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# Determination of fungicide residues in fruits and vegetables by liquid chromatography–atmospheric pressure chemical ionization mass spectrometry☆

C. Blasco, Y. Picó*, J. Mañes, G. Font

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

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**Abstract**

A liquid chromatography (LC) method for the quantitative determination of five fungicide residues (dichloran, flutriafol, *o*-phenylphenol, prochloraz and tolclofos methyl) in oranges, lemons, bananas, peppers, chards and onions is described. The residues were extracted by matrix solid-phase dispersion (MSPD) using $C_8$. Quantitative analysis was performed by isocratic LC coupled to quadrupole mass spectrometer using atmospheric pressure chemical ionization in the negative ionization mode. The limit of quantification was 0.01 mg $kg^{-1}$ for flutriafol, *o*-phenylphenol and dichloran, and 0.1 mg $kg^{-1}$ for prochloraz and tolclofos methyl. The MSPD method is also suitable for LC–UV analysis but higher limits of quantification (between 1 and 5 mg $kg^{-1}$) were obtained. Validation of the method was performed between 0.01 and 25 mg $kg^{-1}$. Recoveries for fungicides ranged from 52.5 to 91.1% with relative standard deviations between 6.1 and 11.9%. The method was applied to the determination of residues in samples taken from agricultural cooperatives. The fungicides most often detected were *o*-phenylphenol and prochloraz. 

*Keywords:* Fruits; Vegetables; Food analysis; Pesticides

## 1. Introduction

A great portion of the pesticide residues found in fruits and vegetables are fungicides [1]. Dichloran (nitro derivative), flutriafol (triazole), *o*-phenylphenol (biphenyl), prochloraz (imidazole) and tolclofos methyl (thiophosphate) are a variety of the chemical structures commercially available as fungicides for different crops.

Several analytical methods for determining these pesticides in fruits and vegetables have been reported. Most of them were developed to analyze dichloran, *o*-phenylphenol, prochloraz or tolclofos methyl separately, and to our knowledge no analytical methods for flutriafol or for the simultaneous determination of the five compounds in crops have been reported in the literature. These methods are based on conventional schemes that generally involve liquid–liquid extraction (LLE) [2–7] or supercritical fluid extraction (SFE) [8,9] followed by gas

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*Corresponding author. Tel.: +34-96-3864-958; fax: +34-96-3864-954.

*E-mail address:* pico@uv.es (Y. Picó).

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chromatography (GC) with electron-capture detection (ECD) [2,7] or mass spectrometry (MS) [3–5] or liquid chromatography (LC) with fluorescence detection [6].

However, some state of the art procedures based on solid-phase extraction (SPE), solid-phase microextraction (SPME) and LC–MS have been applied to determine some of these compounds in water. SPE followed by LC–MS [10,11] as well as SPME with GC–ECD [12] have been proposed for monitoring pesticides, including prochloraz. Even flutriafol, which is the less frequently determined pesticide, was extracted by SPE and analyzed by laser desorption Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR–MS) [13] or by LC coupled to diode array detection [14].

LC–MS has been widely accepted as the preferred technique for the identification and quantification of polar and thermally labile compounds in pesticide residue determination. One of the most versatile LC–MS interfaces is atmospheric pressure chemical ionization (APCI). In the last few years, APCI has been applied to the analysis of a large variety of compounds because it is very effective in analyzing medium- and low-polarity molecules [15]. The suitability of LC–APCI-MS for pesticide residue determination has been demonstrated by analyzing compounds such as carbamates [16,17], benzoylureas [18], and insecticides [19] in fruits and vegetables.

Extraction procedure methods need to be simplified not only to shorten the working times but also to increase controls. The matrix solid-phase dispersion (MSPD) process requires simple devices and permits the miniaturization of the extraction step. It has also proved to be suitable for the isolation of several classes of compounds. Application of MSPD in fruits and vegetables can greatly decrease analysis time, increase sample throughput and reduce the use of solvent volumes [20].

The purpose of this work was to develop an MSPD method for microextraction of dichloran, flutriafol, *o*-phenylphenol, prochloraz and tolclofos methyl in oranges, lemons, bananas, peppers, chards and onions followed by LC–APCI-MS using the negative ionization (NI) mode. The method is also suitable for rapid screening of residues using UV detection with a compromise in sensitivity.

