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Journal of Chromatography A, 949 (2002) 359–366

JOURNAL OF CHROMATOGRAPHY A

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Analysis of thiabendazole and procymidone in fruits and vegetables by capillary electrophoresis–electrospray mass spectrometry

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Abstract

A capillary electrophoresis–mass spectrometry method for determining procymidone and thiabendazole in apples, grapes, oranges, pears, strawberries and tomatoes is described. Separation is achieved using a buffer of formic acid–ammonium formate at pH 3.5 with 2% of methanol. Fungicide residues present in the sample are preconcentrated by both solid-phase extraction and injection of large sample volumes into the capillary by a stacking technique, to obtain lower detection limits. Ionization is performed at atmospheric pressure in an electrospray type source and detection is carried out using positive ionization and selected ion monitoring modes. The quantitation limits are 0.005 and 0.05 mg kg^{-1}, and the mean recoveries are 64 and 75% for thiabendazole and procymidone, respectively, with relative standard deviations below 12% ($n=5$). Real fruit and vegetable samples are analyzed by the proposed method showing that residues of both fungicides are frequently present.

Keywords: Fruits; Vegetables; Food analysis; Thiabendazole; Procymidone; Pesticides

1. Introduction

Fungicide residues present in fruits and vegetables from pre- and post-harvest treatments are a topic of public concern due to the toxic properties of these compounds [1,2]. Different social sectors are interested in controlling fungicides content on foods, because pesticide residue data are increasingly being used for risk assessment or for making regulatory or economic decisions that can affect the availability of pest control chemicals to agriculture or the food safety [3,4].

Although pesticide residue analysis methodologies are well established, there is still a need for more rapid and cost-effective procedures to satisfy the knowledge-quest officials and consumers [5,6]. Many analytical procedures are based on the use of liquid–liquid extraction [7–10], solid-phase extraction (SPE) [11,12] or combinations of both [13], followed by an analytical separation method, typically liquid chromatography (LC) [8–10,12], gas chromatography (GC) [7,11] or capillary electrophoresis (CE) [13]. Method selection for a particular problem is a question of individual preference, derived from experience and techniques available, as well as analyte and matrix properties. However, the popularity of CE has increased in latter years since it is a good technique for the analysis of ionic compounds, presents superior separation efficiency and requires low solvents cost [14,15]. Recent reports have demonstrated that CE with UV detection is applicable for an accurate analysis of thiabendazole and

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0021-9673/02/$ – see front matter
PII: S0021-9673(01)01395-4

procymidone in fruit and vegetables in routine work [13,16–18].

Nevertheless, CE has the disadvantages of insufficient sensitivity and the lack of selectivity, especially using UV detection, but its efficiency can be additionally enhanced by performing an off-line or on-line preconcentration step. Off-line SPE includes simultaneous sample enrichment, which contributes to low detection limits, and clean up with elimination of interferences from the matrix [19,20]. On-column preconcentration techniques involve several sample staking procedures [21], the simplest of which consists of the injection of large sample volumes dissolved in lower conductivity buffer matrix. Stacking is one of the desirable features of CE that has not been fully examined.

Presently, the coupling of CE to mass spectrometry (MS) is an attractive approach, which is generating growing interest because the acquisition of mass information facilitates the identification and characterization of the analytes and overcome a large number of interfering substances [14,15,20].

The ionization technique most successfully used for CE–MS is electrospray ionization (ESI) which has proven to be sensitive, versatile and relatively easy to use in combination with CE [14,15,19,20]. CE–ESI-MS procedures have been described for determining acidic herbicides and related compounds [22], triazines [23,24] and ammonium quaternary herbicides [25] in water. Surprisingly, no CE–MS method is available for the determination of fungicides in fruits and vegetables.

In this report a method based on SPE followed by an on-line sample stacking technique in a CE system coupled to MS by ESI was investigated to enhance sensitivity and selectivity for the analysis of thiabendazole and procymidone. Quality parameters were obtained and the method was applied to the analysis of apples, grapes, oranges, pears, strawberries and tomatoes.

