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# Liquid chromatographic–mass spectrometric determination of post-harvest fungicides in citrus fruits

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**Abstract**

Liquid chromatography (LC)–atmospheric pressure ionisation (API)–mass spectrometry (MS) has been used to determine residues of five fungicides in oranges with a minimum sample cleanup. Atmospheric pressure chemical ionisation (APCI) and electrospray (ES) were compared and both gave similar results in terms of sensitivity and structural information. The main ions were $[M+H]^+$ for carbendazim, imazalil, thiophanate methyl and thiabendazole, and $[M+H-C_4H_9NHCO]^+$ for benomyl. Samples were extracted with sodium sulphate and ethyl acetate. Although benomyl and thiophanate methyl were transformed through the extraction procedure to carbendazim, the method showed good precision (<13%) and recovery (>70%), except for thiophanate methyl (50%), whilst also yielding limits of detection (<0.03 mg $kg^{-1}$) that are adequate for the determination of the studied fungicides in oranges. 

*Keywords:* Citrus fruits; Pesticides

## 1. Introduction

Imazalil, thiabendazole, benomyl, thiophanate methyl and carbendazim are five fungicides widely used to control postharvest decay caused by various fungal pathogens in citrus fruit [1]. Because of possible toxic effects, widespread use and insufficient data, monitoring of their residues has become priority in pesticide control and health care [2,3]. The main drawback is that different sample preparation and determination methods are usually required to analyze these compounds, and as a result, a great deal of time and money are consumed in their analysis. However, all of them are basic compounds, with one or more nitrogen atoms in their molecules and slightly soluble in water. Then, it seems possible to develop a common procedure to analyse them simultaneously [4,5].

The first HPLC–MS method reported to determine one of these compounds in fruit and vegetables was developed for benomyl analysis in apples, peaches, and tomatoes using thermospray (TSP) ionization [6]. Residues of benomyl were determined after its quantitative conversion to carbendazim. The presence of carbendazim in the extract of samples, obtained from treated beehives, was also confirmed by HPLC–particle beam (PB)–MS in SIM mode [7].

Recently the HPLC–MS ionization techniques of atmospheric pressure ionization (API) have shown in

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several reports to be viable for the determination of various classes of pesticides in fruit and vegetables [8–14]. API includes a group of interfaces, the most common of which are atmospheric pressure chemical ionization (APCI) and electrospray (ES) ionization. In ESI, the nebulization and ionization of the mobile phase is effected by an applied electrical field, whereas in APCI, the ionization is performed by a combination of heated capillaries and a corona discharge. They are highly sensitive, show greater ionization stability and are more universally applicable than other HPLC–MS techniques. Barnes et al. [8] evaluated APCI and ES for the analysis of ten selected pesticides (including carbendazim and thiabendazole). Both interfaces were comparable for most compounds although APCI was the more sensitive technique for some ones. This fact, and the greater flexibility regarding liquid chromatography (LC) flow-rates associated with this interface, led to choice of APCI as the technique on which to base the multiresidue methods development. However, Lacassie et al. [9] reported a method for the analysis of eight pesticides (including carbendazim, thiabendazole and thiophanate methyl) involving HPLC–ES–MS with enough sensitivity to ensure a reliable determination at levels lower than the respective maximum residue levels (MRLs). In addition to these limited and little decisive data, no result on imazalil behavior in LC–MS or the possibility to determine the intact benomyl molecule has been reported until now.

The present study compares APCI and ES interfaces as regards to their applicability to the determination of fungicides in citrus fruits. The next aim is to elaborate a fast, selective and simple method for the simultaneous analysis of these five post-harvest fungicides as intact molecules in oranges, which can be applied for the identification and quantitation of these compounds in long term monitoring programs.

## 2. Experimental

### 2.1. Chemicals

The fungicides (benomyl, carbendazim, imazalil, methyl thiophanate and thiabendazole) were supplied by Aldrich (Madrid, Spain) with a minimum certified purity of at least 98%. Table 1 shows the chemical characteristics of the selected compounds. The oranges were purchased from an agricultural cooperative sited in the surroundings of Valencia City.

