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Application of matrix solid-phase dispersion to the determination of a new generation of fungicides in fruits and vegetables ☆

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Abstract

A method based on matrix solid-phase dispersion (MSPD) and gas chromatography to determine eight fungicides in fruits and vegetables is described. Fungicide residues were identified and quantified using nitrogen–phosphorus detection and electron-capture detection connected in parallel and confirmed by mass spectrometric detection. The method required 0.5 g of sample, C_{18} bonded silica as dispersant sorbent, silica as clean-up sorbent and ethyl acetate as eluting solvent. Recoveries from spiked orange, apple, tomato, artichoke, carrot and courgette samples ranged from 62 to 102% and relative standard deviations were less than 15% in the concentration range 0.05–10 mg kg^{-1}. Detection and quantitation limits ranged 3–30 μg kg^{-1} and 10–100 μg kg^{-1}, respectively, with linear calibration curves up to 10 mg kg^{-1}. The analytical characteristics of MSPD compared very favourably with the results of a classical multiresidue method, which uses ethyl acetate and anhydrous sodium sulphate for the extraction.

Keywords: Matrix solid-phase dispersion; Extraction methods; Food analysis; Fruits; Vegetables; Pesticides

1. Introduction

Classical active ingredients used to control fungal pathogens in fruits and vegetables belong to the chemical class of chlorinated phthalimides, such as captan and folpet. Recently, pesticides of other chemical families have been marketed for pre- and postharvest applications. To take an example, carboxin and flutolanil are anilines, fludioxonil is a phenylpyrrole, pyrimethanil is an anilinopyrimidine, quintozene is a chlorinated nitrobenzene and tebuconazole is a triazole. The structures of these compounds are illustrated in Fig. 1.

Teratogenic, carcinogenic and toxic properties of these compounds have been reported in the literature [1,2]. The presence of their residues in fruits and vegetables can be a significant route to human exposure. As a consequence, the European Union Commission established maximum residue limits (MRLs) in fruits and vegetables to ensure that they are not present at levels that may pose a health risk to the public [3]. When these fungicides are applied according to good agricultural practices, MRLs are not exceeded, but their incorrect application may

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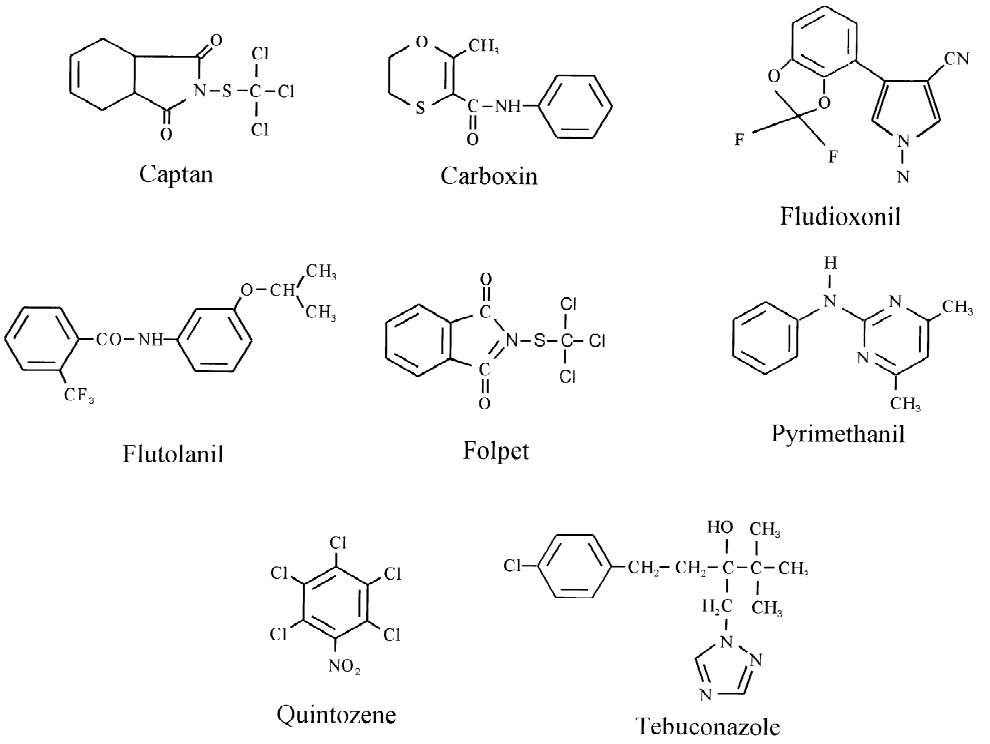

Fig. 1. Structures of the studied compounds.

leave harmful residues, which involve possible health risk. The development of easy analytical methods for them is very important to monitor these fungicide residues in fruits and vegetables.