## 2. Experimental

### *2.1. Chemical*

Fungicides (dichloran, flutriafol, *o*-phenylphenol, prochloraz and tolclofos methyl) were supplied by Riedel-de Haën (Seelze, Germany). Physical, chemical and toxicological properties of the studied pesticides are shown in Table 1. Individual stock solutions were prepared by dissolving 100 mg of each compound in 100 ml of methanol and stored in glass-stopper bottles at 4 °C. Standard working solutions at various concentrations were prepared daily by appropriate dilution of aliquots of the stock solution in methanol.

HPLC-grade methanol and dichloromethane were purchased from Merck (Darmstadt, Germany). Deionized water (>18 MΩ cm resistivity) was obtained from the Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA). All the solvents were passed through a 0.45 μm cellulose filter from Scharlau (Barcelona, Spain) before use.

The silica-based sorbent with octyl functional groups (particle diameter in the range of 45–55 μm) was acquired from Análisis Vínicos (Tomelloso, Spain).

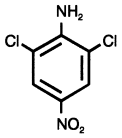
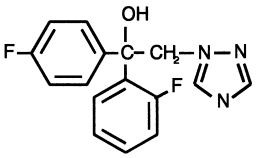
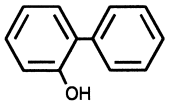
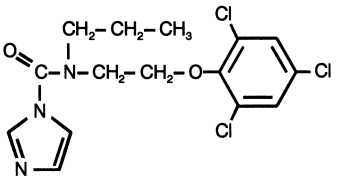
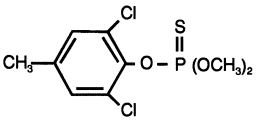
### *2.2. Liquid chromatography with UV detection*

A Merck–Hitachi LC system equipped with an L-7100 pump, a Rheodyne Model 7125 injector (20 μl loop), an L-4250 UV–Vis detector and Millennium software was used. The UV–Vis detector was operated at 210 nm.

### *2.3. Liquid chromatography with mass spectrometry*

A Hewlett-Packard (Palo Alto, CA, USA) HP-1100 Series LC–MS system equipped with a binary solvent pump, an autosampler, and a mass-selective detector coupled with an analytical work station was employed. The mass-selective detector consisted of a standard atmospheric pressure ionization (API) source configured as APCI. Typical operating conditions of the APCI interface in the negative mode were: vaporizer temperature, 450 °C; nebulizer gas,

Table 1
Physical, chemical and toxicological properties of the pesticides studied

| Common name | Chemical structure | Chemical name of IUPAC | $S_w$[a] (g $l^{-1}$) at 20 or 25 °C | $M_r$[b] (g $mol^{-1}$) | Chemical group | ADI[c] (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|
| Dichloran |  | 2,6-Dichloro-4-nitroaniline | $6.3 \cdot 10^{-3}$ in water<br>40 in acetone<br>19 in ethyl acetate<br>2 in ethanol | 207.06 | Nitro derivative | 0.03 |
| Flutriafol |  | (*RS*)-2,4′-Difluoro-α (1H-1,2,4-triazol-1-ylmethyl)-benzhydryl alcohol | $13 \cdot 10^{-2}$ in water<br>190 in acetone<br>150 in dichloromethane<br>69 in methanol | 3012 | Triazole | 0.002 |
| *o*-Phenylphenol |  | Biphenyl-2-ol | 0.7 in water<br>soluble in most organic solvents | 170.2 | Biphenyl | 0.02 |
| Prochloraz |  | 1-*N*-Propyl-*N*-[2-(2,4,6-trichlorophenoxy)ethyl] carbamoylimidazole | $55 \cdot 10^{-3}$ in water<br>3500 in acetone | 376.5 | Imidazole | 0.01 |
| Tolclofos methyl |  | *O*-(2,6-Dichloro-*p*-tolyl *O*,*O*-dimethyl phosphorothioate | $11 \cdot 10^{-3}$ in water<br>389 in acetone<br>389 in ethyl acetate<br>59 in methanol | 301.1 | Thiophosphate | 0.15 |

[a] $S_w$: Solubility in water.
[b] $M_r$: Molecular mass.
[c] ADI: Acceptable daily intake.

nitrogen at a pressure of 60 p.s.i. (1 p.s.i.=6894.76 Pa); drying gas, also nitrogen, at a flow-rate of 4 l $min^{-1}$ and temperature of 350 °C; capillary voltage, 4000 V; and corona current 25 μA.