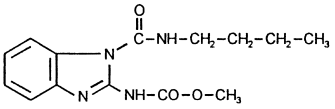
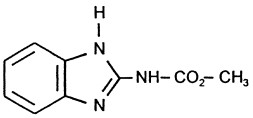
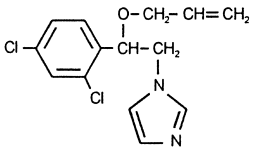
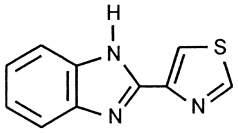
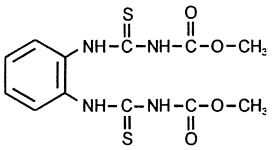
HPLC-grade methanol and acetonitrile and organic trace analysis-grade ethyl acetate were purchased from Merck (Darmstadt, Germany). Deionized water of 18 MΩ cm resistivity was obtained from a Milli-Q water purification system. Ammonium formate 97% was obtained from Aldrich. All the solvents and solutions were passed through a 0.45-μm cellulose filter from Scharlau (Barcelona, Spain) before use. Granular AR anhydrous sodium sulphate was obtained from Panreac (Barcelona, Spain) and was heated at 400 °C for 10 h prior to use.

### 2.2. Preparation of standard and spiked samples

The standard solutions of thiabendazole, thiophanate methyl and imazalil were prepared in methanol at a concentration of 1000 μg $ml^{-1}$ and those of benomyl and carbendazim in 10 *M* HCl at 1000 μg $ml^{-1}$. They were stored in glass-stopper bottles at 4°C. Working solutions of pertinent concentrations (0.01–25 μg $ml^{-1}$) were made daily by appropriate combination and dilution of the standard solutions with 2 m*M* ammonium formate–methanol (50:50 v/v). Using extracts (obtained as described below) of orange samples that had only been post-harvest treated with waxes (without fungicide addition in any step), a set of matrix-matched standards was prepared.

The recovery test was carried out by adding 250 μl of each individual standard solution to 50 g of chopped and homogenized orange samples in blender jar. The concentrations of pesticide in the standard solutions were 10, 100 and 1000 μg $ml^{-1}$, so the oranges were spiked at 0.05, 0.5 and 5 mg $kg^{-1}$, respectively. The spiked sample was allowed to stand for a 15 min before extraction to allow the pesticide distribution in the orange. The recovery was performed for each pesticide individually to avoid overestimation of the carbendazim residues. Each recovery test was repeated five times. Untreated control samples were analyzed with each fortification level.

Table 1
Chemical characteristics of the studied compounds

| Chemical structure | Name | Molecular mass | Elemental composition |
|---|---|---|---|
|  | Benomyl | 290 | $C_{14}H_{18}N_4O_3$ |
|  | Carbendazim | 191 | $C_9H_9N_3O_2$ |
|  | Imazalil | 297 | $C_{14}H_{14}CI_2N_2O$ |
|  | Thiabendazole | 201 | $C_{10}H_7N_3S$ |
|  | Thiophanate methyl | 342 | $C_{12}H_{14}N_4O_4S_2$ |

### 2.3. Extraction procedure

Fifty grams of chopped orange samples were weighed in a 200-ml glass beaker and were homogenized with 100 ml of ethyl acetate and 60 g of anhydrous sodium sulphate using a Waring blender for 3 min. The homogenate was filtered through 50 g of sodium sulphate into a 500-ml rotary-evaporation flask. The glass beaker was rinsed with ethyl acetate (approximately 200 ml) and the rinsings used to wash the filter cake remaining on the sodium sulfate.

The extract was evaporated in a rotary evaporator to a volume of less than 2 ml. After addition of acetonitrile (10 ml) the extract was evaporated near dryness and then was dissolved in a mixture acetonitrile: 2 m*M* ammonium formate (50:50, v/v) to a final volume of 10 ml. This solution was filtered through an acrodisk (0.2 μm of cellulose) and 5 μl were injected into the LC–MS system.

### 2.4. Instrument and conditions

A Hewlett-Packard (Palo Alto, CA, USA) HP-1100 Series LC/MSD system equipped with a binary solvent pump, an autosampler, and a MSD coupled with an analytical workstation was used. The MSD consisted of a standard API source that can be configured as APCI or ES.

A Zorbax Rapid Resolution SB-$C_{18}$ (30×4.6 mm I.D., 3.5 μm) column and a guard column LiChrosorb RP-8 (10×4.6 mm, 5 μm) were both from

Supelco (Madrid, Spain). The solvents used in the mobile phase were methanol and a solution of 2 m*M* ammonium formate in water. The gradient for LC–APCI–MS at the flow-rate of 1 ml $min^{-1}$ was methanol 30%, increased linearly to 50% in 4 min and then increased to 80% in 11 min, and held at 80% for 7 min. A return to the initial conditions was carried out in 3 min. Typical operating conditions of the APCI interface in positive mode were vaporizing temperature=325°C; nebulizer gas=nitrogen at a pressure of 4.0 bar; 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=4 μA.