2. Experimental

2.1. Chemicals

Methanol and acetonitrile of gradient grade for liquid chromatography, and dichloromethane for organic trace analysis were from Merck (Darmstadt, Germany). Ammonium formate and formic acid were of analytical grade from Fluka (Madrid, Spain). Octylsilica sorbent (particle diameter in the range of 45–55 μm) was acquired from Análisis Vínicos (Tomelloso, Spain). Procymidone and thiabendazole standards were obtained from Aldrich (Madrid, Spain). A stock standard solution of two fungicides was prepared in methanol and stored in the refrigerator at 4°C. Deionized (<18 MΩ) water (Milli-Q water system; Millipore, Bedford, MA, USA) was used in the preparation of the sample and buffer solutions. Standard solutions, samples and buffers were filtered through a 0.22-μm membrane filter (Análisis Vínicos), and degassed before using.

2.2. Capillary electrophoresis

CE was carried out with a P/ACE MDQ capillary electrophoresis system (Beckman, Fullerton, CA, USA). System Gold Software was used for CE instrument control. All the experiments were carried out in the anionic mode (anode at the inlet and cathode at the outlet) and using a special external detector adapter (EDA) cartridge (Beckman) to accommodate the capillary. It was equipped with an untreated 150 cm×75 μm I.D. fused-silica capillary (Beckman). A scheme of the instrument system detailing each component and voltage is shown in Fig. 1.

Before its first use, the fused-silica capillary was washed with 0.1 *M* sodium hydroxide for 20 min, deionized water for 10 min and separation buffer for 80 min. Between analyses, the capillary was flushed with the running buffer for 2 min.

The buffer selected was 12 m*M* ammonium formate–20 m*M* formic acid in water at pH 3.5 with 2% methanol. The separation was performed with positive power supply of 30 kV (average current 5.5 μA) at a temperature of 25°C.

For sample stacking, the fungicides were dissolved in CE separation buffer diluted 1:8 (4 m*M*). The capillary was filled 2 min at 20 p.s.i. with the carrier electrolyte and then a plug of sample was introduced hydrodynamically by pressure (0.5 p.s.i.) during 0.25 min (1 p.s.i.=6894.76 Pa). A high voltage (+30 kV) was then applied. When the current was 95% of the original carrier electrolyte value, the voltage was

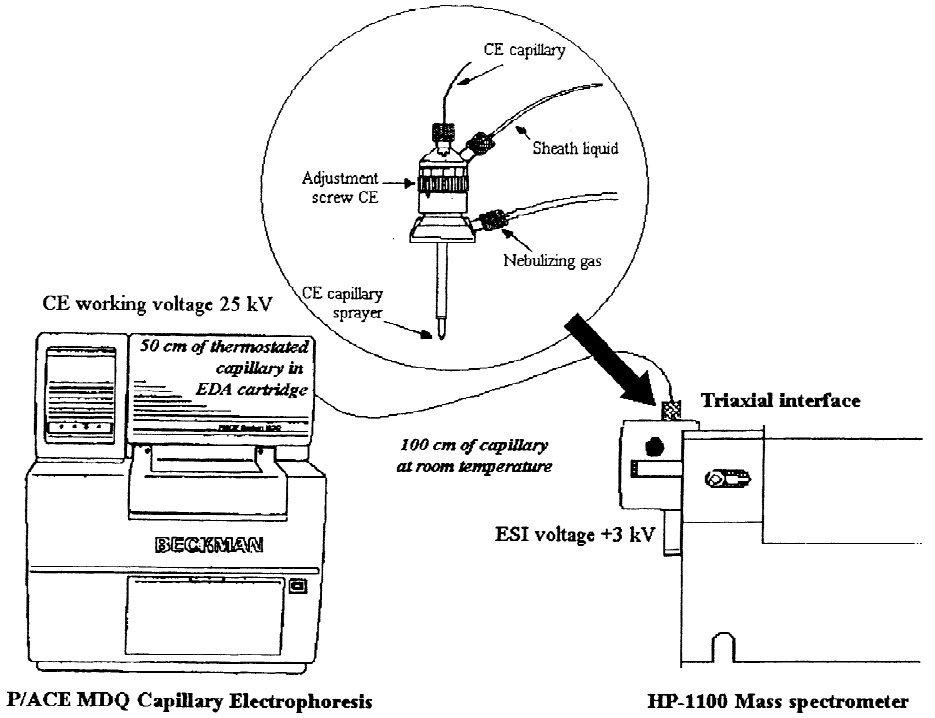

Fig. 1. Scheme of the instrument system.

turned off and the electrodes were switched to the separation configuration.

