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Determination of Postharvest Fungicides in Fruit Juices by Solid-Phase Extraction Followed by Liquid Chromatography Electrospray Time-of-Flight Mass Spectrometry

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A multiresidue method using liquid chromatography−time-of-flight mass spectrometry (LC−TOFMS) has been developed for the quantitative analysis of five widely used postharvest fungicides (carbendazim, thiabendazole, imazalil, prochloraz, and iprodione) and two of their transformation products (imazalil and prochloraz metabolites) in fruit juices. LC−TOFMS in positive electrospray ionization mode was used to quantify and confirm trace levels of these fungicides in fruit juices. The proposed method consists of a sample treatment step based on solid-phase extraction using hydrophilic–lipophilic-balanced polymer-based reverse-phase SPE cartridges (Oasis HLB) and methanol as an eluting solvent. Fruit-juice extracts spiked at different fortification levels (10 and 20 μg L^{-1}) yielded average recoveries in the range of 71–109% with RSD (%) below 15%. Subsequent identification, confirmation, and quantitation were carried out by LC−TOFMS analysis. The confirmation of the target species was based on accurate mass measurements of protonated molecules ($[M + H]^+$) and fragment ions, obtaining routine accuracy errors lower than 2 ppm in most cases. The obtained limits of detection (LODs) of the proposed method were in the range of 0.08–0.45 μg L^{-1}. Finally, the proposed method was successfully applied to the analysis of 23 fruit juice samples collected from different European countries and the United States, showing the potential applicability of the method in routine analysis. Over 50% of the samples tested contained pesticide residues, but relatively low concentration levels were found.

KEYWORDS: Pesticides; juice; liquid chromatography; mass spectrometry; electrospray; time-of-flight

1. INTRODUCTION

Pesticides are widely used at various stages of cultivation and during postharvest storage to protect fruit and vegetables against a range of pests and fungi and provide quality preservation. As a consequence, residues of these substances can be found in food, thus constituting a potential risk for human health considering their toxicity and the exposure to these compounds, particularly for children because they consume a higher proportion of fruits and vegetables in relation to their body weight. For this reason, maximum residue limits (MRLs) in foodstuffs have been set by government agencies and the European Union Commission to guarantee consumer safety and regulate international trade (*1–3*). In the case of processed food, such as juices, MRLs corresponding to the original matrices are normally considered.

In general, pesticides are often found at higher concentrations in the peel of fruits than in the fruit juice, because it is assumed that the production of juice does not involve the squeezing of the peel (*4*). However, in some types of juices (i.e., orange), the entire fruit is squeezed without removing the peel. In this case, the presence of trace amounts of pesticides in juice may occur. Anyhow, the expected concentration levels of pesticides in fruit juices are low, which increases the difficulty for analysis. For this reason, analytical methodologies employed for the trace determination of these compounds must be capable of residue measurement at very low levels and must also provide unambiguous evidence to confirm both the identity and quantity of any residues detected. Several methods have been developed for the analysis of pesticide residues in fruits and vegetables (*5–9*), but few methods are available in the scientific literature for their determination in juice (*10, 11*).