The analytical separation for the LC–ES–MS was performed using methanol 50%, isocratic for 15 min, then increased to 70% in 5 min, held for 5 min, then increased to 90% in 5 min, and held at 90% for 5 min. The flow-rate of the mobile phase was 0.5 ml $min^{-1}$. The ES–MS interface in positive mode was operated at 350°C gas temperature, 13.0 l $min^{-1}$ drying gas flow, 30 p.s.i. nebulizer gas pressure and 4000 V of capillary voltage.

Full-scan LC–MS chromatograms were obtained by scanning from $m/z$ 100 to 350. Time-scheduled SIM of the most abundant ion of each compound was used for quantification.

## 3. Results and discussion

The LC separation was carried out using a Zorbax rapid resolution column and gradient elution of methanol and ammonium formate that allowed us to obtain the highest separation efficiency in short columns. Fig. 1 shows the scan chromatogram of a standard solution (10 mg $l^{-1}$, 5 μl injected) using APCI. The proposed conditions generated narrow and reproducible peaks, the volatility of the ion pair reagent allows coupling LC–MS, and the separation of the five fungicides was accomplished within 12 min.

### 3.1. Mass spectrometry optimization

The early LC–MS experiments to select the optimum MS parameters and the appropriate ions were carried out by flow injection analysis (FIA) of the individual solutions of the fungicides. Fragmentor voltage affects both sensitivity and fragmentation and resulted in being compound class specific. It must be optimized for each compound. The fragmentor voltage was varied from 10 to 200 V. Table 2 list the relative abundances of protonated molecules and fragment ions observed with the fragmentor at 20, 70 and 140 V. The highest responses were obtained at 70 V for all the compounds using both interfaces, so, this value was chosen for identification and quantitation purposes. Using this fragmentor, ES and APCI sources provided mass spectra where $[M+H]^+$ was the most abundant ion for all the compounds, except benomyl, for which the most abundant ion was the fragment $[M+H-C_4H_9NHCO]^+$.

Benomyl was the compound that presented the most differences in the mass spectra obtained by both interfaces. Its molecular mass is 290, however,

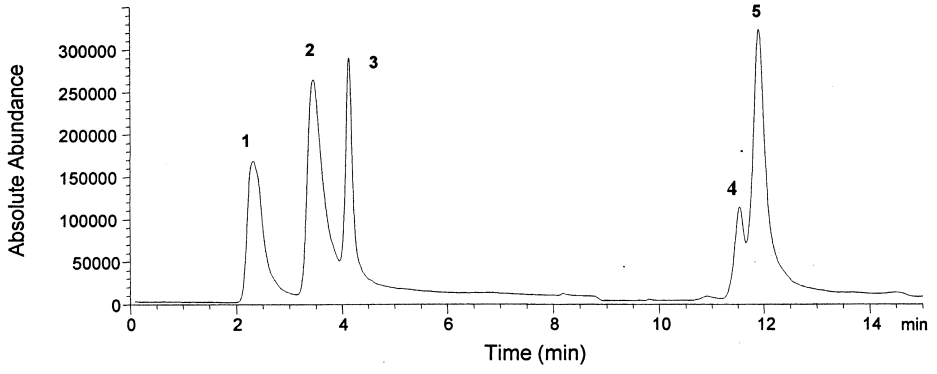



Fig. 1. Scan mode LC–APCI–MS chromatogram of a standard solution (10 mg $l^{-1}$) using Zorbax SB-$C_{18}$ (3 cm×4.6 mm I.D., 3.5 μm). Peak identification: (1) Carbendazim, (2) thiabendazole, (3) thiophanate methyl, (4) benomyl and (5) imazalil.

