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Analysis of post-harvest fungicides by micellar electrokinetic chromatography

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Abstract

A method based on solid-phase extraction (SPE) and micellar electrokinetic chromatography (MEKC) was developed for the simultaneous determination of carbendazim, imazalil, methylthiophanate, *O*-phenylphenol, prochloraz, procimidone, thiabendazole and triadimefon residues in grape, lettuce, orange and tomato. Selectivity and resolution were studied changing the pH and the concentration of the buffer, the type and concentration of surfactant and the methanol content in the mobile phase. A buffer consisting of 4 m*M* borate with 75 m*M* sodium cholate (pH 9.2) gave the best results. The recoveries of the fungicides in spiked fruit and vegetable samples ranged from 30 to 105%, and the limits of detection were between 0.1 and 1 mg kg^{-1}. The reproducibility and repeatability of the combination of SPE pretreatment and MEKC were good for all the compounds, except for imazalil and *O*-phenylphenol in oranges, due to some matrix compounds interfering with the separation. The method was applied to post harvest treated samples, and the fungicides were sometimes detected at concentration levels lower than maximum residue limits (MRLs). © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Fruits; Vegetables; Food analysis; Pesticides

1. Introduction

Modern agricultural production in major agriculture countries depends heavily on the use of pesticides. Fungicides represent about 20% of all pesticides used (in the USA and Spain the figure is about 25%). Carbendazim, imazalil, methylthiophanate, *O*-phenylphenol, prochloraz, procimidone, thiabendazole and triadimefon are eight fungicides widely used to control postharvest decay caused by various fungal pathogens in fruits and vegetables because of this residues of these fungicides are frequently found in agricultural commodities [1,2].

Methods use to analyse fungicides and their residues are similar to those used for other pesticides. Gas chromatography (GC) is a classical method, and presently GC–MS is often used [3–5] and liquid chromatography (LC) is more widely recommended for thermally and unstable labile compounds, usually in conjunction with UV, fluorimetric detection or with a mass spectrometry system [6,7]. However, no attempt has been shown to analyse the selected compounds simultaneously using state of the art techniques. This is due perhaps to the limitations of the techniques, notably varying analyte volatility of GC, need for gradient elution in LC, large solvent and variable molecule charges and masses.

Capillary electrophoresis (CE) becomes an important practical analytical technique for the analysis of pesticides [8]. Its main advantages for this type of

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analysis are simple equipment, the possibility of varying a large number of the experimental parameters, fast analysis, economy and low solvent consumption. Correspondingly, CE procedures for the separation of fungicide have been described [9–17]. A few studies determined triadimefon [12,17], carbendazim [11,13] and thiabendazole [16], but none imazalil, methylthiophanate, *O*-phenylphenol and prochloraz. In addition most of these applications are restricted to demonstrations of high-resolution separation of specific compounds, and only a few refer to the determination of fungicides in real matrices. The lack of CE applications to real-word samples has been principally caused by the insufficient sensitivity, limited by the small sample volumes typically injected (e.g. 1–10 nl). To enhance the detection capability of CE several sample preconcentration techniques or improved detection systems were developed [8,11–13]. Among various sample preconcentration techniques there is one major approach: concentration by solid-phase extraction (SPE) [11,12].

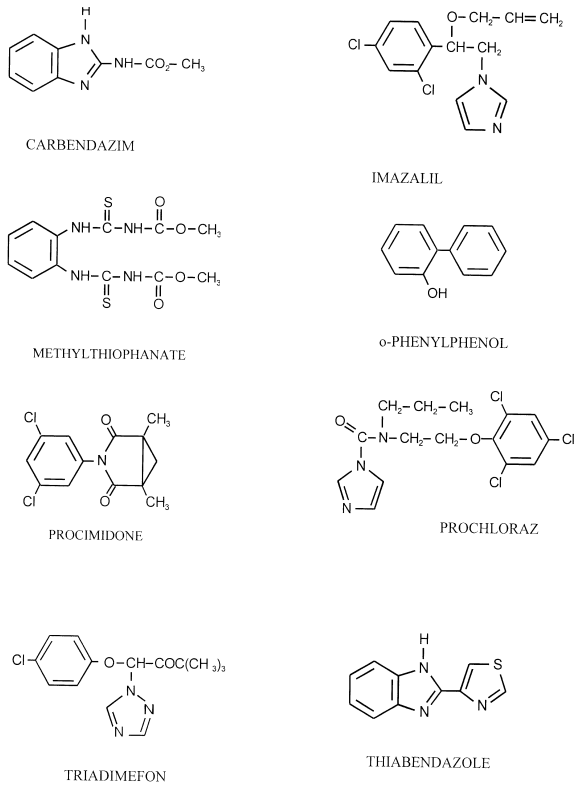

Fig. 1. Structures of the studied fungicides.