Full-scan LC–MS chromatograms were obtained by scanning from $m/z$ 100 to 400. Time-scheduled selected-ion monitoring (SIM) of the most abundant ion of each compound was used for quantification.

### 2.4. Chromatographic conditions

Chromatographic conditions were the same for both set-ups. The analytical column, a $C_{18}$ (250×4.6 mm I.D., 5 μm) and a Securityguard cartridge $C_{18}$ (4×2 mm I.D.) were both from Phenomenex. The isocratic mobile phase was methanol–water (85:15) with a flow-rate of 0.6 ml $min^{-1}$.

### 2.5. Sample preparation

The samples analyzed, bananas, chards, onions, oranges, lemons and peppers, were obtained from agricultural cooperatives. All samples were taken in accordance with the guidelines of the European Union (EU) Directive 79/700/CEE [21]; that is, as far as possible, the sample was taken at various places distributed throughout the lot (size ca. 50 kg). The sample weigh at least 1 kg and consisted of at least 10 individual fruits or vegetables.

A representative portion of the sample (200 g of whole fruit or vegetable) was chopped and homogenized in a Bapitaurus food chopper (Taurus, Berlin, Germany). Portions of 0.5 g were weighed and placed in a mortar.

For the preparation of fortified samples, volumes between 30 and 50 μl of the standard working solutions were added to 0.5 g of sample. Then, they were allowed to stand at room temperature for 3 h. The samples were spiked with pesticides at concentration levels between 0.01 and 25 mg $kg^{-1}$.

A sample of 0.5 g placed into a glass mortar (50 ml capacity) was gently blended with 0.5 g of the adsorbent ($C_8$) for 5 min using a pestle, to obtain a homogeneous mixture. This mixture was introduced into a 100×9 mm I.D. glass column and conditioned with 0.2 ml of distilled water; then, 10 ml of dichloromethane was added to the column and the sample was allowed to elute dropwise by applying a slight vacuum. The eluent, which does not contain water, was collected in a graduated conical tube (15 ml). A 1-ml volume of methanol was added to the eluent to avoid evaporate to dryness and then, it was concentrated, under a stream of nitrogen, to 0.5 ml. A 5-μl volume of the final extract was injected into the LC–MS system.

## 3. Results and discussion

### 3.1. General remarks on mass spectrometry

Preliminary evaluations were carried out using a 10 μg $ml^{-1}$ solution of each pesticide in the flow injection analysis (FIA) mode, and data were acquired in the scan mode (ca. $m/z$ 100–400). The fragmentor voltage was adjusted to obtain the best compromise between enough fragmentation for identification purposes and sensitivity.

The fragmentor voltage was varied between 10 and 130 V. For the compounds studied the fragmentor has more influence on sensitivity than on the fragmentation pattern. Fig. 1 shows the mass spectra of the selected fungicides at 70 V fragmentor voltage, which was selected for the analytical procedure. The only ion obtained for dichloran and *o*-phenylphenol at any fragmentor voltage was the deprotonated molecule $[M-H]^-$ (see Fig. 1). Flutriafol gave a molecular ion $[M-H]^-$ and a fragment at $m/z$ 204 that can be interpreted as $[M-C_6H_6F-H]^-$. The deprotonated molecule disappeared at fragmentor voltages higher than 90 V. The prochloraz spectrum presents two predominant fragments at $m/z$ 356 and 196 that can be elucidated as $[M-Cl+O]^-$ and $[C_6H_2Cl_3O]^-$, respectively. The molecular ion $[M-H]^-$ was also observed with low intensity. Tolclofos methyl produced the main fragments at $m/z$ 285 and 219 corresponding to $[M-CH_3]^-$ and $[M-Cl+O-(OCH_3)_2]^-$, respectively. Moreover, characteristic fragment ions at $m/z$ 255 and 175 were observed with low abundance.

