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Combined Use of GC-TOF MS and UHPLC-(Q)TOF MS To Investigate the Presence of Nontarget Pollutants and Their Metabolites in a Case of Honeybee Poisoning

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The combined use of gas chromatography (GC) and ultrahigh-pressure liquid chromatography (UHPLC), both coupled to time-of-flight mass spectrometry (TOF MS), has been explored in this work for the investigation of several cases of honeybee poisoning. The procedure applied involves a previous extraction with acetone followed by liquid−liquid extraction with dichloromethane. Both techniques, GC-TOF MS and UHPLC-(Q)TOF MS, have been applied to discover the presence of compounds that might be responsible of honeybee deaths. The application of a nontarget methodological approach to a first case of poisoning allowed the detection of the insecticide coumaphos at high concentration levels in the samples. The presence of possible metabolites of this organophosphorus insecticide was investigated by using both techniques. UHPLC-(Q)TOF MS showed its higher applicability in this case, as most of the metabolites were more polar than the parent compound. Four metabolites were identified by UHPLC-(Q)TOF MS, whereas only two of them were found by GC-TOF MS. The developed methodology was applied to other subsequent poisoning cases in which insecticides such as coumaphos, thiamethoxam, pyriproxyfen, and chlorfenvinphos were identified by both techniques, whereas GC-TOF MS also allowed the detection of fenitrothion and methiocarb. In all positive cases, the confirmation of the presence of the compound detected was feasible by means of accurate mass measurements of up to five ions together with their ion ratio evaluation.

KEYWORDS: Gas chromatography; ultrahigh-pressure liquid chromatography; time-of-flight; pesticides; honeybee poisoning; metabolites

INTRODUCTION

The honeybee (*Apis mellifera*) is an important insect worldwide. Its pollinating activity is crucial for the production of high-quality commercial seeds and fruits (*1*). However, the extensive use of pesticides in agricultural activities is resulting in more and more frequent honeybee poisoning. Within the pesticides that have caused more incidents of honeybee poisoning, insecticides are the main group of concern (*2*). Toxic compounds are retained and bioaccumulated in honeybee bodies, being therefore good bioindicators of the type of pesticides applied in the area surrounding their hives (*3*).

In recent years, massive honeybee death has been an issue of increasing concern in several European countries. Although pesticides and agricultural management may play an important role in these losses, it is also recognized that several other factors might be involved, including colony management, diseases, or global climate change. The submission of samples of dead bees is therefore necessary for this forensic investigation. This work involves both field and laboratory assessment and analytical research to look for pesticide residues (*4*). For this purpose advanced analytical instrumentation is needed.

Determination of pesticides in honeybees has been traditionally carried out using gas chromatography (GC) with electron capture detection (ECD) or nitrogen−phosphorus detection (NPD) (*2*, *5*). In the past decade, a tendency toward the use of more polar pesticides was observed due to their lower persistence and human hazard. For the analysis of these semipolar and polar pesticides and/or metabolites, liquid chromatography (LC) has been traditionally the technique of choice, in combination with UV or diode array detection.

In the past few years, conventional detectors have been replaced by mass spectrometry (MS) analyzers due to their inherent higher selectivity, good sensitivity, and useful information for an appropriate confirmation. Target analysis has been traditionally carried out by gas chromatography−mass spectrometry (GC-MS) or liquid chromatography−mass spectrometry (LC-MS), typically using quadrupole instruments, ion traps (IT) or, more recently, triple-quadrupole analyzers in environmental, food, and biological samples (*6*−*13*). Qualitative information that supports the recognition and structural elucidation of

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compounds other than the target is still needed to obtain more information on sample composition. To obtain an unbiased data set, full-spectrum acquisition techniques are required. The time-of-flight mass spectrometer (TOF MS) seems to be more appropriate for qualitative purposes, as it provides the selectivity and sensitivity required for an efficient and wide-scope screening. TOF MS combines high full-spectral sensitivity with high mass resolution, allowing any LC ionizable component in the sample (in the case of LC-TOF MS) or GC-amenable (in the case of GC-TOF MS) to be accurately mass-measured. Elemental compositions can be proposed with this technique with low mass errors (typically below 5 ppm, according to the manufacturer's specifications). TOF MS can provide a notable amount of chemical information in a single experiment, so this technique is very attractive for searching for a high number of compounds in a "post-target way", that is, compounds are selected and searched after MS acquisition (*14*−*17*).