In this work, a CE procedure using UV detection for the separation of the eight previously listed fungicides was developed. The structures of the compounds being investigated are shown in Fig. 1. The applicability of combined off-column SPE for the enrichment of these fungicides from oranges, grapes, tomatoes and lettuces, prior to micellar electrokinetic chromatography (MEKC) analysis was also studied. Furthermore, the optimised method was applied to the determination of fungicides in real samples taken from different local markets, with excellent resolution between the compounds.

2. Experimental

2.1. Chemicals and reagents

Carbendazim, imazalil, methylthiophanate, *O*-phenylphenol, procimidone, prochloraz, thiabendazole and triadimefon were purchased from Aldrich (Madrid, Spain). Standard solutions at 1 mg/ml of each fungicide were prepared in methanol, except carbendazim that was prepared in 1 *M* HCl, stored at 4°C, and mixed and diluted with the buffer to appropriate concentrations before use.

All the chemicals used were of analytical grade. Ultrapure water (>18 MΩ cm resistivity) prepared with the Milli-Q SP reagent water system (Millipore, Bedford, MA, USA) was used through the experiment. All solutions used in the present study were filtered through a 0.22-μm membrane filter before use.

Octylsilica sorbent (particle diameter in the range of 45–55 μm) was acquired from Análisis Vínicos (Tomelloso, Spain). A 1-g aliquot of C_8 was transferred into a 100 mm×9 mm I.D. glass column fitted with a coarse frit (No. 3) and covered with a plug of silanised glass wool.

The samples analysed — grapes, lettuces, tomatoes and oranges — were obtained from a local market. All the samples were taken in accordance with the guidelines of the EU Directive 79/700/CEE [18]; that is, as far as possible, the sampling at various places distributed throughout the lot (size ca. 50 kg). The sample weighed at least 1 kg and

consisted of at least ten individual pieces of fruit or vegetable.

2.2. CE analysis

A P/ACE 2050 capillary electrophoresis system (Beckman, Fullerton, CA, USA) with α-diode array detection (DAD) system and with System Gold Software was used. It was equipped with an untreated fused-silica capillary (Beckman) of 60 cm (50 cm effective length×75 μm I.D.). Electrokinetic injection, typically 5 s at 10 kV, was used, and detection was performed in the range between 190 and 320 nm. The single wavelength electropherograms depicted are those registered for 210 nm. Before each experiment the capillary was rinsed with 0.1 *M* NaOH for 2 min, water for 2 min and running buffer for 2 min. After a carefully optimisation of the different parameters, the buffer selected was 4 m*M* borate (pH 9.2) containing 75 m*M* sodium cholate and the separation was performed with positive power supply of 15 kV (average current 34 μA) at a temperature of 25°C.

2.3. Extraction procedure

A representative portion of sample (200 g of whole fruit or vegetable) was chopped and homogenised in a Bapitaurus food chopper (Taurus, Berlin, Germany). Then, a 5-g portion was weighed and placed into an Erlenmeyer flask and homogenised with 5 ml of methanol and 5 ml of water by sonication over 15 min. The resulting suspension was filtered through Whatman 40-μm filter, and the filter cake was washed twice with 5 ml of deionised water.

The filtrated was adjusted to a volume of 100 ml with distilled water and passed under vacuum through a C_8 solid-phase column that was preconditioned with 10 ml of methanol and 10 ml of distilled water. Pesticides were eluted with 10 ml of dichloromethane. The eluate was evaporated to dryness with nitrogen at 50°C. The dry residue was reconstituted in 500 μl of buffer and thoroughly mixed in an ultrasonic bath for 5 min.

Extraction recoveries were determined spiking fresh samples (5 g) with a fortification solution at different levels: 1–100 mg/kg.

3. Results and discussion

These fungicidal compounds are mainly nonpolar compounds (Fig. 1) because of this MEKC was investigated. The separation conditions including, pH, buffer nature and concentration, surfactant and its concentration, and percentage of organic modifier were varied in order to achieve the optimum separation conditions for the eight fungicides selected. The optimum conditions chosen for this separation were those reported in experimental section.

3.1. Buffer characteristics

The effect of buffer pH (7–11) on migration time for the studied fungicides is illustrated in Fig. 2. In the range of pH (7–9.2) a slight decrease of the migrations time of fungicides was observed, whereas above pH 9.2 an increase in the migration time was noted. The resolution for standards was complete in the range 7.00–11.00. Under pH 7 some peaks disappeared and resolution was not complete in some cases and over pH 11 the capillary material can be partially dissolved in the buffer. Based on these data, pH 9.2 was selected for routine MEKC because it provided the shorter analysis time with good resolution.