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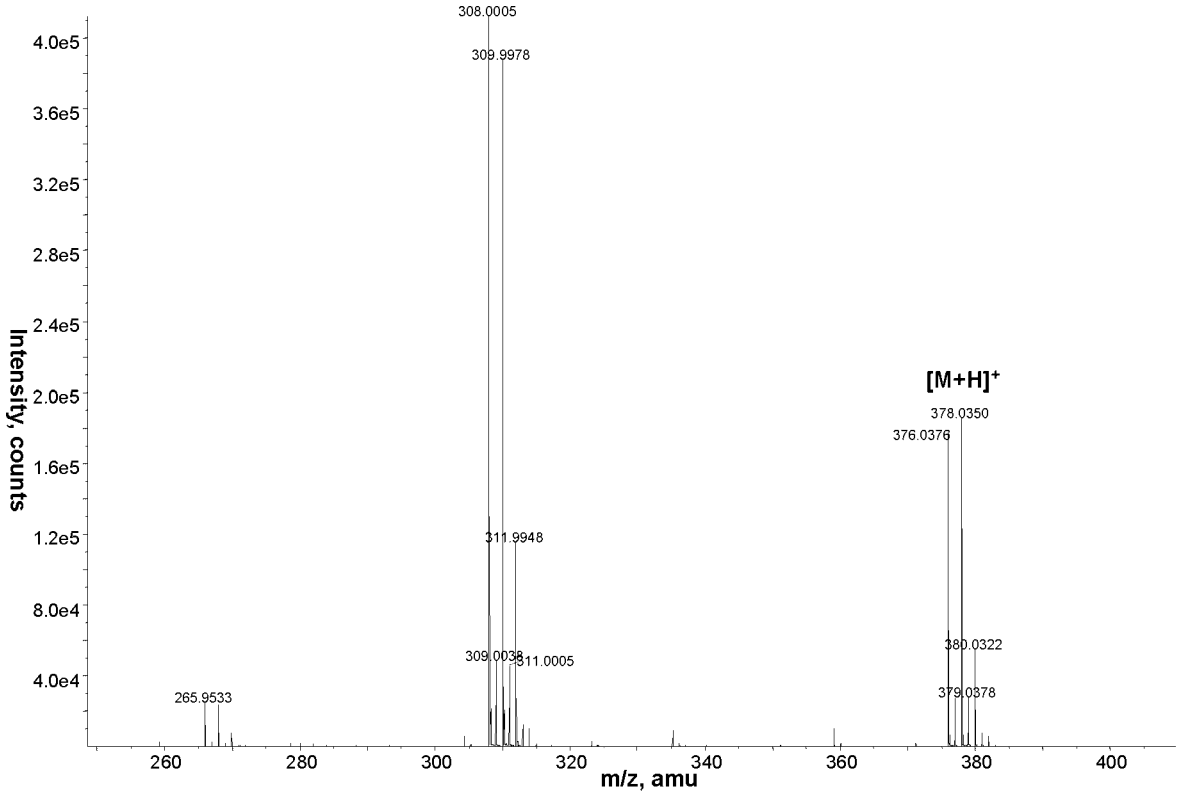
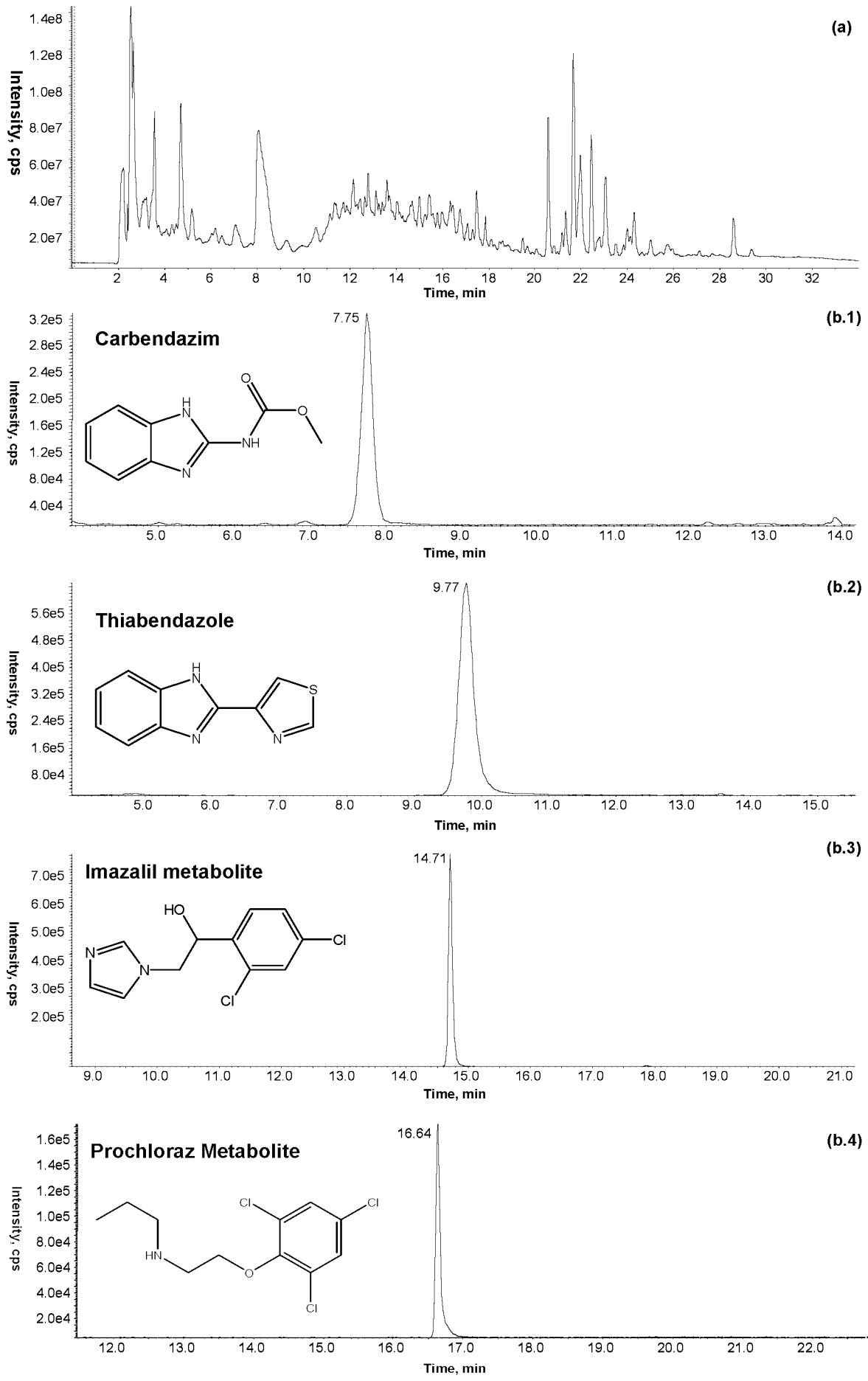


Figure 1. Accurate mass spectrum obtained for the confirmation of prochloraz in a spiked orange juice extract at a concentration of 10 μg L^{-1}.

Because trace amounts of pesticides are usually found in juice samples, preconcentration and purification steps are required. The presence of pesticides in fruit juices generally requires the concentration of these compounds in a clean extract before they can be determined. The development of appropriate methods to monitor pesticide residues in juices is demanding, because it requires simple and fast sample treatment procedures that may be easily implemented in routine laboratories. Classical analytical methodology is based on liquid–liquid extraction (*4*). Nowadays, sample treatment strategies based on solid–liquid extraction are the more widely used approach because of the simplicity and robustness of these extraction procedures, together with the low requirement of organic solvents. These techniques are mainly based on the extraction of pesticides in a solid phase, which allows for the concentration of analytes in the sorbent and their subsequent elution or desorption, frequently in a selective way. Solid-phase extraction (SPE) (*12–14*), matrix solid-phase dispersion (MSPD) (*15–19*), solid-phase microextraction (SPME) (*11,20–22*), and stir-bar sorptive extraction (SBSE) are the main examples of these extraction techniques applied for multiclass pesticide analysis in juices (*4*). In these cases, a simultaneous extraction and cleanup of extracts occurs, which often allows for the direct analysis.