In a nontarget analysis, the objective is that all compounds eluting from the analytical chromatographic column can be detected and identified without any kind of selection (with the obvious limitations derived from the chromatographic and ionization processes). Here, the analyst is searching for unknown compounds actually, as no previous information about the analytes is taken into account.

On the basis of these improved characteristics, GC has been combined with high-resolution TOF-MS (GC-HR-TOFMS) for nontarget screening of GC-amenable organic (micro) pollutants in water (*16*, *18*, *19*), anthropogenic contaminants in biological matrices (*20*), or flavor research (*21*).

With regard to LC, very few applications using ultrahigh-pressure LC (UHPLC)-(Q)TOF MS have been reported in the nontarget field. This technique has been successfully applied for a nontarget screening of organic pollutants in water (*22*) and in the metabolite-profiling field (*23*). Some applications have been recently reported in other fields, such as impurity profiling of pharmaceutical drug substances (*24*), metabonomics (*25*), or food safety (*26*).

With regard to honeybee analysis, the complexity of the sample matrix together with the presence of wax residues adhered to honeybee bodies may lead to important chromatographic interferences (*3*). Several analytical procedures for the determination of target pesticides in bees have been published in the past few years. Most of these methods involve an extraction with organic solvent followed by a cleanup step (*3*, *27*, *28*). Alternative procedures are based on solid-phase extraction (SPE) (*29*), solid-phase microextraction (SPME) (*5*), or matrix solid-phase dispersion (MSPD) (*2*, *5*, *30*), among others.

The aim of this work is to investigate the presence of toxic compounds in several honeybee poisoning episodes by the combined use of GC-TOF MS and UHPLC-QTOF MS. The application of a nontarget approach has allowed the detection and safe confirmation of several parent pesticides in samples. Then, the presence of their main metabolites has been investigated by both techniques.

EXPERIMENTAL PROCEDURES

Chemicals and Solvents. Reference materials (thiamethoxam, pyriproxyfen, promecarb, fenitrothion, chlorfenvinphos, methiocarb, coumaphos) with purities of 97−99.7% were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany) for standard preparation. Stock solutions (around 500 mg/L) were prepared by dissolving reference standards in acetone and stored in a freezer at −20 °C. Working solutions were prepared by diluting stock solutions with acetone for sample fortification, with ethyl acetate for GC injection, and with methanol/water (10:90) for LC injection.

Acetone (pesticide residue analysis), GC-ultra trace analysis grade dichloromethane (DCM), ethyl acetate (ultratrace quality), HPLC-grade methanol, reagent-grade formic acid (HCOOH, content 98−100%), sodium hydroxide, and ammonium acetate (NH_4OAc, >98%) were purchased from Scharlab (Barcelona, Spain). HPLC-grade water was obtained by distilled water passed through a Milli-Q water purification system (Millipore, Bedford, MA). Celite was purchased from Merck (Darmstadt, Germany). Anhydrous sodium sulfate of pesticide residue quality (Baker, Deventer, The Netherlands) was dried for 18 h at 300 °C before use. Sodium chloride of analytical grade from Scharlab was used after purification by heating at 300 °C overnight.

Leucine enkephalin and heptacose, used as LC and GC lock masses, respectively, were purchased from Sigma (St. Louis, MO).

Samples. Five honeybee samples (samples 1−5) from different sites of the Valencia area (Spain) suspected to be intoxicated by insecticide applications, together with one sample of nectarine flowers and leaves (sample 6) (possibly related to the sample 3 honeybee intoxication) were received at our laboratory to investigate the reason for the massive intoxications. Additionally, one sample of a supposedly blank honeybee was also provided. After reception of the samples at the laboratory, they were immediately frozen at −18 °C. Analyses were performed after 1 week.