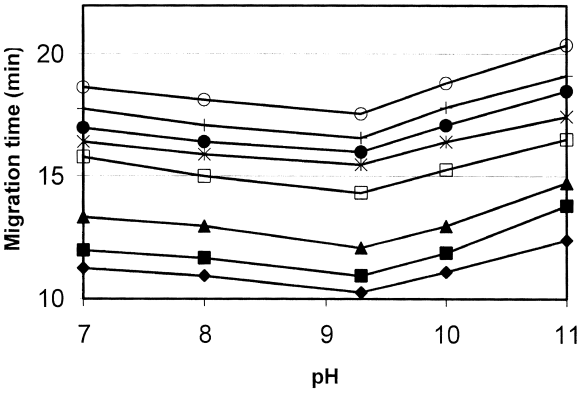

Fig. 2. Graph of migrations times of the eight fungicides as a function of buffer pH. Conditions: 16 m*M* phosphate buffer; 75 m*M* sodium cholate; separation voltage: 15 kV; detection: 210 nm; capillary: 50 cm×75 μm I.D.; hydrodynamic injection: 5 s; injection in methanol–electrophoretic buffer (20:80). (♦) Methylthiophanate; (■) carbendazim; (▲) thiabendazole; (□) procimidone; (✳) *O*-phenylphenol; (●) imazalil; (✦) triadimefon; (○) prochloraz.

