1} levels. Peak identification: 1, flutriafol; 2, cyproconazole I; 3, cyproconazole II; 4, myclobutanil; 5, tebuconazole; 6, acrinathrin; 7, bitertanol; 8, fludioxonil; 9, pyriproxyfen.

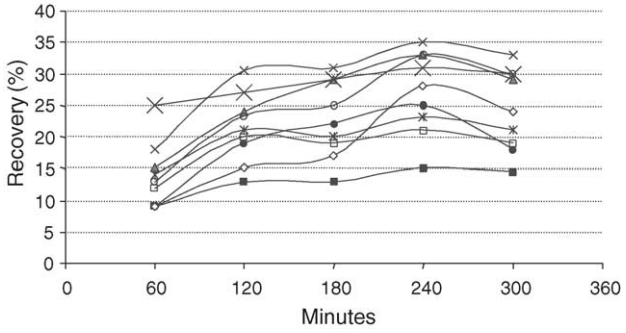

Fig. 5. Effect of the extraction time on the recovery (each solution was added of 40% NaCl). Curve identification as in Fig. 2.

[12]. The identification and further confirmation of this pesticide showed the suitability of the method to determine these residues in real samples and the difficulty to find control samples without residues.

3.3. SBSE procedure

The optimum SBSE desorption conditions were obtained by exposure of the stir bar to 1 ml of methanol for 15 min using a 1.5 ml volume glass vial; this volume enables the stir-bar to be completely immersed in the solvent. Those parameters were selected not only due to the higher quantitative desorption obtained, but also because of the minimum carryover observed under this experimental conditions.

The matrix pH effect on the extraction efficiency of the pesticides was tested using pH values between 4 and 9 adjusted with potassium phosphate buffers. This range of pH values does not have influence in the extraction efficiency, and all subsequent analyses were performed without pH modification.

As expected, the addition of NaCl to the samples had a significant influence on the amount of pesticides adsorbed on the stir-bar. The addition of salt to the matrix altering the ionic strength and consequently decreasing the solubility of the pesticides in solution, can favor adsorption onto the stir-bar. The effect of NaCl concentration was investigated in the range 10–40%. The saturated solution (40% of NaCl) provided the best recoveries.

Fig. 5 shows the time profile of the extraction for the pesticides. The extraction equilibrium time was reached at 4 h for all studied pesticides. So far, it has been reported that the shorter extraction, which could be of the order of minutes, is one of the advantages of the SBSE [9,11]. However, for the studied pesticides, extraction times of over 240 min were required, enlarging the analysis more than practical convenience.

The influence of the matrix on the extraction efficiency of the SBSE was negative for all compounds. A sample size of 5 g provided acceptable recoveries whereas higher amounts avoid the extraction of the flutriafol and cyproconazole. The quantity of matrix that can be used (no more than 5 g) is one limitation of this procedure.

Specificity of the method is demonstrated by representative electropherograms of tomato sample in Fig. 6. Blank tomato sample showed no significant interference at the retention times of the analytes.

The linearity was determined using spiked samples between 1 and 100 mg kg^{-1}. The evaluated interval was linear with correlation coefficients higher than 0.996. The precision and the recovery of the studied pesticides at LOQ levels for all the matrices tested are outlined in Table 3. The recoveries

Table 3
Accuracy and precision at LOQ (amount of sample processed 5 g) by SBSE

Pesticide	Concentration (mg kg^{-1})	Recovery, % (R.S.D., %, $n = 5$)			
		Lettuce	Tomato	Grape	Strawberry
Flutriafol	1	12 (14)	13.4 (8)	17 (7)	14.6 (16)
Cyproconazole I	1	25 (9)	30.5 (3)	28 (5)	24 (17)
Cyproconazole II	1	29 (10)	33 (3)	34 (15)	31 (3)
Myclobutanil	1	20 (8)	24 (16)	26 (9)	19 (13)
Tebuconazole	1	32 (5)	35 (11)	25 (4)	31 (8)
Acrinathrin	1	47 (4)	45 (3)	43 (6)	41 (9)
Bitertanol	1	26 (13)	22 (4)	24 (4)	21 (4)
Fludioxinyl	1	22 (15)	24 (12)	27 (12)	23 (11)
Pyriproxyfen	1	24 (9)	23 (6)	32 (8)	33 (12)

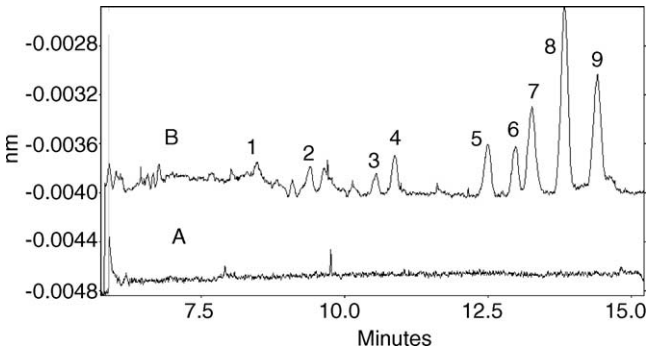

Fig. 6. Electropherograms of SBSE extracts of (A) tomato blank sample, and (B) tomato sample spiked at 1 mg kg^{-1} of each pesticide. Peak identification as in Fig. 4.