Instrumentation. *GC-TOF MS.* An Agilent 6890N GC system (Palo Alto, CA) equipped with an Agilent 7683 autosampler was coupled to a time-of-flight mass spectrometer, GCT (Waters Corp., Milford, MA), operating in electron ionization (EI) mode (70 eV). The GC separation was performed using a fused silica HP-5MS capillary column with a length of 30 m × 0.25 mm i.d. and a film thickness of 0.25 μm (J&W Scientific, Folsom, CA). The oven temperature was programmed as follows: 90 °C (1 min); 5 °C/min to 260 °C; 40 °C/min to 300 °C (2 min). Splitless injections of 1 μL of sample were carried out. Helium was used as carrier gas at 1 mL/min. The interface and source temperatures were both set to 250 °C, and a solvent delay of 3 min was selected. The time-of-flight mass spectrometer was operated at 1 spectrum/s acquiring the mass range m/z 50−650 and using a multichannel plate (MCP) voltage of 2700V. TOF-MS resolution was about 8500 (fwhm) at m/z 612.

Heptacosa, used for the daily mass calibration as well as lock mass, was injected via syringe in the reference reservoir at 30 °C for this purpose. The m/z ion monitored was 218.9856.

UHPLC-QTOF MS. An ultraperformance liquid chromatography (UPLC) Acquity system (Waters) was interfaced to a QTOF mass spectrometer (QTOF Premier, Waters) using an orthogonal Z-spray-electrospray interface. The LC separation was performed using an Acquity UPLC BEH C_{18} 1.7 μm particle size analytical column 2.1 × 50 mm (Waters) at a flow rate of 300 μL/min. The mobile phase used was a time-programmed gradient using H_2O and MeOH, both 0.1 mM ammonium acetate. The percentage of organic modifier changed linearly from 5 to 90% in 5 min. The injection volume was 10 μL. Desolvation gas as well as nebulizing gas was nitrogen, obtained from a nitrogen generator. The desolvation gas flow was set at 800 L/h. TOF-MS resolution was ~10000 fwhm (V-mode) and 17500 fwhm (W-mode) at m/z 556. MS data were acquired over an m/z range of 50−1000 Da. The MCP detector potential was set to 1750 V in both positive and negative ionization modes. Capillary voltages of 3.5 and 3.0 kV were used in positive and negative ionization modes, respectively. A cone voltage of 20 V was selected. The interface temperature was set to 400 °C and the source temperature to 120 °C. A scan time of 0.1 s was chosen. An auto MS profile was performed. In this work, the automated attenuated function (dynamic range enhancement, DRE) was selected to correct possible mass peak saturations, allowing the exact mass measurement accuracy to be maintained within a wide concentration range. A collision energy ramp from 10 to 30 eV was used to perform MS/MS acquisitions.

Leucine enkephalin (approximately 2 mg/L, in 50:50 methanol/water) was introduced via the lock spray needle at a flow rate of 30 μL/min. The m/z ions monitored were 556.2771 and 554.2615 in positive and negative ionization modes, respectively. A cone voltage between 70 and 80 V was selected to obtain adequate signal intensity (around 400 counts/s) for this compound.

Calibration experiments are performed monthly using the built-in single-syringe pump, directly connected to the interface. Calibration from m/z 50 to 1000 was conducted in both ionization modes, with a mixture of NaOH 0.05M/HCOOH 10% (50:50) plus imazalil (m/z 297.0561) at 500 μg/L.

Analytical Procedure. The analytical procedure applied to the samples was based on that of ref *3*. Briefly, 1.5 g of honeybees (fresh weight) was homogenized with 15 g of anhydrous sodium sulfate and 0.5 g of Celite and extracted with 50 mL of acetone in a high-speed blender during 2 min (Ultraturrax T25, Janke and Kunkel, Germany). After filtration by gravity, a 25 mL aliquot was diluted with 50 mL of 2% aqueous NaCl (w/v) and extracted twice with 25 mL of dichloromethane. Organic extracts were preconcentrated in a turbo evaporator under a nitrogen stream at 40 °C until 5 mL. Then, 2 mL aliquots were evaporated to dryness under a gentle nitrogen stream at 40 °C. The final residue was dissolved in 1 mL of ethyl acetate (GC-MS analysis) and in 1 mL of methanol for (LC-MS analysis). In the case of LC-MS, the extract was 10-fold diluted with water before injection in the system to decrease the percentage of organic content.