In the case of volatile and thermally stable pesticides, so that gas chromatography (GC) analysis is feasible, the most frequently used technique for the determination of pesticides in juices is GC with different selective detectors, such as an electron-capture detector (ECD), a nitrogen phosphorus detector (NPD), and a flame photometry detector (FPD) (*4*). The confirmation of residue identity is usually performed by gas chromatography/mass spectrometry (GC−MS) (*23*). However, the number of compounds that cannot be determined by GC because of their poor volatility and thermal instability has grown dramatically in the past few years. For this task, liquid chromatography coupled with mass spectrometry (LC−MS) has recently become a powerful analytical technique for the identification and quantitation of residues in crops. However, scarcely any literature has been reported on LC−MS methods for the determination of pesticides in fruit juices (*14, 19, 24, 25*).

A relatively new technique for the control of pesticides in food is liquid chromatography−time-of-flight mass spectrometry (LC−TOFMS) (*26*). TOFMS instruments provide high specificity (because of both high mass accuracy and mass resolution), without limiting the number of simultaneously observed target compounds. Its high full-scan speed and acceptable sensitivity have made TOF an attractive alternative to quadrupole LC−MS (/MS) instruments. In addition, accurate mass capabilities and high mass resolving power provide a great degree of chemical noise reduction and thus enhanced selectivity. Recently, LC−TOFMS has been proven to be a sensitive and selective method for the determination and confirmation of pesticide residues in vegetables and fruits obtaining limits of quantitation in compliance with established MRLs (*27, 28*). Linearity of 3 orders of magnitude and limits of detection (LODs) at low picogram levels injected are features of LC−TOFMS for quantitative target pesticide residue analysis. In this work, we have exploited these features to develop a new method for the multianalyte determination of fungicides in fruit juices based on SPE followed by LC−TOFMS analysis. The proposed method includes the most common postharvest fungicides (i.e., thiabendazole, imazalil, prochloraz, carbendazim, and iprodione) together with two of their metabolites (from imazalil and prochloraz, *29*) in fruit juices. The method has been validated and applied to the analysis of 23 market-purchased fruit-juice samples from different European countries and the United States. To the best of our knowledge, this is the first paper that evaluates the potential of LC−TOFMS for the confirmation and quantitation of pesticides in fruit juices.


(a)
Intensity, cps
Time, min
(b.1)
Carbendazim
7.75
(b.2)
Thiabendazole
9.77
(b.3)
Imazalil metabolite
14.71
(b.4)
Prochloraz Metabolite
16.64