Table 4
Methods performance comparison

	SPE	SBSE
Spiking concetrations (mg kg^{-1})	0.2–50	1–50
Accuracy (% recovery)	40–106	12–47
Repeatability (R.S.D., %)	8–19	3–17
Linearity (r^2)	>0.994	>0.996
Sensitivity (LOQ)	0.3–0.5	1
Analysis time (h)	1.5	5

obtained were between 12 and 47% and the R.S.D.s ranged from 3 to 17%. The LOQ was 1 mg kg^{-1} for all the studied pesticides.

3.4. Comparison of methods

Analytical parameters of both procedures are compared in Table 4. Although SBSE extraction is less reliable than SPE to carry out the routine analyses, this procedure also presents some advantages over SPE such as better precision and cleaner electropherograms (as can be deduced from comparing Table 4 and Figs. 4 and 6).

SPE recoveries are, at least, twice higher than those obtained by SBSE. In addition, SPE showed better LOQs (twice) that can be improved for some matrices processing larger amounts of samples. The LOQs obtained mark the difference between both procedures because SPE combined with MEKC–UV achieves the detection of the studied pesticides at levels lower than MRLs established by the Spanish and EU legislations. Unfortunately, LOQs obtained by SBSE are too high to met the MRLs. Because of this, the procedure is only useful in some cases. Other advantage of SPE, that consolidates it as that of choice, is speediness (the complete analysis of each sample to obtained results are ca. 1.30 h in front of 5 h required by SBSE). Disadvantages described in the literature for the SPE such as breakthrough and plugging [17,20] have not been observed in the present study, after optimization of the conditions.

4. Conclusions

Comparing the SPE with SBSE, the first one is more sensitive, robust, and rapid than SBSE as well as it provides higher extraction efficiencies and sample throughput. The results of this work demonstrate that MEKC is useful, simple, and rapid for separating, identifying, and determining eight pesticides with sufficient sensitivity in fruits and vegetables. The LOQs show that the developed method can be used to detect the pesticides at concentrations below the MRLs established by the European Union, Spanish legislations, and other recommendations. SPE combined with MEKC achieves the analysis of a large number of fruit and vegetable samples in a short period of time attaining a fast and inexpensive method for routine pesticide residue monitoring in laboratories.

Acknowledgements

This work has been supported by the Spanish Ministry of Science and Technology together with the European Regional Developments Funds (ERDF) (project No. AGL2003-01407). A.J.G. thanks the Ministry of Science and Education for the FPI grant (2004FPI).

References

[1] L. Nasreddine, D. Parent-Massin, Toxicol. Lett. 127 (2002) 29.
[2] F.E. Ahmed, Trends Anal. Chem. 20 (2001) 649.
[3] W.G. Fong, H.A. Moye, J.N. Seiber, J.P. Toth, Pesticide Residues in Food, Wiley–Interscience, New York, 1999, p. 1335.
[4] D. Stajnbaher, L. Zupancic-Kralj, J. Chromatogr. A 1015 (2003) 185.
[5] F.J. Schenck, S.J. Lehotay, V. Vega, J. Sep. Sci. 25 (2002) 883.
[6] M. Michel, B. Buszewski, J. Liq. Chromatogr. Relat. Technol. 25 (2002) 2293.
[7] V.J. Barwick, S.L.R. Ellison, S.J. Lacey, C.R. Mussell, C.L. Lucking, J. Sci. Food Agric. 79 (1999) 1196.
[8] C. Blasco, M. Fernández, Y. Picó, G. Font, J. Chromatogr. A 1030 (2004) 77.
[9] P. Sandra, B. Tienpont, F. David, J. Chromatogr. A 1000 (2003) 299.
[10] C. Bicchi, C. Cordero, P. Rubiolo, P. Sandra, Eur. Food Res. Technol. 216 (2003) 449.
[11] P. Sandra, B. Tienpont, J. Vercammen, A. Tredoux, T. Sandra, F. David, J. Chromatogr. A 928 (2001) 117.
[12] C. Blasco, G. Font, Y. Picó, J. Chromatogr. A 970 (2002) 201.
[13] Y. Picó, R. Rodríguez, J. Mañes, Trends Anal. Chem. 22 (2003) 133.
[14] F. Menzinger, Ph. Schmitt-kopplin, D. Freitag, A. Kettrup, J. Chromatogr. A 891 (2000) 45.
[15] Z. El Rassi, Electrophoresis 20 (1999) 3280.
[16] R. Rodríguez, J. Mañes, Y. Picó, Anal. Chem. 75 (2003) 452.
[17] M. Khrolenko, P. Dzygiel, P. Wieczorek, J. Chromatogr. A 975 (2002) 219.
[18] E.W.J. Hooijschuur, C.E. Kientz, J. Dijksman, U.A.Th. Brinkman, Chromatographia 54 (2001) 295.
[19] R. Rodríguez, Y. Picó, G. Font, J. Mañes, J. Chromatogr. A 949 (2002) 359.
[20] R. Rodríguez, Y. Picó, G. Font, J. Mañes, J. Chromatogr. A 924 (2001) 387.

[21] M. Molina, M. Silvia, Electrophoresis 21 (2000) 3625.
[22] J. Hau, S. Riediker, N. Varga, R.H. Stadler, J. Chromatogr. A 878 (2000) 77.
[23] The European Union “on-line” http://europa.en.int/, 2004.
[24] Codex Alimentarius, http://www.fao.org/, 2004.
[25] Food and Drug Administration of the United States, http://www.cfsan.fda.gov, 2004.
[26] Ministerio de Agricultura, Pesca y Alimentación, http://www.mcx.plaguicidas.plagas/, Límites Máximos de Residuos (MRLs) Oficales, 2004.