### 3.2. Fruits and vegetables analysis

For fast LC–MS analysis, isocratic elution was found satisfactory. Interferences from the eluting matrix components from previous sample injection in the APCI ionization process were not noted, probably because the high percentage of organic solvent used in the mobile phase removed all matrix components from the system.

The matrix components can provide variations in the MS response of fungicides. This phenomenon was studied comparing the calibration graphs obtained for each compound in a standard solution with those obtained in a spiked blank extract. Calibration graphs for the SIM mode were plotted using six points (0.01, 0.05, 0.1, 1, 5 and 10 μg $ml^{-1}$). The presence of matrix leads to signal enhancement (ranging from 0 to 15%, depending on the matrix and the compound). This led to inaccurate quantification of the fungicide concentration in some samples. No correlation between matrix type and enhancement was found. Hence, for consistency and accurate

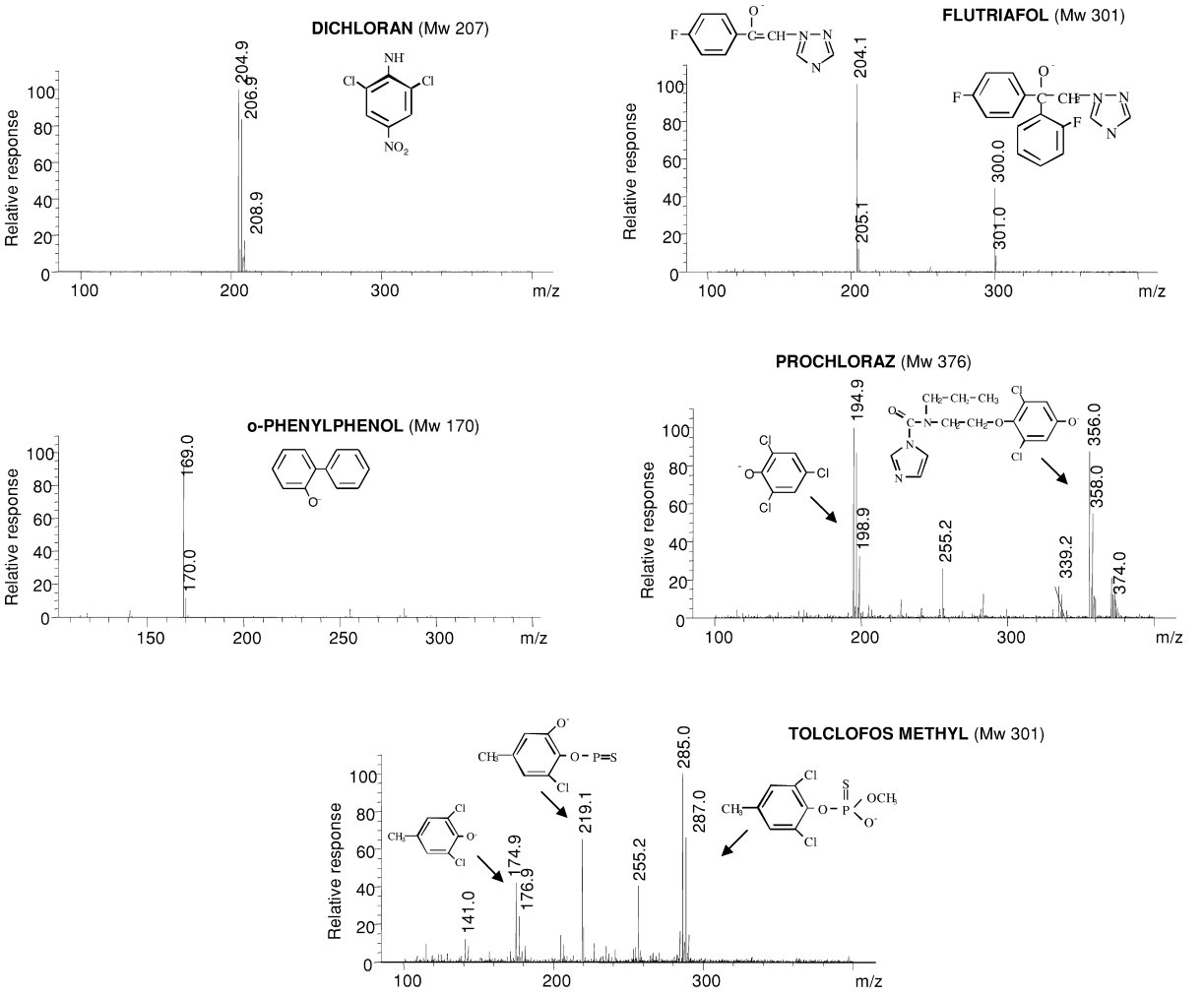



Fig. 1. Full scan mass spectra of dichloran, flutriafol, *o*-phenylphenol, prochloraz and tolclofos methyl, obtained by LC–APCI-MS using NI mode (fragmentor voltage, 70 V).

quantification, matrix matched standards were used in all analyses.

Untreated control and fortified orange samples in the range of 0.5 to 25 mg $kg^{-1}$ were analyzed by LC–UV and LC–MS. Similar recoveries were obtained for both detection techniques and summaries of the resultant data are shown in Table 2. Examples of representative chromatograms for LC–MS and LC–UV are given in Figs. 2 and 3, respectively. The LC–MS chromatograms clearly demonstrate a better sensitivity and selectivity compared to those obtained using LC–UV.

Under the experimental conditions using LC–MS, the limits of quantitation (LOQs) ranged from 0.01 to 0.1 mg $kg^{-1}$. These values correspond to the lowest concentration of a compound that gives a response that could be quantified with an inter-assay relative standard deviation (RSD) of less than 26%. Data for the six matrices spiked at the LOQ levels are shown in Table 3.