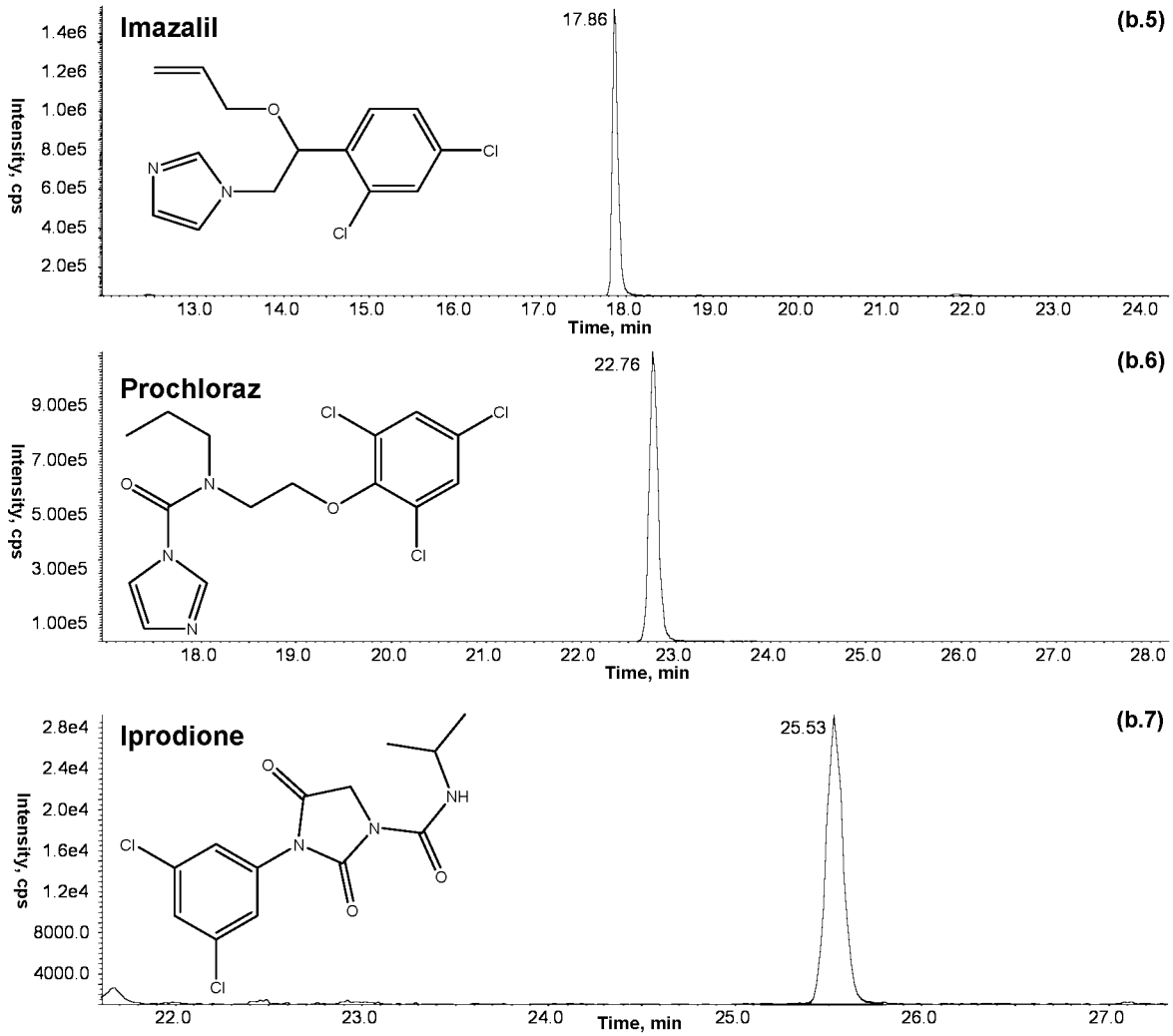


Figure 2. (**a**) TIC corresponding to the LC−TOFMS analysis of a spiked orange juice sample with the studied fungicides (fortification level = 10 μg L^{-1}). (**b**) XIC for each corresponding protonated molecule of the studied fungicides (mass-window width = 20 mDa).

2. EXPERIMENTAL PROCEDURES

2.1. Chemicals and Materials. HPLC-grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Formic acid was obtained from Fluka (Buchs, Switzerland). A Milli-Q-Plus ultrapure water system from Millipore (Milford, MA) was used throughout the study to obtain the HPLC-grade water used during the analyses. Fungicide analytical standards were purchased from Dr. Ehrenstorfer (Ausburg, Germany) and from Riedel de Haën, Pestanal quality (Seelze, Germany). Individual fungicide stock solution (200–300 μg mL^{-1}) were prepared in methanol and stored at −20 °C. Prochloraz metabolite was synthesized in our laboratory from acid hydrolisis of prochloraz. Oasis HLB SPE cartridges (200 mg, 6 mL) purchased from Waters (Milford, MA) and a Supelco (Bellefonte, PA) Visiprep SPE vacuum system were also used.

2.2. Sample Treatment. Different brands of juices were purchased in different European countries and in the United States. The fungicides were extracted using off-line SPE. Before the SPE was performed, a sample aliquot of 50 mL was centrifuged (3700 rpm) for 3 min to avoid blockage of the cartridges. The SPE procedure involves a preconditioning step of the cartridges with 5 mL of MeOH and 5 mL of milli-Q water at a flow rate of 2 mL min^{-1}. After that, aliquots of 30 mL of centrifuged juice sample (without pH adjustment) were loaded at a flow rate of 3 mL min^{-1}. The retained analytes were eluted with 5 mL of MeOH at 1 mL min^{-1}, and this eluate was collected in a 15 mL centrifuge tube, evaporated until near dryness by a gentle nitrogen stream, and taken up with 1 mL of MeOH and 2 mL of milli-Q water (preconcentration factor of 10:1). Prior to LC−TOFMS analysis, this extract was filtered through a 0.45 μm PTFE filter (Millex FG, Millipore, Milford, MA) and transferred into a vial.

For quantitation purposes, matrix-matched standards were prepared by spiking the extracts with an appropriate volume of working standard solutions of the studied analytes. For recovery studies, fruit juice samples were spiked before the extraction procedure with the mixture of the studied fungicides at two concentration levels of 10 and 20 μg L^{-1}.

2.3. LC−TOFMS. The separation of the species from the SPE juice extracts was carried out using a HPLC system (consisting of a vacuum degasser, autosampler, and a binary pump) (Agilent Series 1100, Agilent Technologies, Santa Clara, CA) equipped with a reverse-phase C_8 analytical column of 150 × 4.6 mm and 5 μm particle size (Zorbax Eclipse XDB-C8). A total of 20 μL of extract was injected in each study. Mobile phases A and B were water with 0.1% formic acid and acetonitrile, respectively. The chromatographic method held the initial mobile-phase composition (10% B) constant for 5 min, followed by a linear gradient to 100% B at 30 min. The flow-rate used was 0.6 mL min^{-1}. Then, the mobile-phase composition was kept constant for 5 min (100% B), and finally, a 12 min postrun time at initial mobile-phase composition (10% B) (0.4 mL min^{-1}) was included to re-equilibrate the column.

The HPLC system was connected to a time-of-flight mass spectrometer Agilent MSD TOF (Agilent Technologies, Santa Clara, CA) equipped with an electrospray interface operating in positive-ion mode, using the following operation parameters: capillary voltage, 4000 V; drying gas, 9 L min^{-1}; gas temperature, 325 °C; nebulizer pressure, 40 psig; skimmer voltage, 60 V; octapole DC 1, 37.5 V; octapole RF, 250 V; fragmentor voltage, 190 V. LC−TOFMS accurate mass spectra were recorded across the range of *m*/*z* 50–1000. Accurate mass measurements of each peak from the total ion chromatograms were