Table 2
Important mass spectra fragments and their relative abundances (*R*%) obtained by FIA–APCI–MS at different fragmentor voltages

| Compound | *M*/*z*, tentative ion | APCI (V) | | | ES (V) | | |
|---|---|---|---|---|---|---|---|
| | | 20 | 70 | 140 | 20 | 70 | 140 |
| Benomyl | 291 $[M+H]^+$ | | | | 100 | 30 | |
| 290 | 192 $[M+H-C_4H_9NHCO]^+$ | 100 | 100 | 5 | 15 | 100 | |
| | 160 $[M+H-C_4H_9NHCO-CH_3O]^+$ | | 5 | 100 | | 10 | 100 |
| | 134 $[M+H-C_4H_9NHCO-CH_3OCO]^+$ | 15 | 10 | 25 | | | 10 |
| Carbendazim | 192 $[M+H]^+$ | 100 | 100 | | 100 | 100 | 100 |
| 191 | 160 $[M-CH_3O]^+$ | | 10 | 100 | 8 | 8 | 30 |
| | 134 $[M+H-CH_3OCO]^+$ | | | 25 | | | |
| Imazalil | 297 $[M+H]^+$ | 100 | 100 | 100 | 100 | 100 | 100 |
| 296 | | | | | | | |
| Thiabendazole | 202 $[M+H]^+$ | 100 | 100 | 100 | 100 | 100 | 100 |
| 201 | 175 $[M-CN]^+$ | | | 20 | | | 40 |
| Thiophanate methyl | 343 $[M+H]^+$ | 100 | 100 | | 100 | 100 | |
| 342 | 311 $[M-CH_3O]^+$ | | 5 | 20 | 8 | 12 | |
| | 268 $[M+H-CH_3NHCOO]^+$ | | 5 | 10 | | 5 | |
| | 226 $[M+H-CH_3OCONHCS]^+$ | | 5 | 5 | | 5 | |
| | 192 $[M+H-CH_3OCONHCS_2]^+$ | | | 5 | | 5 | 10 |
| | 151 $[M+H-CH_3-OCONH-CH_3OCONHCS]^+$ | | 5 | 100 | | 5 | 100 |

the APCI mass spectrum of benomyl does not exhibit an $[M+H]^+$ ion at *m*/*z* 291, but has always the base peak at *m*/*z* 192 due to loss of $C_4H_9NHCO$ and its transformation in carbendazim. The ES mass spectrum obtained at 20 V fragmentor shows the molecular ion of benomyl as the base peak, but the sensitivity was half than that achieved at the fragmentor voltage of 70 V. The resulting ES mass spectra of benomyl at increased values of fragmentor voltages was similar to those obtained with APCI as is shown in Fig. 2 (variations in the absolute abundance of the base peak give an idea of the changes in sensitivity).

SIM mode was used to obtain the maximum sensitivity for quantitative analysis. The following mass-to-charge (m/z) values were chosen: m/z 192 (carbendazim and benomyl), m/z 202 (thiabendazole), m/z 343 (thiophanate methyl), and m/z 297 (imazalil). The ion groups 192, 202 and 343 were monitored during the first 9 min of the chromatographic run (dwell time of 192 ms), and then the ion groups 192 and 297 were monitored until the end of the chromatographic run (dwell time 289 ms). The same monitoring ion program was used for APCI and ES.

The interfaces showed approximately the same performance with detection limits of 0.01 μg $ml^{-1}$ for carbendazim, thiophanate methyl, thiabendazole and imazalil (equivalent to 50 pg injected) and 0.1 μg $ml^{-1}$ for benomyl (equivalent to 500 pg injected).

From comparing these results with detection limits in the literature, it can be seen that the values obtained are in agreement with those by Barnes et al. [8]. Nevertheless, these authors concluded that ES–MS is generally less adequate than APCI–MS, its disadvantages being the formation of alkali metal adduct ions and the lower flexibility regarding LC flow-rates. On the contrary, in the present study was observed that, using the ES–MS, adduct ions were virtually absent and the present day interfaces allow us to use high flow-rates (up to 0.5 ml $min^{-1}$), which are compatible with conventional size LC, especially when short analytical columns were used.

### *3.2. Sample extraction procedure*

Benomyl and thiophanate methyl were recovered as carbendazim and not as the parent compounds. It is well known that thiophanate methyl and benomyl