The recoveries were between 40 and 99% and RSDs between 6.3 and 24%. They seem to be higher in fruits (banana, lemon and orange) than in vegetables (chard, onion and pepper). The reason may be differences in the composition of the crops studied. The main differences were found in the carbohy-

Table 2
Recoveries and LOQs by LC–MS and LC–UV in oranges

| Pesticide | LC–MS | | | | LC–UV | | | |
|---|---|---|---|---|---|---|---|---|
| | Average recovery (%) | RSD (%) | Range (%) | LOQ (mg $kg^{-1}$) | Average recovery (%) | RSD (%) | Range (%) | LOQ (mg $kg^{-1}$) |
| Dichloran | 73.3 | 11.9 | 69–79 | 0.01 | 60.1 | 10.6 | 45–74 | 0.5 |
| Flutriafol | 78.5 | 9.5 | 73–84 | 0.01 | 87.7 | 11.2 | 75–115 | 0.5 |
| *o*-Phenylphenol | 70.3 | 6.7 | 62–79 | 0.01 | 58.1 | 10.5 | 50–64 | 0.5 |
| Prochloraz | 90.1 | 6.1 | 80–101 | 0.1 | 67.7 | 7.7 | 55–79 | 0.5 |
| Tolclofos methyl | 65.5 | 9.5 | 53–75 | 0.1 | 52.5 | 6.6 | 43–59 | 0.5 |

Table 3
Recoveries at LOQ levels by LC–MS in the different matrices

| Matrix | Dichloran | | Flutriafol | | *o*-Phenylphenol | | Prochloraz | | Tolclofos methyl | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Recovery, % (RSD, %) | MRL (mg $kg^{-1}$) | Recovery, % (RSD, %) | MRL (mg $kg^{-1}$) | Recovery, % (RSD, %) | MRL (mg $kg^{-1}$) | Recovery, % (RSD, %) | MRL (mg $kg^{-1}$) | Recovery, % (RSD, %) | MRL (mg $kg^{-1}$) |
| Banana | 85 (11.6) | 0.01 | 76 (8.9) | 0.01 | 88 (12.9) | 0.1 | 55 (15.5) | 0.05 | 99 (11.2) | 0.01 |
| Chard | 41 (6.3) | 0.01 | 40 (12.3) | 0.01 | 51 (15.7) | 0.1 | 40 (12.8) | 0.05 | 86 (23.9) | 0.01 |
| Lemons | 70 (20.1) | 0.5 | 46 (17) | 0.01 | 52 (12.7) | 12 | 41 (8.5) | 5 | 89 (11.6) | 0.01 |
| Onions | 56 (13.8) | 0.01 | 51 (21) | 0.01 | 73 (13.7) | 0.1 | 42 (9.9) | 0.05 | 65 (16.5) | 0.01 |
| Oranges | 73 (11.3) | 0.5 | 79 (9.5) | 0.01 | 79 (16.7) | 12 | 90 (16.0) | 5 | 66 (14.5) | 0.01 |
| Pepper | 43 (15.2) | 5 | 47 (19.6) | 0.01 | 58 (17.5) | 0.1 | 48 (10.5) | 0.05 | 55 (8.5) | 0.01 |