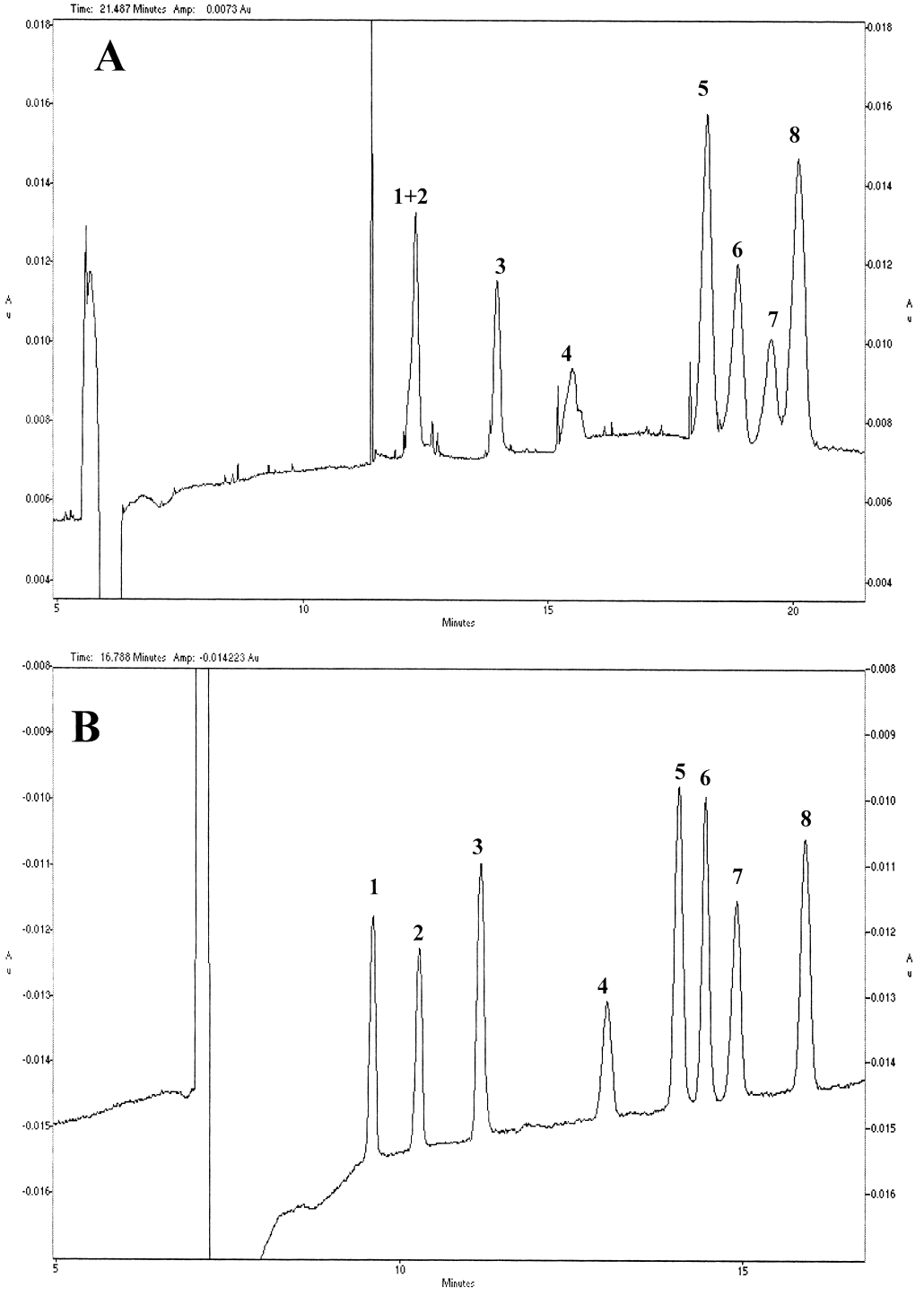

Fig. 3. Graph of migrations times of the eight fungicides as a function of surfactant concentration, (A) SDS and (B) sodium cholate. Conditions: 4 m*M* borate buffer; pH 9.2; other conditions as in Fig. 2. Peak identification: (1) Methylthiophanate; (2) carbendazim; (3) thiabendazole; (4) procimidone; (5) *O*-phenylphenol; (6) imazalil; (7) triadimefon; (8) prochloraz.

At the selected pH values, four different buffers phosphate, borate, carbonate and NH_3–NH_4Cl were evaluated. The results showed that the constituents of the buffer solution did not affect the resolution of the compounds.

The buffer concentration was varied from 4 to 64 m*M*. The migration times of the compounds were greatly increased by the rise in the run buffer concentration without a significant improvement in the resolution of the compounds.

3.2. Variations in surfactants and its concentration

Sodium dodecyl sulphate (SDS) and sodium cholate were tested as surfactants. The migration time of the compounds increased with increased concentration of any of these surfactants as shown in Fig. 3.

When SDS was used, triadimefon and prochloraz were not separated. They were well separated with sodium cholate as show in Fig. 4.

3.3. Organic modifier

Addition of different percentages of methanol was assessed. The methanol addition expanded the migration times and also increased resolution between the compounds. However, the separation among the eight fungicides is very good without adding methanol to the run buffer.

3.4. Separation voltage

Increasing the voltage from 10 to 20 kV reduced the analysis time from 25 to 12 min at 20 kV. However, for 20 kV separation voltage the current becomes higher than 100 μA, resulting in excessive heating of the capillary and frequent falls in current. The optimum voltage applied was 15 kV.

3.5. Separation characterization

The linearity was tested over a concentration range of 1–100 μg ml^{-1}. Regression lines were calculated with the area versus concentration. A good linearity (r>0.99) was obtained for all fungicides.

Table 1 illustrates the reproducibility of this separation including eight compounds in a mixture.

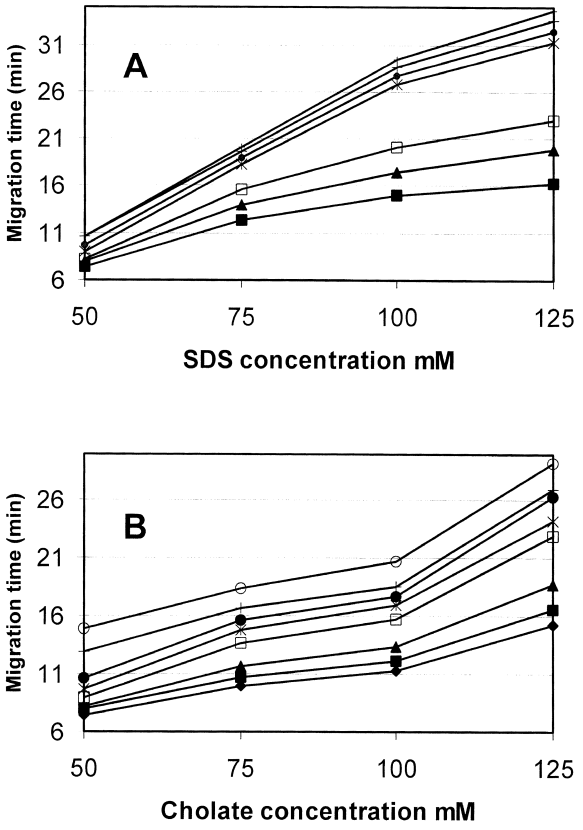

Fig. 4. Electropherogram of fungicides using different surfactants (A) SDS and (B) sodium cholate. Other conditions as in Table 1 and compound identification as in Fig. 2.

Table 1
Reproducibility of MEKC separation of fungicide mixture[a]

Fungicide	Resolution[b]	Average t_m (min)	SD (N=6)
Methylthiophanate	0	10.0	0.3
Carbendazim	1.3	10.6	0.3
Thiabendazole	1.5	11.6	0.4
Procimidone	2.4	13.5	0.5
O-Phenylphenol	1.5	14.5	0.5
Imazalil	1.1	15.0	0.6
Triadimefon	1.1	15.6	0.8
Prochloraz	1.6	16.6	1.0

[a] Conditions: 4 m*M* borate buffer; 75 m*M* sodium cholate; separation voltage: 15 kV; detection: 210 nm; capillary: 50 cm× 75 μm I.D.; hydrodynamic injection: 5 s, 10 kV; injection in methanol–electrophoretic buffer (20:80). t_m=Migration time.