2.3. Mass spectrometry

A Hewlett-Packard HP1100 mass spectrometer (Palo Alto, CA, USA) equipped with an Agilent CE–ESI-MS sprayer kit (G1607A) (Waldbronn, Germany) was used for this study. The interface utilized a triaxial flow arrangement whereby the CE eluate was mixed with a suitable sheath liquid at the probe tip and then nebulizer using nitrogen gas (see Fig. 1). The polyamide coating was removed at the end of the capillary (ca. 5 mm) and the capillary was protruded 0.1 mm of the sprayer to maintain a stable electrospray. Nitrogen was used as drying and nebulization gas. The coaxial sheath liquid was delivered at 13 μl min^{-1} by a Hewlett-Packard 1100 Series pump that was connected to the outlet a sheath flow splitter (included in the Agilent CE–MS sprayer kit). The splitter splits the sheath liquid in the ratio 1:100, so the flow-rate of the pump will be 1.3 ml min^{-1}. HPLC Chemstation was used for MS instrument control, data acquisition and data handling. The coaxial sheath liquid was the solution used as buffer in CE.

The mass spectrometer was operated in the positive ion mode. The spray chamber parameters were: drying gas flow, 11 l min^{-1}; drying gas temperature, 150°C; nebulizing gas pressure, 40 p.s.i.; voltage, 4000 V; and fragmentor voltage, 100 V.

Full scan (from m/z 150 to 350) and selected ion monitoring (SIM) ($[M+H]^+$ ion was monitored) were carried out. The selected masses were acquired with a gain of 3 and a dwell time of 349 ms on each m/z, which were 202 for thiabendazole and 284 for procymidone.

2.4. Sample extraction

The samples analyzed – apples, grapes, oranges, pears, strawberries and tomatoes – were obtained from local markets. All the samples were taken at various places distributed throughout the lot weigh-

ing at least 1 kg and consisted of at least 10 individual pieces.

The sample preparation was done as follows, based on previous papers [13,18]. A representative portion of sample (200 g of whole fruit or vegetable) was chopped and homogenized in a Bapitaurus food chopper (Taurus, Berlin, Germany). Then, a 5-g portion was weighed and placed into an Erlenmeyer flask and homogenized with 5 ml of methanol and 5 ml of water by sonication during 15 min. The resulting suspension was filtered through a Whatman 40-μm filter, and the filter cake was washed twice with 5 ml of deionized water. The filtrate was adjusted to a volume of 100 ml with deionized water and passed under vacuum, through a C_8 solid-phase column that was preconditioned with 10 ml of methanol and 10 ml of water. The column was prepared transferring 1 g of C_8 to a 100 mm×9 mm I.D glass column fitted with a coarse frit (No. 3) and covered with a plug of silanized glass wool. Pesticides were eluted with 10 ml of dichloromethane. The eluate was evaporated to dryness with nitrogen at 40°C. The dry residue was reconstituted in 500 μl of 4 m*M* buffer and thoroughly mixed in an ultrasonic bath for 5 min.

Extraction recoveries were determined spiking fresh samples (10 g) with a fortification solution at different levels: 0.1–10 mg/kg.

3. Results and discussion

3.1. Influence of ESI-MS parameters

The optimization of the CE–ESI-MS parameters were tested for the analysis of both fungicides. Best sensitivity was obtained using the conditions reported in the Experimental section for nebulizer pressure, drying gas flow-rate, drying gas temperature and voltage. Thiabendazole and procymidone can be easily detected by positive ionization mode ESI. The mass spectra are characterized by protonated molecular ion with little fragmentation. The effect of modifying the fragmentor voltage in the production of diagnostic ions is poor. Representative full scan (mass range *m*/*z* 150–350) mass spectra of procymidone and thiabendazole at different fragmentor voltages are shown in Fig. 2. The main detected ions were the protonated molecular ions $[M+H]^+$. Procymidone formed cluster ions with NH_4^+ at