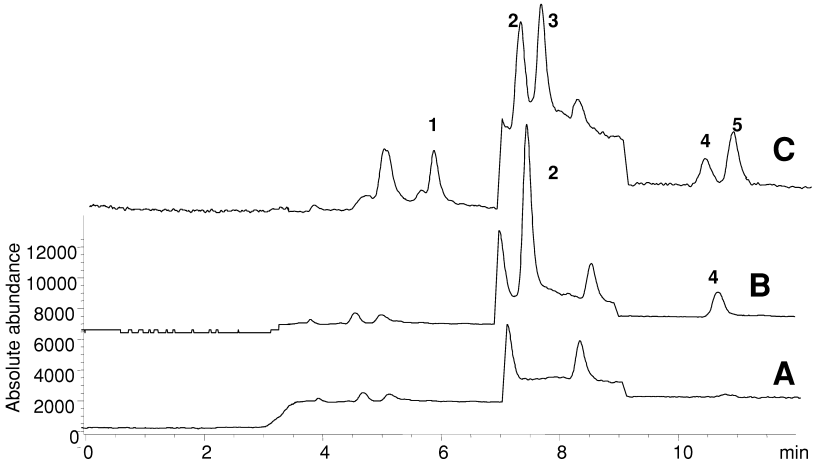



Fig. 2. LC–MS chromatogram of (A) untreated control orange, (B) post-harvest treated orange No. 3, and (C) fortified control (0.05 μg $ml^{-1}$ of flutriafol, *o*-phenylphenol and dichloran, and 0.5 μg $ml^{-1}$ of prochloraz and tolclofos methyl). Peak identification: 1=flutriafol, 2=*o*-phenylphenol, 3=dichloran, 4=prochloraz, 5=tolclofos methyl.

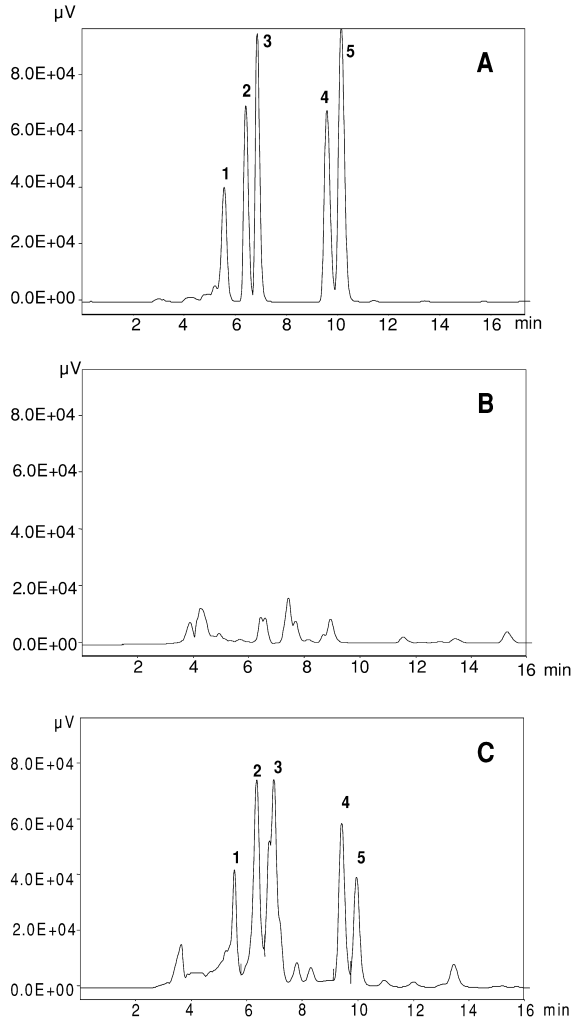



Fig. 3. LC–UV chromatogram at 210 nm of (A) standard (10 mg $ml^{-1}$ of each compound), (B) untreated orange and (C) fortified orange at 10 mg $kg^{-1}$ of each compound. Peak assignment as in Fig. 2.