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

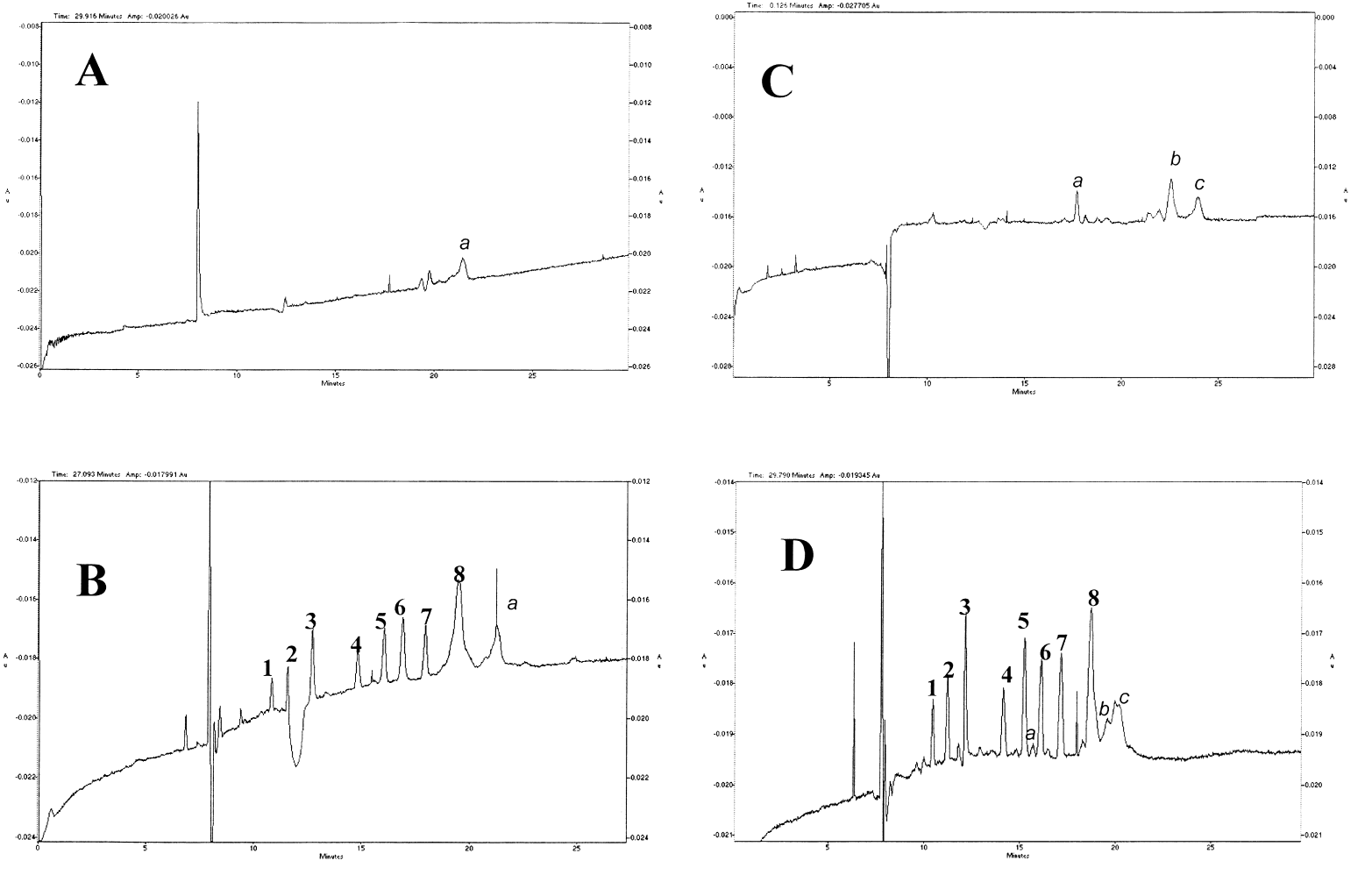

Fig. 5. Electropherograms of (A) grape blank and (B) spiked grape at 1 mg kg^{-1} of each fungicide; (C) tomato blank and (D) spiked tomato at 1 mg kg^{-1}. Analysis conditions as in Table 1 and peak identifications as in Fig. 3, (a), (b) and (c) are peaks from the matrix.

Using MEKC, reproducibility of <0.6% RSD were obtained for all the analytes. This result is based on six repeated separation runs of the analyte mixture.

The inter-day reproducibility, performed over 5 days, for four standards at 10 mg ml^{-1} is lower than 18%. The intra-day reproducibility was performed by analysing the compound mixtures five times on the same day and was lower than 12%.