At present, the separation and quantitation of these fungicides are mainly carried out by gas chromatography (GC) with nitrogen–phosphorus (NPD), electron-capture (ECD) and mass spectrometric (MS) detection since they are volatile and thermally stable chemicals [4–18]. Numerous methods have been published to determine captan and folpet in fruits and vegetables using solvent partitioning [4–9], supercritical fluid extraction (SFE) [10,11], solid-phase extraction (SPE) [12,13] or microextraction (SPME) [14], and matrix solid-phase dispersion (MSPD) [15]. On the contrary, only a few analytical methods have been reported for determining some of these new generation fungicides, which are based on complex solvent extraction methods [16–18]. However, solvent extraction methods have drawbacks related to: (1) time consuming and complexity that avoid the generation of relevant data in time to prevent contaminated foods from entering the market place, (2) environmental contamination by the great amount of chemicals and solvents, and (3) false positives due to the lack of specificity.

The purpose of this work is to develop a rapid MSPD method followed by GC with ECD, NPD and MS for determining captan, carboxin, flutolanil, fludioxonil, folpet, pyrimethanil, quintozene and tebuconazole in oranges, apples, tomatoes, artichokes, carrots and courgettes. Precision and accuracy between MSPD and solvent extraction with ethyl acetate were compared. The method is applied to analyse these compounds in fruit and vegetable samples taken from the market.

2. Experimental

2.1. Reagents

Captan, carboxin, fludioxonil, flutolanil, folpet, pyrimethanil, quintozene and tebuconazole were

obtained from Dr. Ehrenstorfer (Ausburg, Germany). Stock standard solutions of each pesticide were prepared in ethyl acetate (with the exception of captan that was dissolved in hexane) at concentrations of 1 mg ml^{-1}, and stored in stained glass stoppered bottles at 4 °C. Different working standard mixtures were prepared by dilution in the same solvent.

Ethyl acetate was for organic trace analysis (Merck, Darmstadt, Germany) and anhydrous sodium sulphate was of analytical grade (Panreac, Barcelona, Spain).

Octadecylsilica MFE-C_{18} was from Análisis Vínicos (Tomelloso, Spain). The particles are spherical with an average size of 50 μm and a pore diameter of 60 Å.

Silica gel 60 was obtained from Supelco (Madrid, Spain) with a surface area of 500 m^2 g^{-1} and pore diameter of 60 Å.

2.2. Instrumental conditions

A Hewlett-Packard 5890 Series II, equipped with NPD and ECD systems, a HP-7673 automatic sampler and a split-splitless injector connected to a HP ChemStation, was used as the routine instrument. In this system, the column was connected via a Y press-fit connector to both detectors. NPD gases were helium at 20 ml min^{-1} as make-up, and hydrogen and air at flow-rates of 4 ml min^{-1} and 120 ml min^{-1}, respectively. ECD make gas was Ar/CH_4 at a flow-rate of 60 ml min^{-1}.

A Fisons Instrument Series 8000 equipped with a mass spectrometer detector Trio-1000 and a split-splitless injector with data station LAB-BASE and spectra library NBS, was employed to confirm the results.

A DB-5ms fused-silica column (30 m×0.25 mm I.D.) was used, with 5% phenyl–95% methylsilicone (film thickness 0.25 μm). For both systems, the carrier gas was helium at a flow-rate of 1 ml min^{-1}, the injector was operated at 280 °C, the sample (2 μl) was injected in splitless mode (0.8 min) and the oven temperature was programmed 50 °C for 1 min, raised to 180 °C at 30 °C min^{-1}, and held for 2 min, raised to 280 °C at 2 °C min^{-1} and held for 1 min.