Table 1. Identification of Pesticides Studied in Juice Extracts by LC−TOFMS Accurate Mass Measurements of the Protonated Molecules and the Main Fragment Ions Using Juice Matrix-Matched Standards[a]

compound	t_R	ion	elemental compositions	*m/z* theoretical	*m/z* experimental	error (mDa)	error (ppm)
carbendazim	7.7	$[M + H]^+$	$C_9H_{10}N_3O_2$	192.0767	192.0763	−0.45	2.4
		fragment	$C_8H_6N_3O$	160.0505	160.0500	−0.53	3.4
thiabendazole	9.8	$[M + H]^+$	$C_{10}H_8N_3S$	202.0433	202.0434	0.054	0.3
imazalil metabolite	14.7	$[M + H]^+$	$C_{11}H_{11}N_2OCl_2$	257.0242	257.0238	−0.59	2.3
		^{37}Cl ion	$C_{11}H_{11}N_2OCl\ ^{37}Cl$	259.0213	259.0209	−0.44	1.7
prochloraz metabolite	16.7	$[M + H]^+$	$C_{11}H_{15}NOCl_3$	282.0213	282.0210	−0.37	1.3
		^{37}Cl ion	$C_{11}H_{15}NOCl_2{}^{37}Cl$	284.0184	284.0181	−0.32	1.1
		$^{37}Cl_2$ ion	$C_{11}H_{15}NOCl\ ^{37}Cl_2$	286.0154	286.0152	−0.27	1.0
imazalil	17.8	$[M + H]^+$	$C_{14}H_{15}N_2OCl_2$	297.0555	297.0551	−0.50	1.6
		^{37}Cl ion	$C_{14}H_{15}N_2OCl\ ^{37}Cl$	299.0526	299.0518	−0.85	2.8
prochloraz	22.8	$[M + H]^+$	$C_{15}H_{17}N_3O_2Cl_3$	376.0380	376.0376	−0.49	1.3
		^{37}Cl ion	$C_{15}H_{17}N_3O_2Cl_2{}^{37}Cl$	378.0351	378.0350	−0.14	0.4
		$^{37}Cl_2$ ion	$C_{15}H_{17}N_3O_2Cl\ ^{37}Cl_2$	380.0321	380.0322	0.01	0.04
		fragment 1	$C_{12}H_{13}NO_2Cl_3$	308.0006	308.0005	−0.14	0.4
		^{37}Cl ion	$C_{12}H_{13}NO_2Cl_2{}^{37}Cl$	309.9976	309.9978	0.11	0.4
		$^{37}Cl_2$ ion	$C_{12}H_{13}NO_2Cl\ ^{37}Cl_2$	311.9947	311.9948	0.06	0.2
iprodione	25.5	$[M + H]^+$	$C_{13}H_{14}N_3O_3Cl_2$	330.0406	330.0399	−0.77	2.3
		^{37}Cl ion	$C_{13}H_{14}N_3O_3Cl\ ^{37}Cl$	332.0377	332.0375	−0.22	0.7
		fragment 1	$C_{10}H_8N_3O_3Cl_2$	287.9937	287.9930	−0.72	2.5
		^{37}Cl ion	$C_{10}H_8N_3O_3Cl\ ^{37}Cl$	289.9907	289.9908	0.027	0.1
		fragment 2	$C_9H_7N_2O_2Cl_2$	244.9879	244.9880	0.09	0.4
		^{37}Cl ion	$C_9H_7N_2O_2Cl\ ^{37}Cl$	246.9849	246.9853	0.34	1.4

[a] Spiking level = 10 μg L^{-1}.

Table 2. Recovery Studies on Juice Extracts Fortified with the Pesticide Mixture at the 10 and 20 μg L^{-1} Concentration Levels

pesticide	spiking level (μg L^{-1})	recovery (%)	RSD (%) ($n = 5$)
carbendazim	10	77.5	12.7
	20	96.7	8.8
thiabendazole	10	99.3	10.1
	20	104.6	7.8
imazalil metabolite	10	91.8	11.1
	20	85.7	9.0
prochloraz metabolite	10	75.3	11.4
	20	82.0	10.3
imazalil	10	96.3	8.4
	20	108.8	7.8
prochloraz	10	71.1	12.1
	20	81.7	9.3
iprodione	10	74.1	14.0
	20	76.7	11.6

Table 3. Analytical Parameters for the Analysis of Pesticides in Juice Samples by LC−TOFMS

compound	concentration range (μg L^{-1})	linearity (R)	LOD (μg L^{-1})	LOQ (μg L^{-1})	RSD (%) ($n = 6$) intraday	RSD (%) ($n = 6$) interday
carbendazim	0.2−30	0.9963	0.25	1.0	2.5	7.1
thiabendazole	0.2−30	0.9993	0.20	0.8	4.1	8.8
imazalil metabolite	0.2−30	0.9994	0.25	1.0	3.8	9.1
prochloraz metabolite	0.2−30	0.9999	0.15	0.5	5.1	8.0
imazalil	0.2−30	0.9999	0.30	1.0	1.6	6.0
prochloraz	0.2−30	0.9997	0.08	0.25	2.4	8.5
iprodione	0.2−30	0.9981	0.45	1.5	5.8	10.3

obtained by means of an automated calibrant delivery system using a dual-nebulizer electrospray source that introduces the flow from the outlet of the chromatograph together with a low flow of a calibrating solution (calibrant solution A, Agilent Techologies), which contains the internal reference masses [purine ($C_5H_4N_4$ at *m/z* 121.050 873 and HP-921 [hexakis-(1*H*,1*H*,3*H*-tetrafluoropentoxy)-phosphazene] ($C_{18}H_{18}O_6N_3P_3F_{24}$) at *m/z* 922.009798]. Besides, a software package is autocalibrating and recording continuously the results of the internal reference masses along with the raw data. The instrument worked providing a typical resolution of 9700 ± 500 (*m/z* 922). The full-scan data recorded were processed with Applied Biosystems/MDS-SCIEX Analyst QS software (Frankfurt, Germany) with accurate mass application-specific additions from Agilent MSD TOF software.