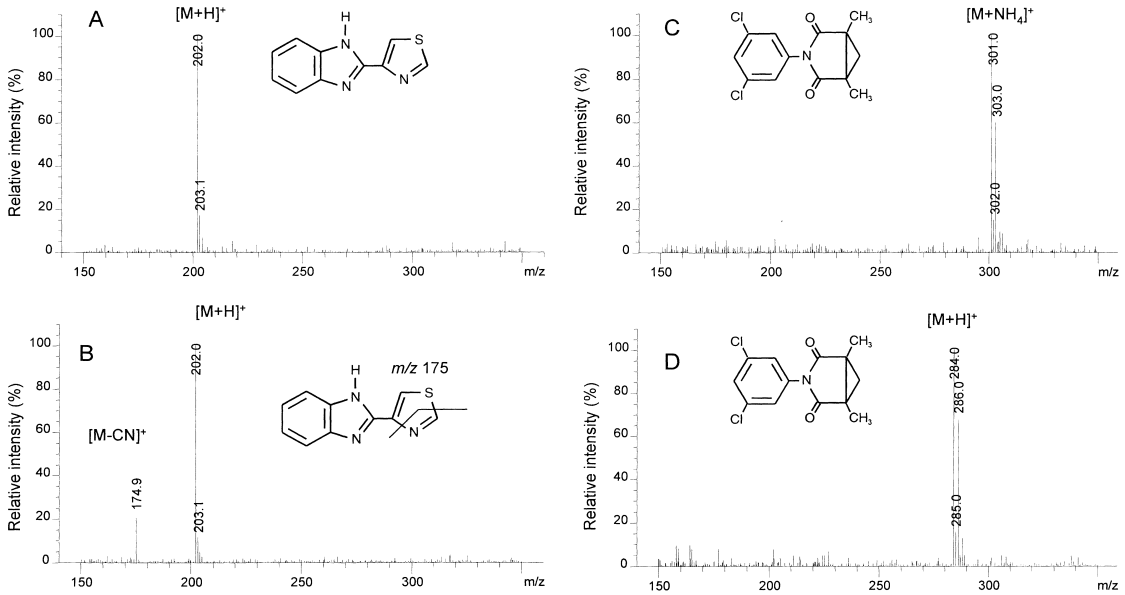

Fig. 2. ESI mass spectra of (A) thiabendazole at fragmentor of 100 V, (B) thiabendazole at fragmentor of 140 V, (C) procymidone at fragmentor of 40 V, and (C) procymidone at fragmentor of 100 V.

fragmentor voltages lower than 40 V and thiabendazole was fragmented at extraction voltages of >140 V producing a fragmented ion in low abundance at m/z 175.

The composition of sheath liquid was found to affect ESI sensitivity and CE resolution. The effect of methanol percentage, change of ammonium formate into ammonium acetate or buffer concentration was tested. The best results were obtained when the composition of sheath liquid was similar to the running buffer.

The sheath liquid flow-rate was also studied. Low flow-rates affect negatively spray stability and peak shape that result in low signal intensity, whereas too high flow-rates cause the dilution of the separated compounds that also generates low signal intensity. As shown in Fig. 3, a flow-rate of 13 μl min^{-1} gives the highest peak area for both procymidone and thiabendazole, as well as the most stable conditions.

3.2. CE–ESI-MS quality parameters

A limited number of buffers were compatible with ESI-MS determination because a volatile buffer is required to prevent plugging of the dielectric capillary between the spray chamber and the mass spectrometer. In addition, the conductivity of the buffer must not be too high to obtain a stable electrospray. Ammonium formate proved to be an appropriate buffer regarding the separation of procymidone and thiabendazole. As has been previously reported [18], the better separation between these compounds is obtained at pH value of 3.5.