ECD and NPD were operated at 300 °C. The MS operating conditions were: interface temperature, 250 °C, source temperature 150 °C, electron energy 30 V, ion energy 3 V and detector temperature 500 °C. The acquisition modes used were: scan (mass range 50–400) and selected ion monitoring (SIM). The time scheduled and ions monitored for quantitation are given in Table 1.

2.3. Extraction procedures

Laboratory samples were prepared as described in the council directive 90/642/EEC [3] to obtain very small analytical portions (0.5 g when MSPD is used) representative of the entire sample. A representative portion of sample (200 g of whole fruit or vegetable) was chopped into small pieces and then was homogenised for 3 min at high speed using a Bapitaurus Bapiplus Dual CP/CM (Taurus, Berlin, Germany) food processor. The amount of matrix per ml of the final extract solution is 5 g ml^{-1} for the ethyl acetate extraction and 1 g ml^{-1} for the MSPD.

2.3.1. Matrix solid-phase dispersion procedure

An aliquot of the sample (0.5 g) was gently blended with 0.5 g of C_{18} into a glass mortar (50 ml capacity) using a glass pestle, until a homogeneous mixture was obtained (ca. 1 min). This homogenized sample was introduced into a glass chromatographic column (100 mm×9 mm I.D.) with a coarse frit of 40 μm, which already contained 1 g of silica. Ten ml of ethyl acetate were added to the column and the sample was allowed to elute dropwise by applying a slight vacuum. The effluent was collected into a graduated conical tube (15 ml) and concentrated, under a nitrogen stream to 0.5 ml.

Table 1
Time scheduled and ions monitored to determine the selected fungicides

Time (min)	SIM ion (m/z)	Compound
0.0–16.1	237, 249, 295	Quintozene
16.3–18.2	198, 199, 200	Pyrimethanil
18.4–23.0	114, 116	Captan
	114, 116, 260	Folpet
23.2–26.0	173	Flutolanil
	248	Fludioxonil
	143, 235	Carboxin
26.2–29.0	250, 252	Tebuconazole

2.3.2. Ethyl acetate extraction procedure

Fifty grams of chopped sample were placed in a 250-ml glass beaker and mixed thoroughly with 100 ml of ethyl acetate and 50 g of anhydrous sodium sulfate using a Waring blender during 2 min. The homogenate was allowed to settle and the supernatant was passed through a filter paper into a 500-ml rotary-evaporation flask. The solid residue was again homogenized with 100 ml ethyl acetate, filtered through the anhydrous sodium sulfate and collected with the first extraction fraction. Twice, 25 ml ethyl acetate were used to rinse the glass beaker and the rinsings were passed through the filter and collected. A rotary evaporator set at 40 °C and 250 mBar was used to evaporate the extract to less than 5 ml and then reconstitute to 10 ml with ethyl acetate in a volumetric flask.

2.3.3. Recovery studies

Recovery studies were carried out by spiking fresh samples (50 g or 0.5 g depending on the extraction procedure) with known volumes of the appropriate working mixtures of fungicides. The mixture was left to stand overnight at 4 °C. The sample was equilibrated to room temperature before following the extraction procedure described.

3. Results and discussion

3.1. Performance of the matrix solid-phase dispersion procedure

Figs. 2 and 3 show chromatograms obtained from spiked and unspiked untreated apples for both ECD and NPD systems. The chromatographic resolution was satisfactory. Fig. 4 illustrates the chromatograms obtained from a spiked untreated courgette sample by GC–MS in SIM mode. Although oven temperature and carrier gas flow were varied, resolution of fludioxonil and flutolanil peaks was not satisfactory with the column used in the GC–MS system. However, these two fungicides can be identified using the SIM program shown in Table 1. As can be seen, the chromatograms were clean without interfering peaks in the areas of interest.