Table 4. Evaluation of the Matrix Effects: Comparison of the Calibration Curve Slopes

analyte	solvent: water/methanol equation	*R* coefficient	slope matrix/slope solvent
carbendazim	$y = 1.43 \times 10^5C + 4.67 \times 10^4$	0.9999	0.68
thiabendazole	$y = 6.97 \times 10^5C + 2.44 \times 10^4$	0.9999	0.72
imazalil metabolite	$y = 4.43 \times 10^5C + 2.53 \times 10^5$	0.9998	0.70
prochloraz metabolite	$y = 2.66 \times 10^5C - 1.18 \times 10^4$	1.0000	0.82
imazalil	$y = 6.61 \times 10^5C + 2.99 \times 10^5$	0.9998	0.81
prochloraz	$y = 3.06 \times 10^5C + 1.88 \times 10^5$	0.9997	0.90
iprodione	$y = 1.04 \times 10^4C + 6.66 \times 10^3$	0.9998	1.00

3. RESULTS AND DISCUSSION

3.1. LC−TOFMS. Electrospray ionization conditions were studied to achieve the best possible sensitivity and selectivity for the selected compounds. We found that these parameters did not significantly affect the signal of the analytes, so that standard values were set for drying and nitrogen flow rates, vaporizer and drying temperatures, and capillary voltage. From previous experience on this kind of compounds (*26*–*28*), the fragmentor voltage was set at 190 V, as a compromise value between sensitivity for quantitation and additional mass spectrum information for confirmation purposes.

For identification and quantitation purposes, we used extracted ion chromatograms (XICs) using a mass-window width of 20 mDa ($[M + H]^+ \pm 10$ mDa). The protonated molecule ($[M + H]^+$) was used for both confirmation and quantitation purposes in most cases, except for prochloraz, where the relative abundance of its characteristic fragment ion (with *m/z* 308) was higher than that of the protonated molecule in the selected conditions (**Figure 1**). In addition, some studied fungicides present chlorine atoms (e.g., imazalil, imazalil metabolite, and prochloraz), which offer an isotopic pattern that yields further information for the unambiguous identification of the target

Table 5. Pesticide Residues Found in Juice Samples Tested[a]

sample number	concentration (μg L^{-1}) carbendazim	thiabendazole	imazalil	prochloraz metabolite	rest of the studied pesticides	total
1	17.2	1.6	4.6	ND	ND	23.4
2	3.9	ND	1.7	2.1	ND	7.7
3	ND	1.1	10.2	ND	ND	11.3
4	12.2	ND	ND	ND	ND	12.2
14	1.8	ND	ND	ND	ND	1.8
16	4.3	ND	ND	ND	ND	4.3
17	4.0	ND	ND	ND	ND	4.0
18	5.4	4.3	ND	ND	ND	9.7
20	5.90	ND	ND	ND	ND	5.9
21	15.8	ND	ND	ND	ND	15.8
22	10.8	ND	ND	ND	ND	10.8
23	2.10	ND	ND	ND	ND	2.1

[a] Only concentration data of positive samples is included. Corresponding samples: 1, "Solevita" 11-fruits multivitamin juice (Jaén, Spain); 2, "Solevita" 12-fruits multivitamin juice (Jaén, Spain); 3, nectar "Compal" citric (Jaén, Spain); 4, orange and mango juice "Feel Good" (Cambridge, U.K.); 5, "Tropicana" orange juice (Cambridge, U.K.); 6, "Tropicana" orange juice (Edinburgh, U.K.); 7, "Tesco" orange juice (Edinburgh, U.K.); 8, "Tropicana" orange juice (St. Petersburg, Russia); 9, "Tropicana" orange juice (St. Petersburg, Russia); 10, "V8-Splash" multifruit (Orlando, FL); 11, "Sunny Delight" (Orlando, FL); 12, "Sunny Delight" (Orlando, FL); 13, "Minute Maid" orange juice (Orlando, FL); 14, "Vivaris" multifruit juice (Berlin, Germany); 15, "Libehna" multifruit juice (Berlin, Germany); 16, "Tropicana" orange juice (Jaén, Spain); 17, "Don Simon" orange juice (Jaén, Spain); 18, "Hero Fruit 2day" strawberry−orange (Jaén, Spain); 19, "Zumosol" orange juice (Jaén, Spain); 20, "KAS Fruit" orange juice (Jaén, Spain); 21, "Granini" orange juice (Jaén, Spain); 22, "Minute Maid Classic" orange juice (Jaén, Spain); and 23, "Granini" pear juice (Budapest, Hungary).

compounds (*27*). As an example, **Figure 1** shows the accurate mass spectrum of prochloraz in a juice extract at 10 μg L^{-1} and **Figure 2** shows the total ion chromatogram (TIC) of a juice sample spiked with 10 μg L^{-1}, together with the XICs for the studied fungicides.

Table 1 shows the results obtained for accurate mass analysis of the selected fungicides in a matrix-matched standard, spiked with 10 μg L^{-1}. In the case of chlorinated compounds, accurate mass analysis was also performed on isotope signals corresponding to ions with ^{37}Cl. From the data obtained, it can be concluded that the method offers a high degree of confirmation because of its very high mass accuracy, enabling routine accurate mass measurements, with mass accuracy below 2 ppm error in most cases.