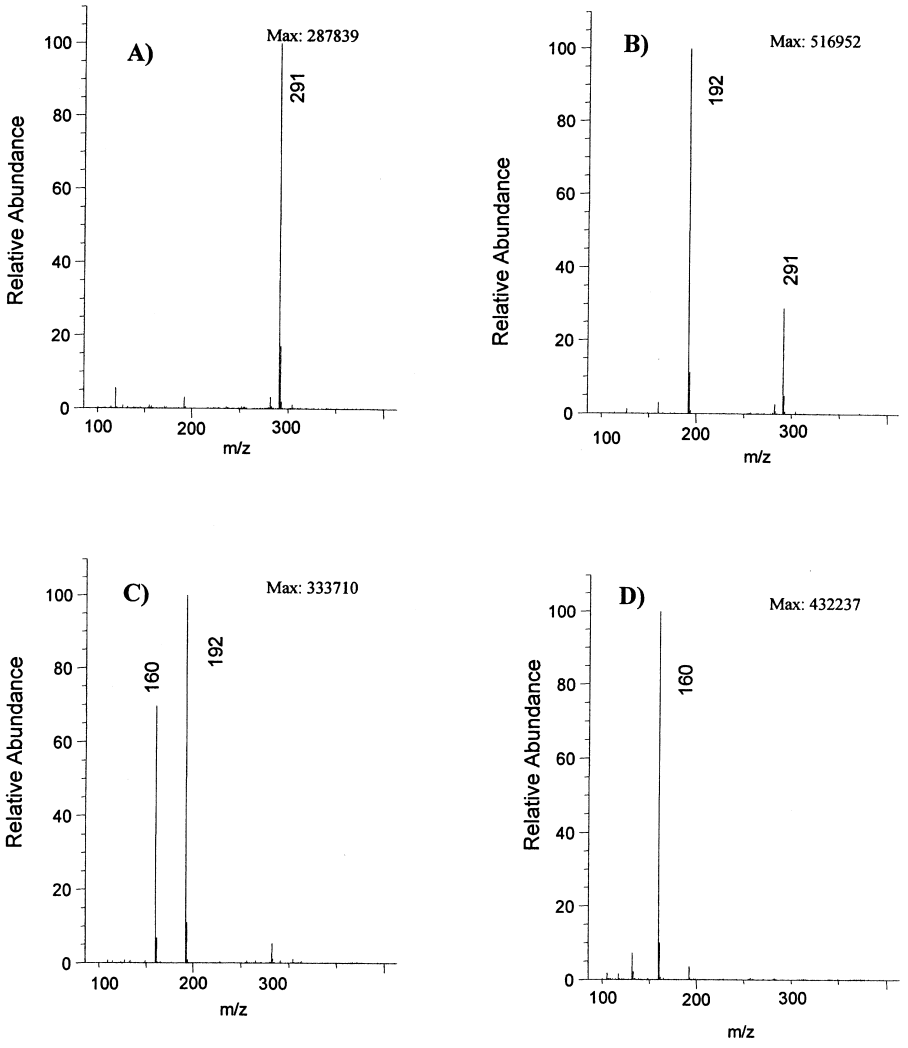



Fig. 2. Variation of the ES–MS spectra vs. the fragmentor voltage for benomyl. (A) 20 V; (B) 70 V; (C) 100 V and (D) 140 V.

can be easily converted to carbendazim; the conversion rate depend on the pH value and the extraction conditions [15,16]. Although, the determination of the intact molecules of thiophanate methyl and benomyl has also been reported by Lacassie et al. [9] and Bernal et al. [7], respectively, both experiences revealed that extraction with organic solvents did not provide good recoveries due likely to a relatively hydrolysis rate to carbendazim in the operation conditions.

Residue extraction was achieved with ethyl acetate and sodium sulphate without further clean up, because of this a matrix interference study was carried out comparing the area obtained for each compound in a standard solution with those obtained in a spiked blank extract at three concentration values: 0.05, 0.5 and 5 mg $kg^{-1}$ for each of the interfaces. For these purpose, calibration graphs of standard solutions and spiked blank extract peak–area ratios of quantitation ion versus pesticide concentration were constructed using a least-square linear regression. The equations obtained with standards and with spiked extracts

were similar and the covariance analysis showed that the calculated $F$ values were lower than the tabulated ones indicating that both straight lines were parallel ($P<0.05$). Figs. 3A and 4A show the chromatogram of a spiked orange extract using APCI and ES, respectively. Both figures show an unknown peak (around 9 min in Fig. 3A and around 14 min in Fig. 4A), but it does not interfere with the chromatographic determination of the five fungicides monitored in the present study.