RESULTS AND DISCUSSION

A First Case of Honeybee Poisoning. Two dead honeybee samples (samples 1 and 2), suspected to be poisoned by insecticide treatment, were received at our laboratory in January 2008. Additionally, one sample of alive honeybees collected from a surrounding area was also provided to be used as a blank sample. As there was no suspicious specific contaminants thought to be responsible for the honeybee poisoning, we applied a nontarget methodology to identify the potential compounds that might be present in the samples. This indicates that we did not work on a list of target compounds. For this purpose, as no information on selected analytes was introduced, specialized deconvolution software was required to detect the components in the sample. In this case ChromaLynx Application Manager was employed (see refs *16* and *22* for more information).

GC-TOF MS Nontarget Screening. Accurate mass GC-TOF MS data were submitted to an automatic nontarget screening by applying the previously mentioned software. This software automatically detected all peaks with a response over user-defined parameters, displayed their deconvoluted mass spectra to be searched in the library, and produced a hit list with positive matches (library match > 700 was used as criterium). To perform accurate mass confirmation/rejection of the library findings, the formula from the library hit was submitted to an elemental composition calculator and up to the five most intense ions were scored by exact mass measurement. Following the described methodology, a large list of compounds was identified. Within this large list, a positive finding of the insecticide coumaphos was detected in both samples (**Table 1**). Two nominal mass libraries were used for this search, the NIST library and a homemade library, which includes around 1000 compounds, many of them pesticides. **Table 2** shows the accurate mass confirmation of

Table 1. Compounds Detected in the Honeybee/Nectarine Samples by GC-TOF MS and UHPLC-(Q)TOF MS

sample	sample type	pesticides identified by GC-TOF MS	pesticides identified by UHPLC-(Q)TOF MS
1	honeybees	coumaphos	coumaphos
2	honeybees	coumaphos	coumaphos
3	honeybees	coumaphos	coumaphos
4	honeybees	fenitrothion, coumaphos	coumaphos
5	honeybees	fenitrothion, chlorfenvinphos, methiocarb	chlorfenvinphos
6	nectarine flowers and leaves	pyriproxyfen, thiamethoxam	pyriproxyfen, thiamethoxam

Table 2. GC-TOF MS Confirmation of Coumaphos and Metabolites in Honeybee Samples 1 and 2[a]

					deviation (mDa)	
compd	mol formula	mol mass	elemental composition	theor *m/z*	sample 1	sample 2
1 (coumaphos)	$C_{14}H_{16}ClO_5PS$	362.0145	$C_{14}H_{16}ClO_5PS$	362.0145	−0.2	−0.2
			$C_{14}H_{16}{}^{37}ClO_5PS$	364.0119	−2.0	0.5
			$C_{12}H_{12}O_5SClP$	333.9832	0.3	−1.3
			$C_{10}H_8ClO_5PS$	305.9519	1.2	1.6
			$C_{10}H_7O_2SCl$	225.9855	−2.2	0.2
2 (CMHC)	$C_{10}H_7ClO_3$	210.0084	$C_{10}H_7ClO_3$	210.0084	0.3	nd
			$C_{10}H_7{}^{37}ClO_3$	212.0057	−0.7	
			$C_9H_7ClO_2$	182.0135	1.1	
			C_8H_7ClO	154.0185	−2.2	
			$C_9H_7O_2$	147.0446	2.0	
5 (coumaphos-oxon)	$C_{14}H_{16}ClO_6P$	346.0373	$C_{14}H_{16}ClO_6P$	346.0373	nd	nd
			$C_{12}H_{12}ClO_6P$	318.0060		
			$C_{10}H_8ClO_6P$	289.9747		
			$C_{10}H_7ClO_3$	210.0084		
			$C_9H_7ClO_2$	182.0135		
6 (potasan)	$C_{14}H_{17}O_5P$	328.0543	$C_{10}H_8O_2S$	192.0245	0.0	nd
			$C_{14}H_{17}O_5PS$	328.0543	−4.1	
			$C_{12}H_{13}O_5PS$	300.0221	0.0	
			$C_{10}H_9O_5PS$	271.9908	−1.3	
			$C_{10}H_8O_3$	176.0473	0.0	
7 (4-methylumbelliferone)	$C_{10}H_8O_3$	176.0473	$C_{10}H_8O_3$	176.0473	nd	nd
			$C_9H_8O_2$	148.0524		
			$C_7H_4O_2$	120.0211		