Table 2 presents recoveries of eight fungicides at three concentration levels for all the crops tested. Average recoveries were between 80 and 102%, except for captan and folpet, which gave lower recoveries (<78% and <79%, respectively). RSDs ranged from 5 to 15%, with an average of 9%. Lower recoveries of captan and folpet compared with those other pesticides have been repeatedly reported in the literature [4–9,12,13]. The cause was attribu-

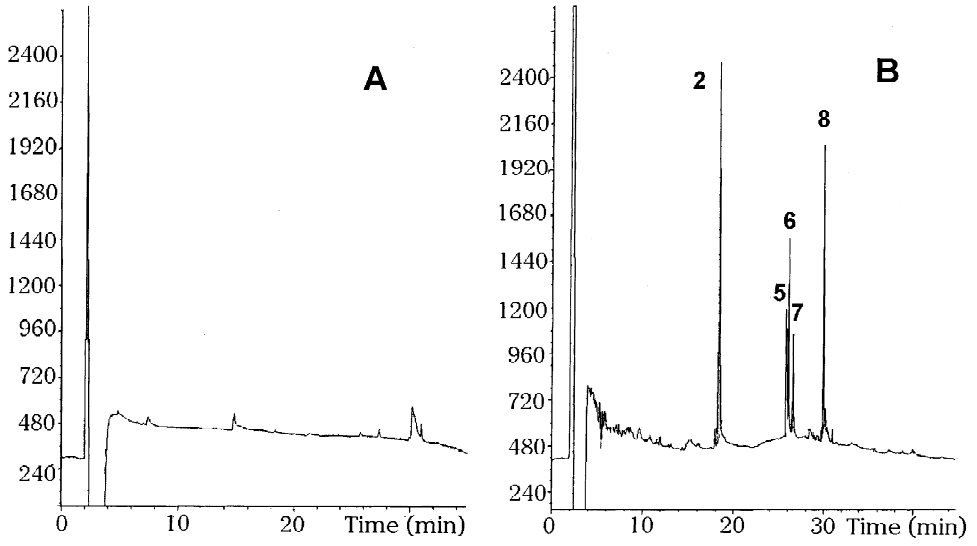

Fig. 2. GC–NPD chromatograms of extracts of (A) untreated apple and (B) untreated apple spiked at 0.5 mg kg^{-1}. Peak identification: 2, pyrimethanil; 5, fludioxonil; 6, flutolanil; 7, carboxin; 8, tebuconazole.

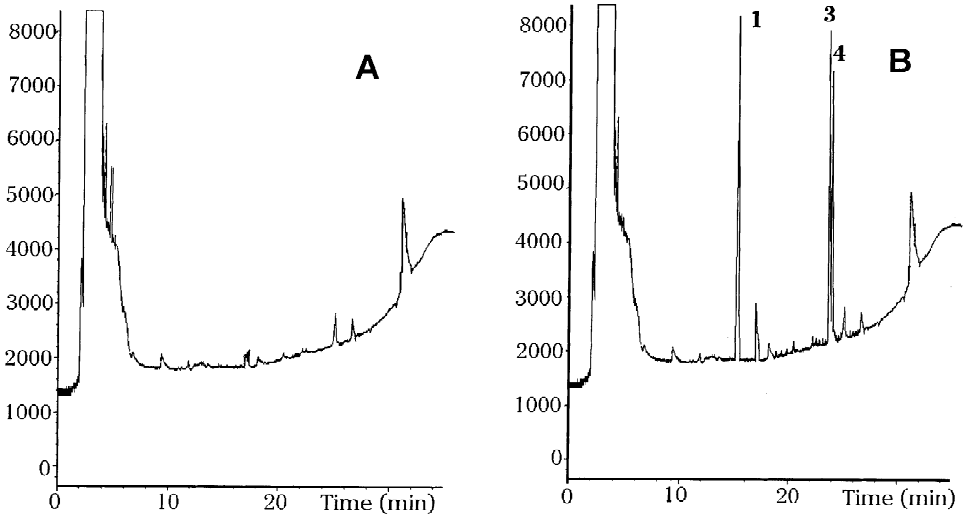

Fig. 3. GC–ECD chromatograms of extracts of (A) untreated apple and (B) untreated apple spiked at 0.5 mg kg^{-1}. Identification: 1, quintozene; 3, captan; 4, folpet.