3.2. Sample Treatment and Recovery Studies. For the SPE step, 30 mL of juice sample was selected as the loaded volume. The preconcentration factor was set at 10:1, because of the complexity of the matrix. It should be noted that the proposed method is based on a direct SPE procedure without further cleanup stages. Therefore, the obtained extracts are relatively dirty to be injected in the LC−MS instrument, so that the use of small preconcentration factors was mandatory. The preconcentration factor of 20:1 or higher involved complex extracts that yielded signal/sensitivity losses and soiled the MS inlet, being necessary for daily cleaning and maintenance of the source. In addition, in these conditions, matrix effects were remarkable (over 35% suppression in all of the studied analytes). In contrast, the use of preconcentration factors of 10:1 (or lower) did not strongly affect the sensitivity and signal stability of the MS source, over large periods of operation. Furthermore, matrix effects were minimized in most cases (see section 3.3). Therefore, if the LC−MS instrument provides sensitivity enough (as was the case of the LC−TOFMS that we used), we recommend to use this preconcentration factor. In this sense, the use of such a preconcentration factor would enable the reduction of the size of the procedure, in terms of, i.e., using smaller SPE cartridges, reduced sample loading volume (5–10 mL), and reduced eluting solvent volumes, thus increasing the throughput of the procedure.

On the other hand, we did not find any significant lost of pesticides using centrifuged juices. Recovery studies were performed by spiking samples before proceeding to centrifuge them. The results were not affected significantly by this step. With regard to the sorbent material used for the SPE step, we also performed experiments with C_{18} cartridges and found that the best suited for this purpose were Oasis HLB cartridges. The recoveries using C_{18} were very poor for most analytes, and the extracts obtained were not particularly clean. For these reasons, the combination of Oasis and methanol was used to isolate and preconcentrate polar pesticides in this matrix, as successfully used in environmental water. Besides, this cartridge is the most suitable one to extend the scope of the method including other classes of pesticides (herbicides, insecticides, etc.) because it offers high recovery rates for a large number of compounds with different physicochemical properties.

To evaluate the effectiveness of the extraction method, different recovery studies were carried out using an orange juice sample of 1 L. We chose the orange juice matrix as the more representative juice matrix, and negligible variations on the method performance in terms of recovery percentage would have been obtained if different fruit juice matrices were used. In this sense, the effect of the sample matrix of a juice does not play a crucial role. The pH value differences between different fruit juices are not very significant, and this value is the main figure to be considered when developing a SPE method. Several portions of 50 mL were spiked at two different concentration levels (10 and 20 μg L^{-1}) with the working standard solution. Then, the spiked samples were centrifuged and extracted with the SPE method described. The obtained extracts were analyzed with the developed LC−MS method, obtaining recoveries between 71 and 109%, as seen in **Table 2**. These results show the feasibility of the studied extraction method for pesticide residue analysis in fruit juices.

3.3. Analytical Features. *(a) Linearity, Calibration, and Analytical Precision.* Calibration curves of the analyzed compounds were constructed at different concentrations, in the range of 0.2–30 μg L^{-1}, using juice extracts to prepare matrix-matched standards. The linearity of the analytical response across the studied range is excellent, taking into account that all the calibration curves of the analyzed fungicides showed correlation coefficients higher than 0.996 as shown in **Table 3**, where these values are summarized together with the LODs and intra- and interday relative standard deviation (RSD) (%). The RSD (n = 6) values for the run−run study were in the range of 1.6–5.8%, and interday RSD (n = 6) values were between 6.0 and 10.3%. These results demonstrate the precision of the developed method and the potential of the proposed approach for quantitative purposes. The LODs obtained were estimated from the injection of matrix-matched standard solutions at concentration levels corresponding to a signal-to-noise ratio (S/N) = 3. Similarly, limits of quantification (LOQs) were estimated on the basis of the 10:1 S/N ratio criterion. The results obtained for each fungicide are shown in **Table 3**. The LODs obtained are as low as 0.08 μg L^{-1} for prochloraz and below 0.5 μg L^{-1} for the rest of the fungicides studied.

(b) Matrix Effects. When using LC−MS atmospheric pressure ionization sources, matrix components can both reduce or enhance the signal given by the analytes when they achieve the detector. The problem is originated in the interface (source)