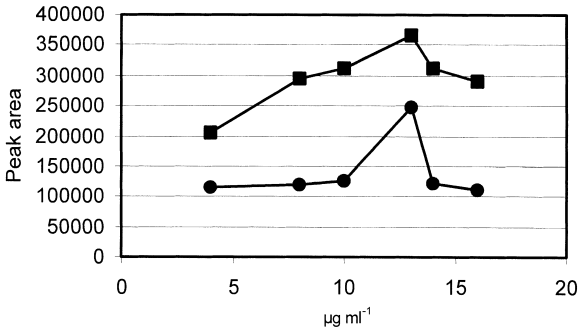

Fig. 3. Ion abundance as a function of the sheath liquid flow-rate. Compound identification: (■) thiabendazole; (●) procymidone.

To optimize the stacking efficiency several factors were taken in consideration, the most important of which is band broadening. Band broadening during the stacking step depends on the injection time, injection plug length and ratio of conductivity between the separation buffer and the sample buffer. Quality parameters using stacking under optimal conditions are outlined in Table 1. The quantities of analytes in the stacked samples were three times greater than those of the standard injection (hydrodynamic injection, 5 s, 0.5 p.s.i.). The stacked procedure did not cause a dramatically improvement in sensitivity, but better detection limits are achieved and high peak efficiencies (N>4 520 000) were obtained for the two analytes. Indeed, in spite of stacking procedure and the large non-thermostated part of the capillary between CE and ESI-MS, no significant band broadening was observed.

Method repeatability was assessed by replicate injections (n=5) of procymidone and thiabendazole mixture. As shown in Table 1, the relative standard deviations (RSDs) of migration time and peak area measurements were lower than 1.35 and 3.21%, respectively. Calibration curves based on the peak area were also obtained and good linearity was observed in the concentration range tested.

In this study, successful coupling of CE with ESI-MS is demonstrated for the separation of thiabendazole and procymidone.

Table 1
Parameters of the CE–ESI-MS method for procymidone and thiabendazole

Parameter	Procymidone	Thiabendazole
t_R (min)	10.63	9.52
t_R repeatability (RSD, %)	1.35	0.97
Plate number ($\cdot 10^4$)	452	567
Resolution[a]	0.616	0.616
Peak area repeatability (RSD, %)	3.21	2.13
Linearity concentration (μg/ml)	10–100	1–10
Slope ($\cdot 10^5$)	0.06	3.13
Intercept ($\cdot 10^5$)	1.94	−1.31
Correlation coefficient	0.9979	0.9989
LOD (μg/ml)[b]	0.1	0.01

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

[b] LOD, Limit of detection defined as signal-to-noise ratio (S/N) of 3.

3.3. Application

The potential of the CE–ESI-MS determination for analyzing thiabendazole and procymidone in fruits and vegetables was assessed. Spiked samples were extracted following an SPE procedure. Fruits and vegetables are complex matrices containing important amount of salt and endogenous compounds. Therefore, it is necessary to apply a sample extraction procedure before analysis. Figs. 4 and 5 show examples of representative electropherograms of orange samples obtained by CE–ESI-MS along with those obtained by CE–UV. The CE–ESI-MS