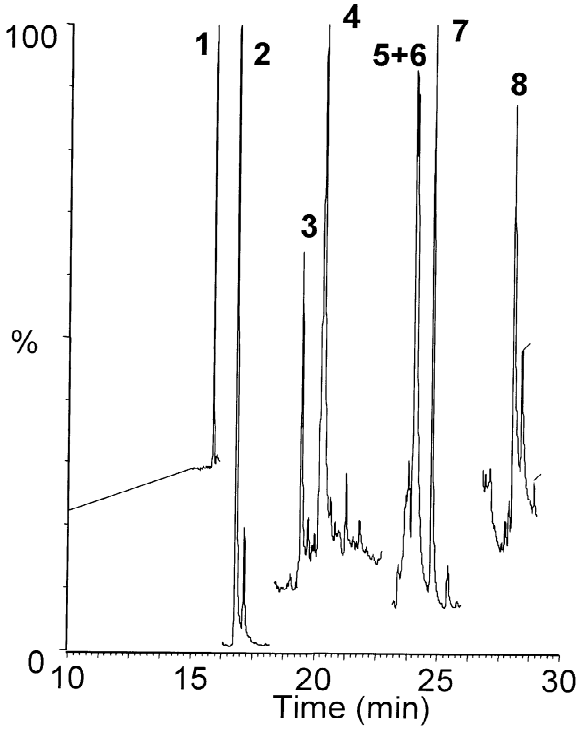

Fig. 4. GC–MS in SIM mode of a courgette extract spiked at 0.5 mg kg^{-1}. Peaks as in Figs. 2 and 3.

ted to irreversible adsorption by the solid-phase [12] or lack of solubility in most organic solvents [6,8,13].

The limits of detection (LODs) were calculated as the lowest concentration giving a response of three-times the average of the baseline noise defined from three unfortified samples. LODs for all the fungicides were in the range of 3–30 μg kg^{-1} for all the crops tested.

The limits of quantitation (LOQs) obtained by the different detectors tested for all the pesticides are summarized in Table 3. These were determined as the lowest concentration of a compound that gives a response that could be quantified with an RSD of less than 26%. Differences between the six matrices tested were not observed. LOQs were lower than or equal to the MRLs set by the European Union.

Linearity was studied for the eight fungicides over a range of spiking levels from the LOQs to 10 mg kg^{-1} on the six matrices. For all the compounds, the calibration curves were linear in this range with regression coefficients >0.994 (Table 3).

MSPD methods have been described for the determination of pesticide residues in fruits and vegetables by GC or liquid chromatography (LC). These include organophosphorus and organochlorine [5,15], carbamates [19,20], ureas [19] and fungicides [21].

Table 2
Recovery of fungicides from the different crops tested

Pesticide	Added (mg kg^{-1})	Average recoveries (%)±RSD (%), $n=5$					
		Apples	Artichokes	Carrots	Courgettes	Oranges	Tomatoes
Captan	0.05	73±7	62±12	69±14	65±7	70±15	71±7
	0.5	74±8	76±8	70±12	74±8	69±10	72±8
	5	78±5	68±5	73±9	72±5	75±11	77±5
Carboxin	0.1	92±10	94±14	93±11	93±7	94±12	95±8
	1	94±7	95±8	92±9	95±5	95±9	99±9
	10	98±9	97±6	94±5	92±7	97±9	94±6
Fludioxonil	0.05	89±11	86±11	85±12	89±11	82±12	88±10
	0.5	90±8	89±10	83±7	86±6	88±7	85±9
	5	92±9	92±9	90±8	93±5	84±5	86±9
Flutolanil	0.05	96±9	93±8	94±12	99±9	102±8	95±10
	0.5	98±9	102±12	101±8	100±8	99±9	97±10
	5	101±5	99±6	102±6	90±7	99±6	96±6
Folpet	0.05	68±12	62±12	67±13	73±9	70±11	68±15
	0.5	75±8	71±9	67±15	74±5	79±11	72±10
	5	79±5	85±7	78±9	70±8	75±8	69±6
Pyrimethanil	0.01	93±10	95±12	92±10	88±6	91±7	90±11
	0.1	94±10	95±11	99±7	92±7	93±7	94±9
	1	99±8	96±8	96±8	94±5	99±6	97±9
Quintozene	0.01	87±5	88±9	93±11	84±8	88±12	88±10
	0.1	88±8	80±8	84±9	87±10	86±7	88±12
	1	92±8	90±9	86±10	91±9	82±5	94±9
Tebuconazole	0.05	98±13	88±11	87±8	88±6	86±14	84±13
	0.5	94±8	93±10	90±10	93±7	89±12	87±8
	5	96±9	92±11	90±5	95±5	92±10	91±9

The results obtained in this study corroborate adequately those presented earlier for the determination of organophosphorus and organochlorine residues [5,15] by MSPD and GC. A column prepared from a C_{18}-matrix blend with a silica layer in the bottom part of the glass column and eluted with ethyl acetate produces an extract that shows minimal interferences and provides the best recoveries. The compounds determined by GC have common physical and chemical characteristics and they are nonpolar, volatile and thermally stable compounds.