Table 3 shows the recovery, precision and detection limits obtained with the APCI interface, which were similar to those obtained with the ES interface. Average recoveries were higher than 70% at the 0.05–5 mg $kg^{-1}$ level. The exception was thiophanate methyl, recovery values for this residue were low, approximately 57%. It may be that the poor response of thiophanate methyl in the orange matrix was due to the formation of other intermediate products different to carbendazim, as suggested by Anastassiades et al. [16]. Detection limits obtained in the present study are comparable to those reported in the literature with LC–ES–MS [9] and with LC–APCI–MS [8]. These detection limits are

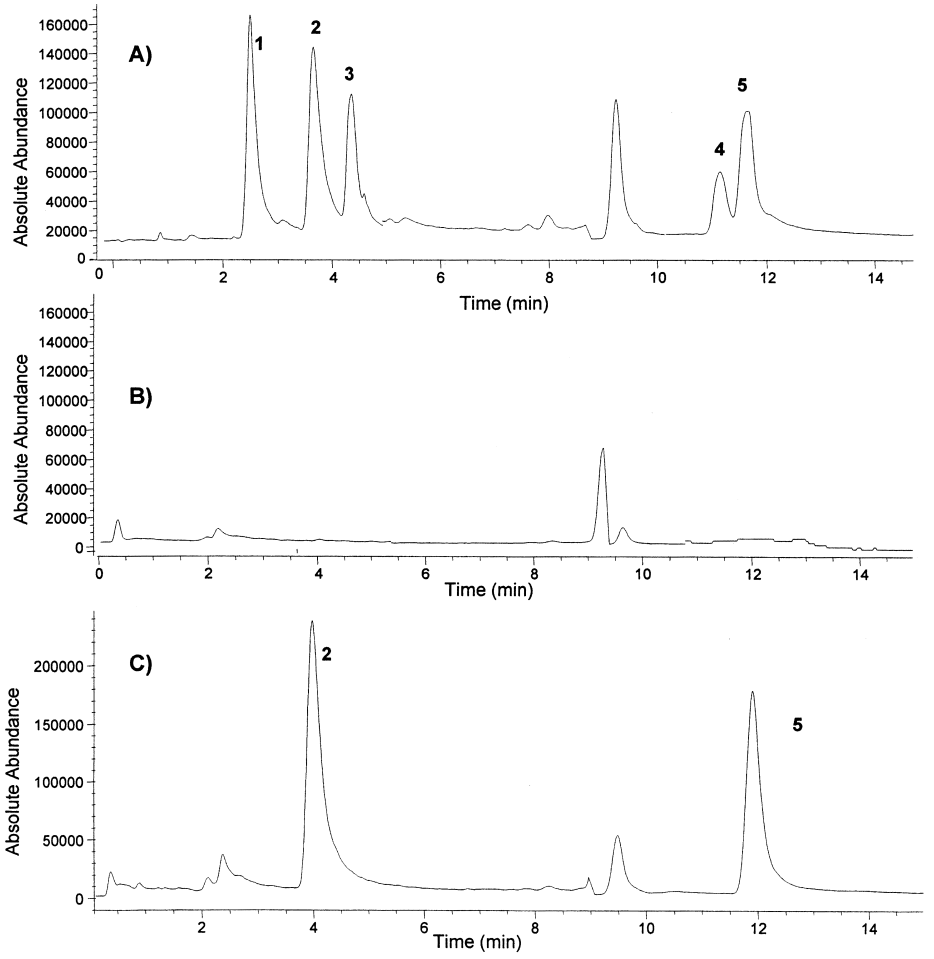



Fig. 3. Time-scheduled SIM mode LC–APCI–MS chromatograms of (A) extract spiked with 2.5 μg $ml^{-1}$ of each fungicide, equivalent to an orange concentration of 0.5 mg $kg^{-1}$, (B) extract of an orange treated only with waxes and (C) extract of the orange sample number 5. Peak assignment as in Fig. 1.