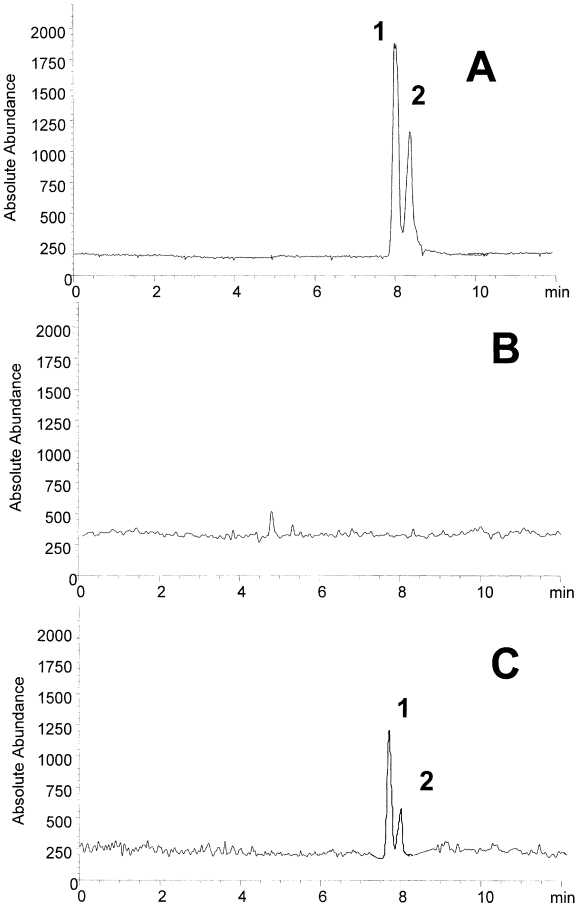

Fig. 4. CE–ESI-MS electropherograms in the SIM mode of (A) thiabendazole and procymidone standard (0.1 and 1 mg kg^{-1}, respectively), (B) untreated control orange sample, and (C) control orange sample spiked at 0.01 and 0.1 mg kg^{-1} of thiabendazole and procymidone, respectively.

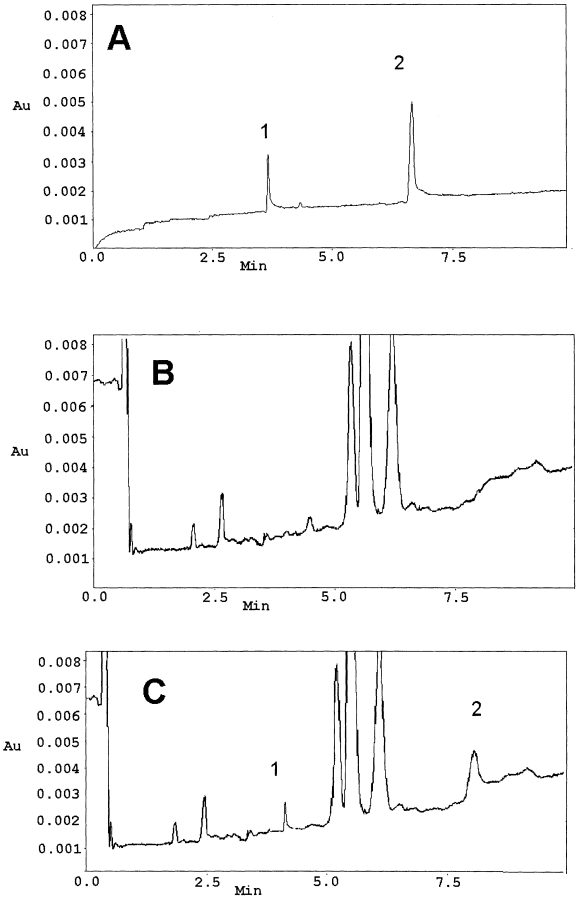

Fig. 5. CE–UV electropherograms at 219 nm of (A) thiabendazole and procymidone standard (10 mg kg^{-1}), (B) untreated control orange sample, and (C) control orange sample spiked at 1 mg kg^{-1}.

electropherograms showed a stable baseline, good peak shapes and no interferences by endogenous compounds, which clearly demonstrate a better sensitivity and selectivity compared to those obtained by CE–UV. Moreover, enhancement or suppression in the MS response caused by the matrix components was not observed for any of the matrices tested.

For method validation, samples of each commodity spiked with different concentrations were extracted by SPE and analyzed by CE–ESI-MS. Recoveries and RSDs are summarized in Table 2. Recoveries were raged from 61 to 68% and from 71 to 80% for thiabendazole and procymidone, respectively, with RSDs between 7 and 12%. Limits of

Table 2
Recovery values from fortified samples

Sample	Procymidone		Thiabendazole	
	Mean recovery[a] (%)	RSD[b] (%)	Mean recovery[c] (%)	RSD[b] (%)
Apple	78	8	65	10
Grape	80	6	63	8
Orange	75	9	64	7
Pear	71	11	62	12
Strawberry	73	9	68	9
Tomato	72	7	61	9

[a] Concentrations tested were 0.1, 1 and 10 mg kg^{-1}. Mean values obtained from quintuplicate measurements for each spiked concentration.

[b] RSD does not show dependence with concentration ($n=5$) for the different spiked levels tested.