When MSPD methods have been developed for

Table 3
Linearity, MRLs and LOQs obtained by different GC detectors

Compound	Linearity[a]		MRLs (mg kg^{-1})	LOQ (mg kg^{-1})		
	Linear range[b] (mg kg^{-1})	r[c]		NPD	ECD	MSD
Captan	0.05–10	0.997–0.999	0.1–3.0	–	0.05	0.05
Carboxin	0.1–10	0.995–0.998	0.2	0.1	–	0.1
Fludioxonil	0.05–10	0.994–0.996	0.05–0.5	0.05	–	0.01
Flutolanil	0.05–10	0.994–0.996	0.05	0.05	–	0.01
Folpet	0.05–10	0.997–0.999	0.1–3.0	–	0.05	0.05
Pyrimethanil	0.01–10	0.996–0.999	0.02–1	0.01	–	0.01
Quintozene	0.01–10	0.999	0.01–0.05	–	0.01	0.01
Tebuconazole	0.01–10	0.994–0.997	0.05–1	0.01	–	0.02

[a] The linearity was calculated using GC–NPD and –ECD.
[b] Five different concentrations were used for the calibration curve.
[c] r interval corresponds to the minimum and maximum value for the different crops tested.

further LC determination the conditions are a little different because LC is preferred for the analysis of polar compounds [19–21]. C_8 was generally the preferred solid material for MSPD because it provides the best recovery averages of the compounds, the lowest variation in the values obtained and the cleanest extract. The clean-up (with a bottom layer of silica, alumina, florisil or cellulose) achieved less coloured extracts than those obtained only by dispersion with C_8, but the recoveries decreased for certain polar pesticides and the chromatographic profiles were not evidently improved even using UV detection, so the clean-up is not considered. Although the elution of pesticides with different solvents produced similar recoveries, dichloromethane was considered best for extraction because it always gave the cleanest extracts.

3.2. Comparison of MSPD and ethyl acetate methods

The developed MSPD method was compared to an established ethyl acetate extraction procedure, which has been previously evaluated to extract a wide variety of pesticide residues in fruit and vegetables before GC determination [4,22]. Table 4 lists the analytical parameters obtained for the ethyl acetate extraction followed by GC–MS, which were calculated by analysing untreated control and spiked orange samples at concentrations between LOQs and 10 mg kg^{-1}. These results are clearly on a par with those shown in Tables 2 and 3 for the MSPD procedure. Similar recoveries, LOQs and linearity were obtained for GC–NPD and –ECD, but the chromatograms obtained by GC–MS clearly demonstrated a better selectivity compared to that by GC–ECD.

Table 5
Fungicide content found in samples from the market using ethyl acetate extraction and the proposed procedure

Sample	Fungicide	Residues[a] (mg kg^{-1})	
		Ethyl acetate extraction[b]	Proposed procedure[c]
Apple	Captan	0.18	0.14
Artichoke	–	–	–
Carrot	–	–	–
Courgette	Fludioxonil	0.15	0.18
Orange	Captan	0.36	0.30
	Captan	0.28	0.25
Tomato	Pyrimethanil	0.43	0.52
	Tebuconazole	0.23	0.18
	Tebuconazole	0.64	0.50
	Captan	1.64	1.65

[a] Triplicate measurements.
[b] RSDs ranged between 10 and 19%.
[c] RSDs ranged between 8 and 16%.

Ten samples of each crop taken from a local market were analysed using both procedures. The results given in Table 5 show satisfactory agreement. Fungicide concentrations in all contaminated samples were above the LOQs and below the MRLs. However, extracts obtained using ethyl acetate extraction typically contain many compounds, which can be the source of interfering peaks at the retention times of the analytes. The MSPD procedure presents some advantages because the column performs several functions in a single step, viz. the removal of water and co-extractives, and the transfer of fungicide residues into an adequate solvent.