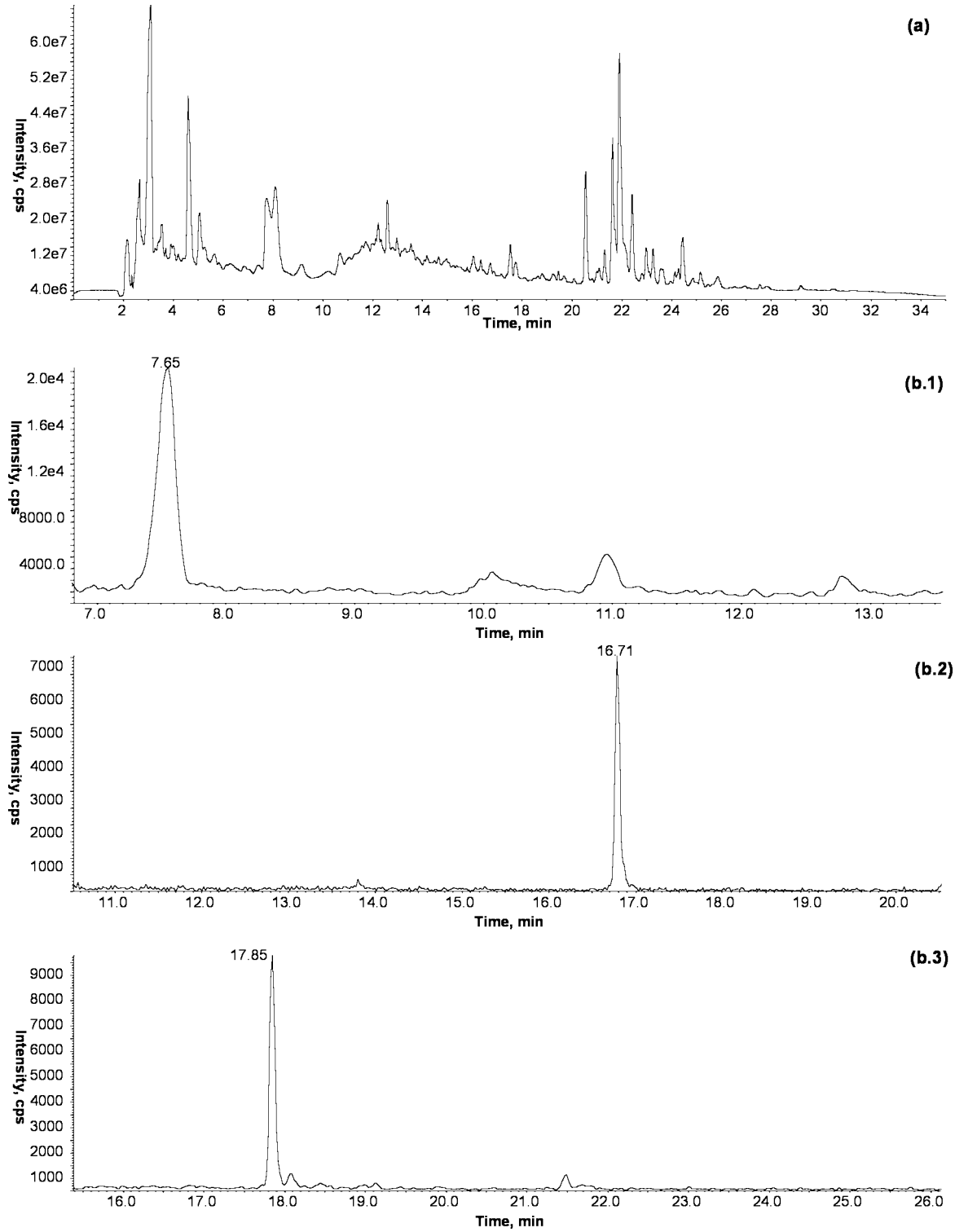


Figure 3. (**a**) TIC corresponding to the LC−TOFMS analysis of a market-purchased 12-fruit multivitamin juice, where carbendazim (3.9 μg L^{-1}), prochloraz metabolite (1.7 μg L^{-1}), and imazalil (2.1 μg L^{-1}) were detected. (**b**) XIC of carbendazim (b.1), prochloraz metabolite (b.2), and imazalil (b.3).

when the matrix constituents influence the ionization of a coeluted analyte, causing ion suppression. Even with the use of a preconcentration factor of 10:1, juice extracts are still very complex, so that a reduction of the response in most of the studied fungicides is usually observed. Matrix-matched standard calibration curves were used throughout the study to take into consideration these effects on the ionization/response of the analytes. The slopes obtained in the calibration with matrix-matched standards were compared to those obtained with solvent-based standards, calculating slope ratios of matrix/solvent for each fungicide. The results are summarized in **Table 4**, where the importance of matrix effects is visible, taking into account the fact that signal suppression equal to or greater than 20% occurs in more than 50% of the studied compounds. Further dilution of the extracts minimizes the matrix effects from previous experience in complex matrices (*30*), although the method detection limits are affected by the dilution factor applied.

3.4. Application to Real Samples. The proposed method was applied to the analysis of 23 juices samples collected in different

European countries and the United States. The results obtained are shown in **Table 5**. Only residues of carbendazim, thiabendazole, imazalil, and prochloraz metabolite were found but at relatively low concentrations (compared to the MRLs for the pesticides in corresponding crops). Over 50% of the juice samples tested contained at least one pesticide. As an example, **Figure 3** shows the analysis of a 12-fruit multivitamin juice sample, which contained carbendazim, prochloraz metabolite, and imazalil. The positive findings of the detected fungicides were confirmed by LC−TOFMS accurate mass analysis (obtaining mass accuracy < 3 ppm error), thus showing the usefulness of LC−TOFMS for the multiresidue analysis of postharvest fungicides in juice samples.

In conclusion, in this work, a new method based on SPE and LC−TOFMS have been described for quantitative analyses of postharvest fungicides in fruit juices. The results shown that the sensitivity obtained with the proposed method is appropriate for multiresidue analysis of pesticide residues in fruit juices. The high sensitivity attained by LC−TOFMS (i.e., LODs as low as 0.08 μg L^{-1} for prochloraz) is in compliance with the current regulations (*11*) and compares well with previous LC−MS/MS methods described for the analyses of pesticides in fruit juices (*14*, *19*, *24*, *25*). The potential of the proposed method was demonstrated by analyzing real samples with excellent selectivity and sensitivity, thus enabling the unambiguous identification, by means of accurate mass analysis, and quantitation of low levels of these pesticides in several juice samples. The potential number of compounds, which could be screened in a single run, is theoretically unlimited. The development of multiresidue methods for the analysis of over 100 multiclass pesticides in complex food matrices is fully feasible (*31*, *32*). Together with the excellent performance for target analysis, LC−TOFMS also offers the possibility of performing *a posteriori* (nontarget) analysis of juice samples, which can be re-analyzed, allowing us to register and save a "fingerprint" of each individual sample (full-scan LC−TOFMS analysis including any analyte covered by the sample treatment and ionization conditions) (*32*). All of the data are saved and can be re-examined to check for compounds that previously were not expected or were not subjected to the control. This fact highlights the potential application of this method based on LC−TOFMS in pesticide residue laboratories worldwide.

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