[c] Concentrations tested were 0.01, 0.1 and 1 mg kg^{-1}. Mean values obtained from quintuplicate measurements for each spiked concentration.

quantification (LOQs), calculated as the lowest fungicide concentration giving a response that could be quantified with a RSD of less than 25%, are given in Table 3 together with the maximum residue limits (MRLs) established by the European Union (EU). The sensitivity of the method was suitable to ensure a reliable determination at levels lower than the respective MRLs.

The method was applied to the determination of procymidone and thiabendazole in real samples obtained from local markets over different days. Seven samples of each fruit or vegetable were analyzed. More than 50% of the analyzed samples contained one or both fungicides at concentrations ranging from 0.05 to 1.00 mg kg^{-1}.

Typical electropherograms of an orange sample containing thiabendazole, a pear sample containing thiabendazole and procymidone and a strawberry sample containing procymidone are shown in Fig. 6. The electropherograms of the real samples demonstrated that for all of the matrices tested no interfering endogenous compounds were observed.

Table 3
Limits of quantitation (LOQs) and maximum residue limits (MRLs) authorized in the different samples

Sample	Procymidone		Thiabendazole	
	LOQ (mg kg^{-1})	MRL (mg kg^{-1})	LOQ (mg kg^{-1})	MRL (mg kg^{-1})
Apple	0.05	0.02	0.005	5.00
Grape	0.05	5	0.005	0.05
Orange	0.05	0.02	0.005	5.00
Pear	0.05	1	0.005	5.00
Strawberry	0.05	5	0.005	0.05
Tomato	0.05	2	0.005	0.05

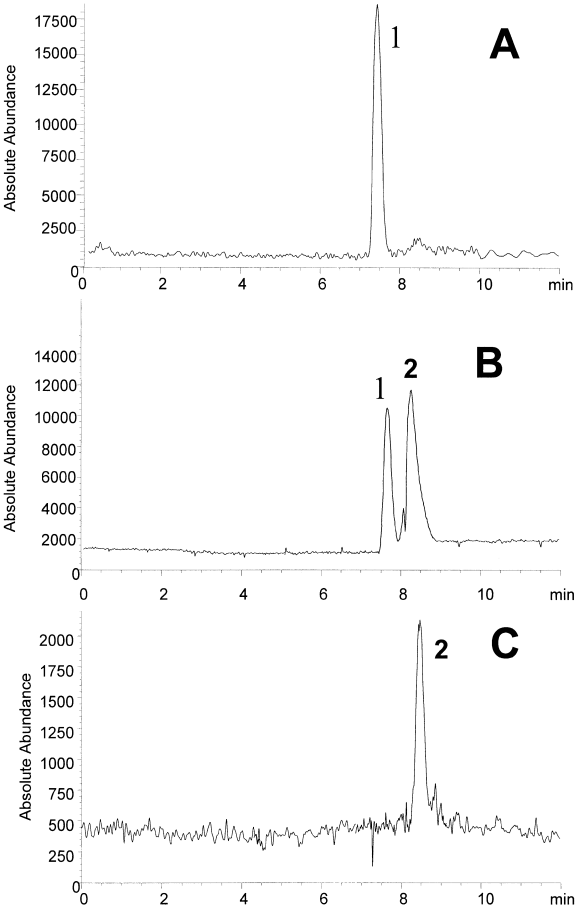

Fig. 6. CE–ESI-MS electropherograms in the SIM mode of (A) orange that contains thiabendazole at 0.95 mg kg^{-1}, (B) pear that contains thiabendazole and procymidone at 0.42 mg kg^{-1} and 0.81 mg kg^{-1}, respectively, and (C) strawberry sample that contains procymidone at 0.2 mg kg^{-1}.

4. Conclusion

A sensitive, selective, linear and reproducible analysis technique for thiabendazole and procymidone has been achieved by coupling CE with

MS. After the SPE procedure and the sample stacking technique, fungicide concentrations lower than 0.01 mg kg^{-1} can be detected. This sensitivity allows determining procymidone and thiabendazole at levels 10-times lower than the MRLs established by the EU. Thus, CE–MS has the potential of becoming an advantageous technique for pesticide residues determination and thereby, an alternative to the widely used GC–MS and LC–MS techniques.

Acknowledgements

This research was funded by the National Program on Food from the Spanish Ministry of Science and Technologies (project No. CAL00-066).

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