The matrices tested (apple, artichoke, carrot,

Table 4
Recoveries, linearity and LOQs of fungicides using ethyl acetate extraction and GC–MS

Fungicide	Linear range (mg kg^{-1})	r	Range (%)	Average recovery (%) $n=5$	RSD (%)	LOQ (mg kg^{-1})
Captan	0.025–10	0.998	50–72	62	6	0.025
Carboxin	0.03–10	0.997	94–113	103	12	0.03
Fludioxonil	0.02–10	0.995	77–94	88	8	0.02
Flutolanil	0.02–10	0.994	87–105	106	10	0.02
Folpet	0.02–10	0.999	45–74	69	11	0.02
Pyrimethanil	0.002–5	0.997	95–108	101	7	0.002
Quintozene	0.005–10	0.999	72–96	89	9	0.005
Tebuconazole	0.005–5	0.995	83–99	90	7	0.005

courgette, orange and tomato) are among the most troublesome to analyse, not only because of the bright colour of the natural pigments they contain, but also because of the many major and minor compounds that are co-extracted with the analytes. The proposed MSPD procedure provided more high-quality clean-up than ethyl acetate extraction because the matrix components were reduced during the dispersion and elution steps and the extracts were much less coloured than those obtained by ethyl acetate extraction. Moreover, MSPD uses amounts of less than 1 g of sample, and as a consequence, the amount of matrix per ml in the final extract is lower than that of the ethyl acetate extraction (1 g ml^{-1} versus 5 g ml^{-1}). This resulted in cleaner chromatograms and an increased DB-5ms fused-silica column lifetime.

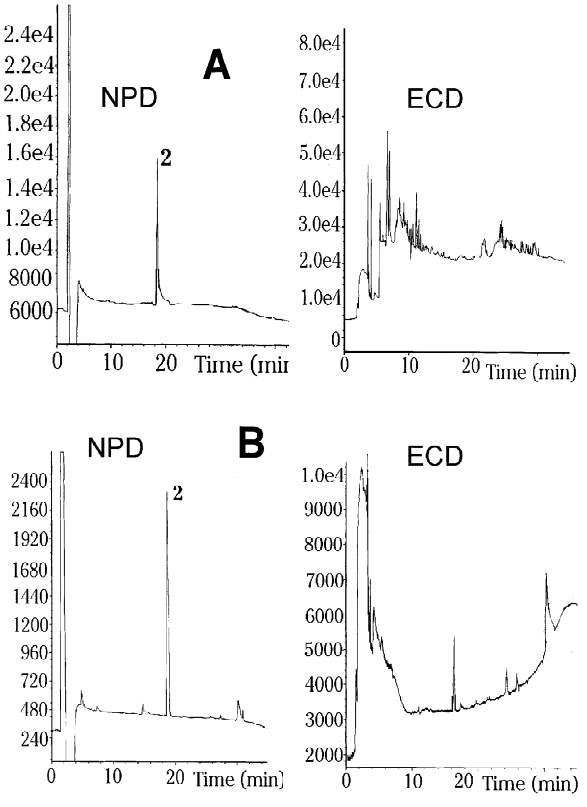

Fig. 5. Chromatograms of a tomato extract obtained by (A) ethyl acetate and anhydrous sodium sulphate extraction (amount of matrix per ml in final extract 5 g ml^{-1}), and (B) MSPD (amount of matrix per ml in final extract 1 g ml^{-1}). Peaks as in Fig. 2.

To corroborate these features, representative GC–NPD and –ECD chromatograms obtained from a real tomato sample containing pyrimethanil (sample 3) are shown in Fig. 5. The co-extractive retention is not easily recognized when the extract is analysed by GC–NPD, but is very obvious when ECD is used, as can be seen by comparing Fig. 5A and B.

4. Conclusion

The proposed MSPD procedure was successfully applied to the extraction of captan, carboxin, fludioxonil, flutolanil, folpet, pyrimethanil, quintozene and tebuconazole from apples, artichokes, carrots, courgettes, oranges and tomatoes. Moreover, further analytes can be added to the repertoire without difficulty. This method allows for the detection, confirmation and quantitation of these compounds in fruits and vegetables down to 0.1 mg kg^{-1}.

Although other multiresidue methods now available offer similar analytical characteristics, from a practical point of view, MSPD presents important advantages because it is simple, rapid and inexpensive.

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