The limit of detection, defined as signal-to-noise ration of 3, was established by injecting serial dilutions of the corresponding 100 μg ml^{-1} standard solution. The limits of detection ranged from 1 to 2 mg ml^{-1}

3.6. Solid-phase extraction validation

Grape, lettuce, orange and tomato samples spiked with a known concentrations of the fungicides were extracted and analyzed by MEKC. Fig. 5 shows typical electropherograms of grape and tomato

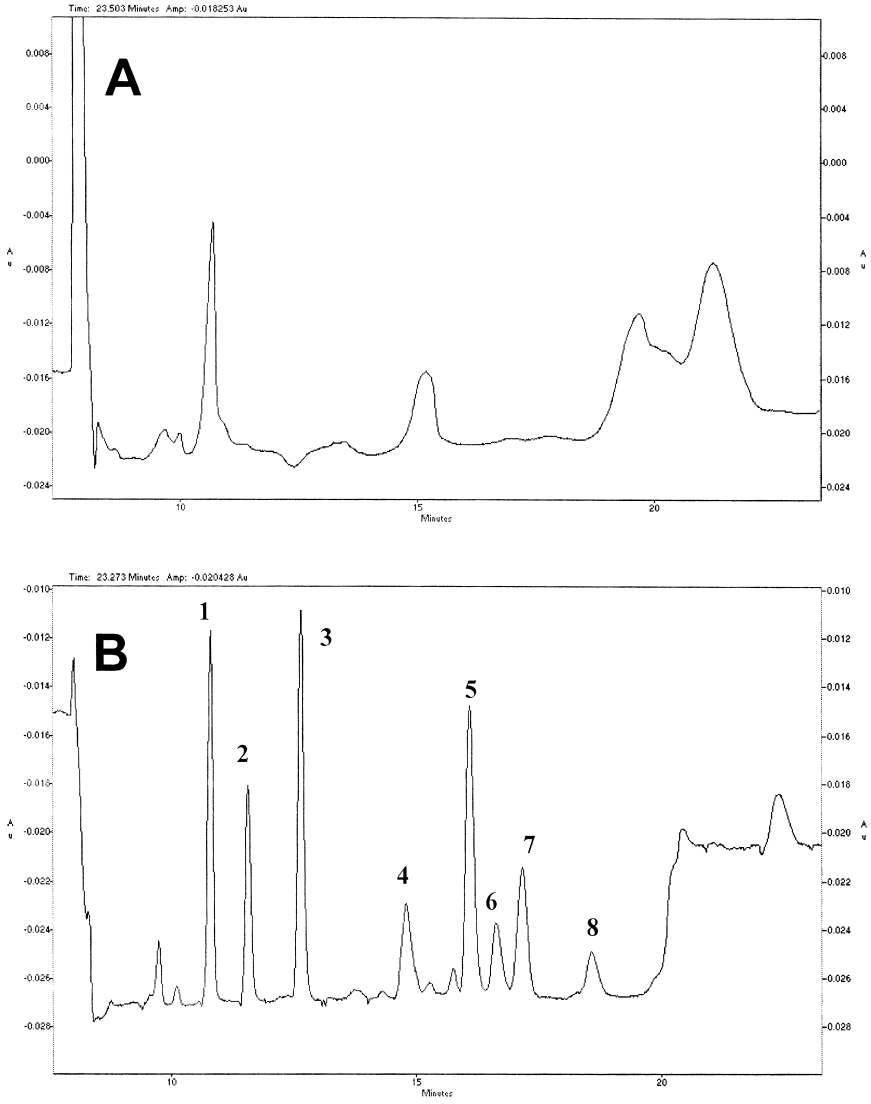

Fig. 6. Electropherograms of (C) lettuce blank and (A) spiked lettuce at 5 mg kg^{-1} of each fungicide. Analysis conditions as in Fig. 5 and peak identifications as in Fig. 3.

Table 2
Fungicide recoveries (%) and relative standard deviation (RSD, %) from grape and tomato samples spiked at 1 mg kg^{-1}

Fungicide	Average recovery (%)[a] (RSD, %)	
	Grape	Tomato
Methylthiophanate	30(4)	35(4)
Carbendazim	58(9)	54(5)
Thiabendazole	56(10)	53(4)
Procimidone	60(13)	60(10)
O-Phenylphenol	36(13)	40(11)
Imazalil	54(4)	47(1)
Triadimefon	65(7)	72(8)
Prochloraz	105(8)	86(3)

[a] $N=5$.