drates and water content, which were, respectively, 9–12% and 86–89% for fruits and 1–4% and 94–95% for vegetables. However, these differences in the extraction efficiency are not statistically significant, probably because the variability of the same fruit or vegetable composition is great and, at the same time, the differences between them are not sufficient to have a significant incidence.

The maximum residue limits (MRLs) established by regulatory authorities are also included in Table 3. The sensitivity of the method was good enough to ensure a reliable determination, except for tolclofos methyl.

### *3.3. Application to real samples*

These fungicides are widely applied, post-harvest, on fruits and vegetables to extend their shelf lives and preserve quality during storage, transport, and marketing. To verify the procedure, 18 samples (three of each fruit or vegetable) taken from an agricultural cooperative were analyzed. Typical chromatograms for banana, chard, lemon, onion and pepper samples containing flutriafol, *o*-phenylphenol, dichloran or prochloraz are presented in Fig. 4.

Table 4 shows the contents of positive samples (15 in all). The five studied fungicides were detected at concentrations ranging from 0.01 to 2.16 mg $kg^{-1}$. Three samples (banana, onion and orange) have over-tolerance residues of procloraz, *o*-phenylphenol and tolclofos methyl. Four samples contained two fungicides and one contained three different fungicides.

*o*-Phenylphenol is the most commonly occurring pesticide (found in 11 samples) followed by prochloraz (six samples) and dichloran (two samples). Flutriafol and tolclofos methyl were found only in one sample. In the case of tolclofos methyl, the reason may be that the quantification limit obtained by this method is too high.

## 4. Conclusions

The present method, which involves an appropriate MSPD extraction and LC–MS, requires only small sample sizes and solvent volumes and provides satisfactory recoveries, repeatability and reproducibility. The extraction method is also suitable for monitoring purposes using less sophisticated instrumentation such as LC–UV. However, the use of LC–APCI-MS for pesticide residues determination