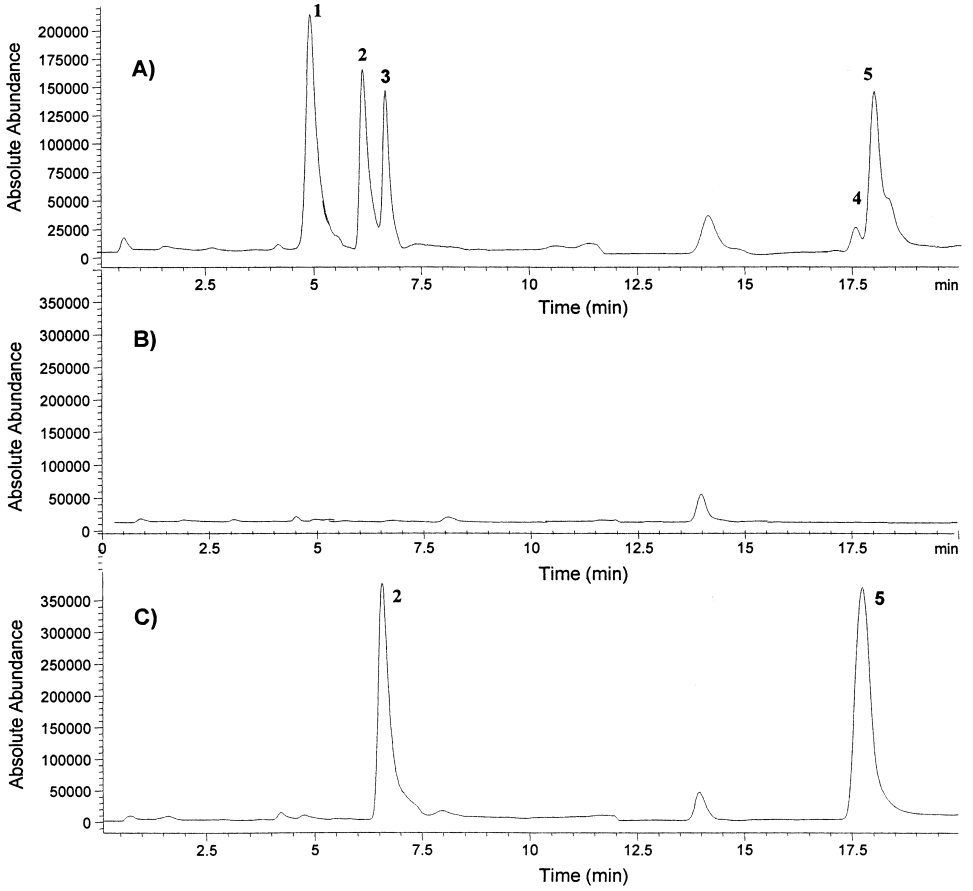



Fig. 4. Time-scheduled SIM mode LC–ES–MS chromatograms of (A) extract spiked with 2.5 μg $ml^{-1}$ of each fungicide, equivalent to an orange concentration of 0.5 mg $kg^{-1}$, (B) extract of an orange treated only with waxes and (C) extract of the orange sample number 5. Peak assignment as in Fig. 1.

Table 3
Recovery, precision and detection limits obtained for post-harvest fungicides in oranges

| Compound | Mean recovery (%)[a] | RSD (%)[b] | LODs (mg $kg^{-1}$) |
|---|---|---|---|
| Carbendazim | 79 | 10 | 0.02 |
| Thiabendazole | 75 | 7 | 0.02 |
| Thiophanate methyl[c] | 56 | 11 | 0.03 |
| Benomyl[c] | 79 | 8 | 0.03 |
| Imazalil | 77 | 8 | 0.02 |

[a] Concentration tested were 0.05, 0.5 and 5 mg $kg^{-1}$. Mean values obtained from quintuplicate measurements for each spiked concentration.

[b] RSD do not show dependence with concentration ($N=5$) for the different spiked levels assayed.

[c] Recovered as a carbendazim.

100 times lower than MRLs admitted in the European Union for citrus fruits, that are 5 mg $kg^{-1}$ for the sum of benomyl, thiophanate methyl and carbendazim expressed as carbendazim; 6 mg $kg^{-1}$ for thiabendazole; and 5 mg $kg^{-1}$ for imazalil.

The method was applied to the determination of fungicide residues in oranges subjected to different treatments. Ten samples without post-harvest treatment, ten treated only with waxes and ten ready for their distribution to the markets were collected from an agricultural cooperative. Only in the ten samples that have been post-harvest treated with fungicides were residues found. Table 4 summarizes the results. It is interesting to note the good agreement between

Table 4
Content[a] of studied fungicides in orange samples expressed as mg $kg^{-1}$

| Sample | Compound | APCI–MS[b] | ES–MS[c] |
|---|---|---|---|
| 1 | Thiabendazole | 1.97 | 1.76 |
| | Imazalil | 1.14 | 1.00 |
| 2 | Thiabendazole | 0.72 | 0.60 |
| | Imazalil | 0.28 | 0.26 |
| 3 | Imazalil | 0.37 | 0.40 |
| 4 | Imazalil | 1.30 | 1.34 |
| 5 | Imazalil | 0.55 | 0.40 |
| 6 | Imazalil | 0.46 | 0.30 |
| 7 | Thiabendazole | 0.30 | 0.20 |
| | Imazalil | 0.80 | 0.54 |
| 8 | Carbendazim[d] | 0.15 | 0.10 |
| | Carbendazim[d] | 0.20 | 0.26 |
| 10 | Thiabendazole | 0.10 | 0.12 |
| | Imazalil | 0.02 | 0.02 |