Table 4
Limits of detection (LODs) and maximum residue limits (MRLs)

Fungicide	LODs (MRLs) (mg kg^{-1})			
	Grape	Lettuce	Orange	Tomato
Methylthiophanate	0.3(5)[a]	1.0(5)[a]	0.5(5)[a]	0.3(2)[a]
Carbendazim	0.2(5)[a]	1.0(5)[a]	0.5(5)[a]	0.3(2)[a]
Thiabendazole	0.1(2)	1.0(3)	0.4(6)	0.2(1)
Procimidone	0.4(5)	1.0(5)	1.0(0.02)	0.5(0.02)
O-Phenylphenol	0.1(0.1)	1.0(0.1)	n.d.[b](12)	0.1(0.1)
Imazalil	0.2(0.02)	1.0(0.02)	n.d.[b](5)	0.2(0.02)
Triadimefon	0.2(1)	1.0(0.05)	1.0(0.05)	0.1(0.5)
Prochloraz	0.3(5)	1.0(5)	1.0(5)	0.2(0.01)

[a] MRL is the sum of carbendazim, benomyl and methylthiophanate, expressed as carbendazim.
[b] n.d. = Compound not detectable because there are some peaks from the matrix.

spiked with a fungicide mixture along with the unspiked control samples. The background obtained from the electropherograms of the samples was low and does not show important interfering peaks from the matrix.

Electropherograms obtained from the lettuce and orange present more interferences and higher background. In the lettuce extract some matrix peaks are observed, but these peaks do not interfere with the selected pesticides, as Fig. 6 illustrates. However, the orange extracts contain a large number of interfering peaks, which avoid identification of *O*-phenylphenol and imazalil.

Samples of each commodity spiked with different concentrations (1–10 mg kg^{-1}) of a standard mixture of fungicides were extracted by SPE and analyzed by MEKC to determine the recoveries. As an example, data of grape and tomato spiked with 1 mg kg^{-1} are shown in Table 2. The recoveries were between 30 and 105% and the RSDs between 1 and 13%. Relatively low recoveries of methylthiophanate and *O*-phenylphenol were noted in all matrices. The poor recovery of methylthiophanate is probably due to its partial degradation to carbendazim during sample processing [19] and the poor recovery of *O*-phenylphenol to its high polarity.

The results of the validation procedure are summarized in Table 3 for the lettuce sample. This commodity was chosen because it presents the worst results. Recoveries and RSD are similar to those reported in Table 2 for grape and tomato. The repeatability and reproducibility of the method are satisfactory (RSD$<$19%) for all compounds at the three levels of concentrations.

The limit of detection (LOD) for every fungicide was determined with spiked samples as signal-to-noise ratio of 3 after the SPE procedure. The results

Table 3
Validation of the method including SPE

Fungicide	Average recovery (%)[a] (RSD, %)			Repeatability average (SD)			Linearity (r)
	2	5	10	2	5	10	
Methythiophanate	24(5)	36(9)	57(3)	2.3(0.1)	5.4(0.3)	10.2(0.4)	0.995
Carbendazim	50(10)	59(11)	67(9)	1.6(0.7)	5.0(1.9)	10.5(2.2)	0.991
Thiabendazole	61(12)	57(8)	69(10)	1.9(0.6)	4.9(2.2)	9.8(3.6)	0.996
Procimidone	58(14)	65(12)	70(8)	2.3(0.8)	5.1(0.9)	10.6(0.7)	0.995
O-Phenylphenol	52(14)	47(13)	51(12)	1.8(0.9)	5.2(0.8)	10.3(0.6)	0.999
Imazalil	48(6)	53(8)	58(5)	2.4(2.8)	4.8(2.5)	9.7(2.0)	0.996
Triadimefon	54(5)	58(6)	68(4)	2.2(0.2)	5.1(0.4)	10.0(0.3)	0.997
Prochloraz	69(11)	72(9)	86(6)	1.9(0.7)	4.9(0.9)	9.3(1.3)	0.998

[a] $N=5$.

are given in Table 4. The maximum residue limits (MRLs) established by regulatory authorities [20] are also included in Table 4. The sensitivity of the method was good enough to ensure a reliable determination at levels lower than the respective MRL, except for imazalil in grape, lettuce and orange; *O*-phenylphenol in lettuce and orange, procimidone in orange and tomato; triadimefon in lettuce and orange and prochloraz in tomato.