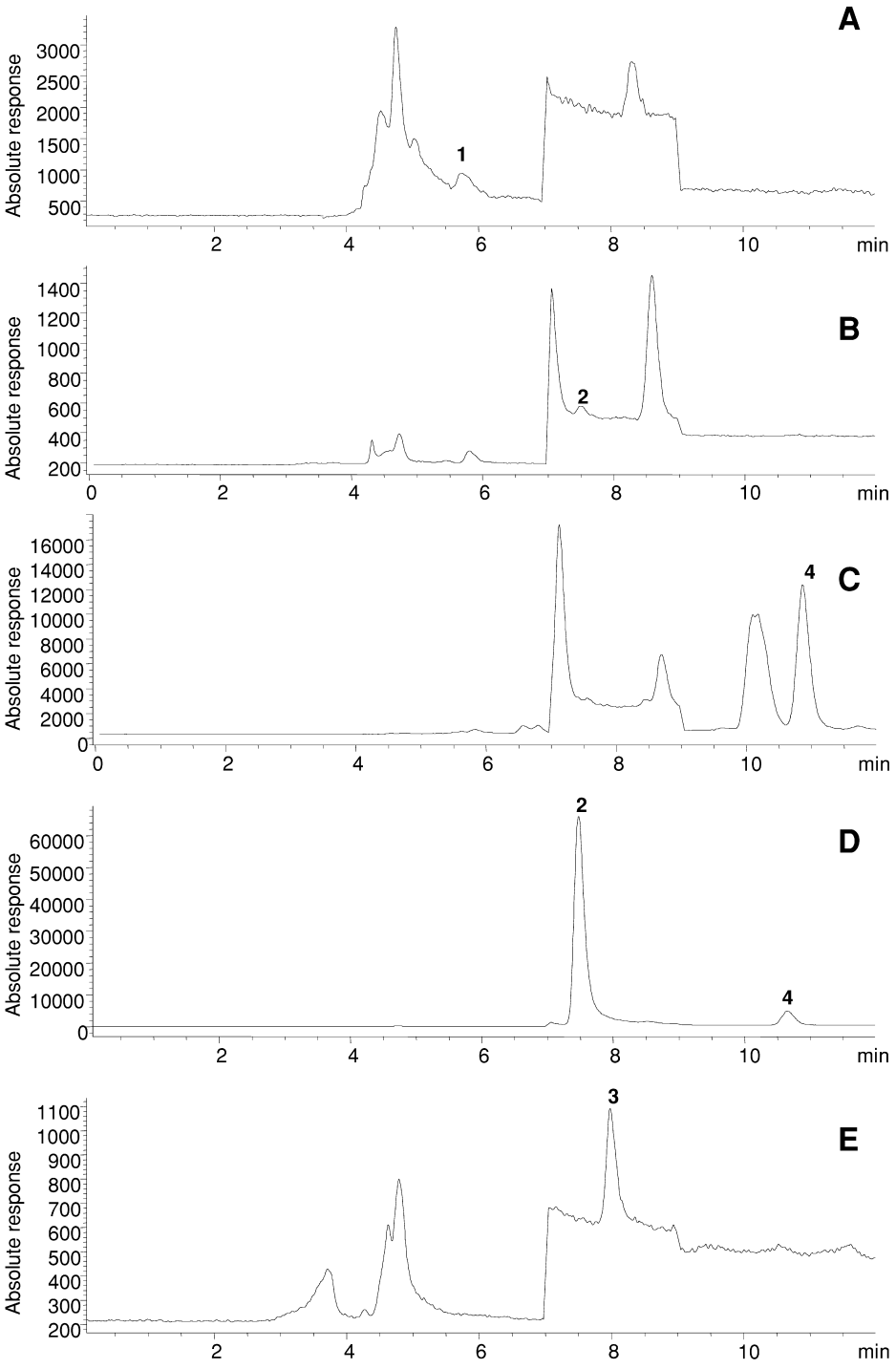



Fig. 4. LC–MS chromatogram of (A) banana No. 1, (B) chard No. 1, (C) lemon No. 2, (D) onion No. 3; and (E) pepper No. 1. Peak assignment as in Fig. 2.

Table 4
Pesticide concentrations in fruits and vegetables taken from Valencian agricultural cooperatives

| Matrix | Sample | Pesticides | Concentration, mg $kg^{-1}$ (RSD, %) |
|---|---|---|---|
| Banana | 1 | Flutriafol | 0.01 (15) |
| | 2 | *o*-Phenylphenol | 0.01 (18) |
| | 3 | *o*-Phenylphenol | 0.06 (12) |
| | | Prochloraz | 0.1 (9) |
| Chard | 1 | *o*-Phenylphenol | 0.01 (19) |
| Lemon | 1 | *o*-Phenylphenol | 0.11 (10) |
| | 2 | Prochloraz | 1.08 (7) |
| | 3 | Dichloran | 0.01 (21) |
| Onion | 1 | *o*-Phenylphenol | 0.01 (17) |
| | 2 | *o*-Phenylphenol | 0.02 (24) |
| | 3 | *o*-Phenylphenol | 1.92 (9) |
| | | Prochloraz | 1.48 (8) |
| Orange | 1 | *o*-Phenylphenol | 1.34 (12) |
| | | Prochloraz | 1.14 (11) |
| | 2 | *o*-Phenylphenol | 2.16 (9) |
| | | Prochloraz | 1.75 (7) |
| | | Tolclofos methyl | 1.44 (13) |
| | 3 | *o*-Phenylphenol | 0.19 (16) |
| | | Prochloraz | 0.19 (17) |
| Pepper | 1 | *o*-Phenylphenol | 0.01 (19) |
| | 3 | Dichloran | 0.01 (20) |

in real matrices eliminate the need for additional confirmatory procedures.

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