[a] Triplicate measurements.
[b] RSD were ranged between 5.6 and 18.4%.
[c] RSD were ranged between 6.4 and 17.8%.
[d] Concentrations found for carbenazim are sum of the three fungicides.

the results obtained with APCI and those obtained with ES and, therefore, both can be used for highly sensitive detection of pesticide residues in oranges. Fungicide content found in all the samples is distinctly below the MRLs established by the EU. These results indicate that the residue levels found are not hazardous but the pesticide residue monitoring programs are necessary to control the consumer's exposure.

As an example, the chromatograms obtained for the orange sample number 5 are presented in Figs. 3C and 4C for APCI and ES, respectively. Thiabendazole and imazalil were safely detected by both interfaces. Fig. 3B and 4B shows the chromatograms obtained from an orange sample treated only with waxes, where no peaks were observed at the retention times of the studied compounds.

## 4. Conclusions

The mass spectra provided in APCI–MS and ES–MS are rather similar for studied fungicides, both interfaces could be indistinctly used for their determination. A combination of LC–MS with a simple liquid multiresidue extraction method may become a highly useful technique to identify and confirm pesticide residues in fruits, becoming a valuable addition to existing analytical tools to determine fungicide residues in oranges.

Another interesting aspect is that benomyl and thiophanate methyl can be analyzed as intact molecules by LC–MS; however, they decomposed through the extraction procedure to the stable compound carbendazim. Moreover, through applying this technique to real samples, we have shown unmistakably the importance of the inclusion of these compounds in general multiresidue procedures to MRLs control and to protect the consumer's health.

## Acknowledgements

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## References

[1] G.J. Holmes, J.W. Eckert, Phytopathology 89 (1999) 716.
[2] Y. Nagayama, K. Tayama, Bull. Environ. Contam. Toxicol. 58 (1997) 402.
[3] M.E. Traina, P. Fazzi, C. Macri, C. Ricciardi, A.V. Stazi, E. Urbani, A. Mantovani, J. Applied Toxicol. 18 (1998) 241.
[4] E. Papadopoulou Mourkidou, J. Assoc. Off. Anal. Chem. 74 (1991) 745.
[5] C.M. Torres, Y. Picó, J. Mañes, J. Chromatogr. A 754 (1996) 301.
[6] C.H. Liu, G.C. Mattern, X. Yu, J.D. Rosen, J. Agric. Food Chem. 38 (1990) 167.
[7] J.L. Bernal, M.J. del Nozal, L. Toribio, J.J. Jiménez, J. Atienza, J. Chromatogr. A 787 (1997) 129.
[8] K.A. Barnes, R.J. Fussell, J.R. Startin, M.K. Pegg, S.A. Thorpe, S.L. Reynols, Rapid Commun. Mass Spectrom. 11 (1997) 117.
[9] E. Lacassie, M.F. Dreyfuss, J.L. Daguet, M. Vignaud, P. Marquet, G. Lachâtre, J. Chromatrogr A 830 (1999) 135.
[10] P. Pleasance, M.R. Anacleto, M.R. Bayley, D.H. North, J. Am. Soc. Mass Spectrom. 3 (1992) 378.
[11] W.H. Newsome, B.P.Y. Lau, D. Ducharme, D. Lewis, J. AOAC Int. 78 (1995) 1312.
[12] A. Di Corcia, C. Crescenzi, A. Laganà, J. Agric. Food Chem. 44 (1996) 1930.

[13] X. Zang, E.K. Fukuda, J.D. Rosen, J. Agric. Food Chem. 46 (1999) 2206.
[14] J. Slobodnik, A.C. Hogenboom, J.J. Vreuls, J. Rontree, B.L.M. van Baar, W.M.A. Niessen, U.A.Th. Brinkman, J. Chromatogr. A 741 (1996) 59.
[15] E. Mallat, D. Barceló, R. Tauler, Chromatographia 46 (1997) 342.
[16] M. Anastassiades, M. Scherbaum, Deuts. Lebbens. Runds. 93 (1997) 316.