The method was applied to the determination of

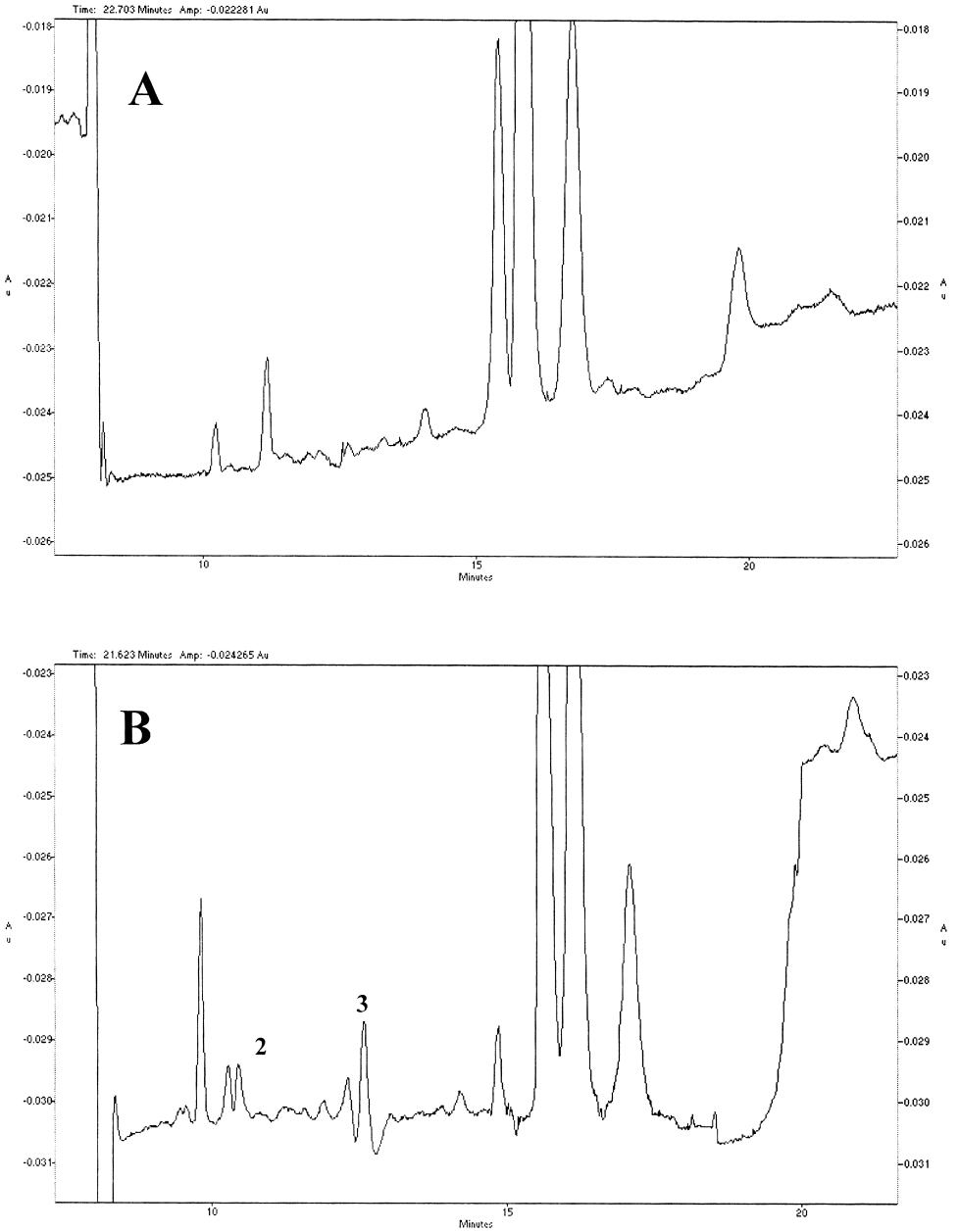

Fig. 7. Electropherograms of (A) orange sample free of the studied fungicides and (B) orange sample that contains thiabendazole at 1 mg kg^{-1}. Analysis conditions as in Table 1 and peak identifications as in Fig. 3.

fungicides in real samples obtained from a local market over different days. Ten samples of each commodity were analysed. Thiabendazole, carbendazim, procimidone and prochloraz were detected in some samples at concentrations ranging from 0.5 to 2.3 mg kg^{-1}. The levels found in the samples were always lower than the MRLs established by the EU.

An example of the results for a real orange sample is presented in Fig. 7 together with a blank. Thiabendazole and carbendazim were clearly detected in the real sample at concentrations of 1.5 and 0.8 mg kg^{-1}, respectively. Although several unknown peaks from the matrix showed up in the electropherogram, these compounds can be determined. The presence of both compounds in the sample were confirmed by LC–MS.

4. Conclusions

The applicability of MEKC to the separation of eight fungicides has been demonstrated. MEKC using sodium cholate provided better separation of the fungicides compared to the SDS micellar solution. The analysis time was found to be dependent on pH, surfactant and buffer concentration, and working voltage. These results improve those reported in the literature describing the separation fungicides.

The present method, involving an appropriate SPE sample enrichment and MEKC requires only small sample sizes and small solvent volumes. It shows satisfactory recovery values, repeatability and reproducibility and is sensible and specific enough.

Although, some additional clean-up would be desirable for a few matrices, the results show that the combination of SPE and CE provides a powerful tool for the rapid and simultaneous determination of fungicide residues in fruit and vegetables.

Acknowledgements

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