[a] Mass fragments and mass errors for the proposed compounds. nd, not detected.

coumaphos in the honeybee samples 1 and 2, for which the mass errors obtained for five representative ions were lower than 2.2 mDa in all cases. Additionally, when the ion intensity ratios of the positive finding in samples were compared with those from a reference standard, all deviations were within tolerances proposed in European Commission Decision 2002/657/EC (*31*). Finally, retention times for the reference standards and peak sample were also compared, presenting a deviation of < 0.5%. As an example, **Figure 1** shows the positive finding of coumaphos in honeybee sample 1 when using the deconvolution process. Accurate mass confirmation automatically performed by the software for five representative ions led to the confirmation of the identity of coumaphos with mass errors normally lower than 2 mDa for every ion.

UHPLC-TOF MS Nontarget Screening. Accurate mass UHPLC-TOF MS data were also processed in a nontarget way by applying the ChromaLynx Application Manager. The only difference with respect to the GC-MS approach was the use of only the two most intense ions (softer ionization in ESI in comparison to EI) and the use of a theoretical homemade library containing around 500 contaminants, including 377 pesticides and 40 transformation products, but also 47 antibiotics, 20 pharmaceuticals, and other emerging contaminants frequently detected in the environment, such as cocaine or caffeine (*22*). This library was built without the need of injected standards, and it shows the theoretical spectrum with information on molecular ion mass and the expected isotopic pattern. The insecticide coumaphos was also found in both samples, by applying UHPLC-TOF MS (see **Table 1**).

The availability of a QTOF instrument made it feasible to perform MS/MS experiments for both standards and samples and to go further in the confirmation of the identity of the compound detected. As an example, **Figure 2** and **Table 3** illustrate the ultimate confirmation achieved in honeybee sample 1 suspected to be positive for coumaphos. Deviations in the measured masses of all product ions were lower than 2.3 mDa. Additionally, when the relative abundances in the suspected positive sample were compared with those of a reference standard, all deviations were within the limits proposed by European Decision 2002/657/EC (*31*). Finally, retention times for the reference standards and peak sample were also compared, presenting a deviation of < 2.5%. Therefore, this sample was confirmed by QTOF to be positive for coumaphos in a highly reliable way.

Metabolite Investigation. The high levels of coumaphos found in both samples encouraged us to investigate the presence of its metabolites by both GC-TOF MS and UHPLC-(Q)TOF MS.

GC-TOF MS Studies. As no specialized software for metabolism studies was available for GC-TOF MS, potential metabolites of coumaphos were investigated in a post-target way, that is, by searching for specific compounds after MS data acquisition, based on information available in the scientific literature.

Several metabolites of coumaphos in human urine, soils, and animals have been described (*32*, *33*) as the *dechlorination* product (potasan, compound **6**), the metabolite resulting after *hydrolysis* of the ester moiety (3-chloro-7-hydroxy-4-methyl-chromen-2-one, CMHC, compound **2**), the *hydrolysis* plus *dechlorination* product (4-methylumbelliferone, compound **7**), and the sulfur atom oxidation product (coumaphos oxon, compound **5**) (see **Figure 3**). To perform an investigation of the presence of these metabolites in honeybee samples, EI spectra of each described analyte were searched for in the NIST library. Up to five *m*/*z* ions

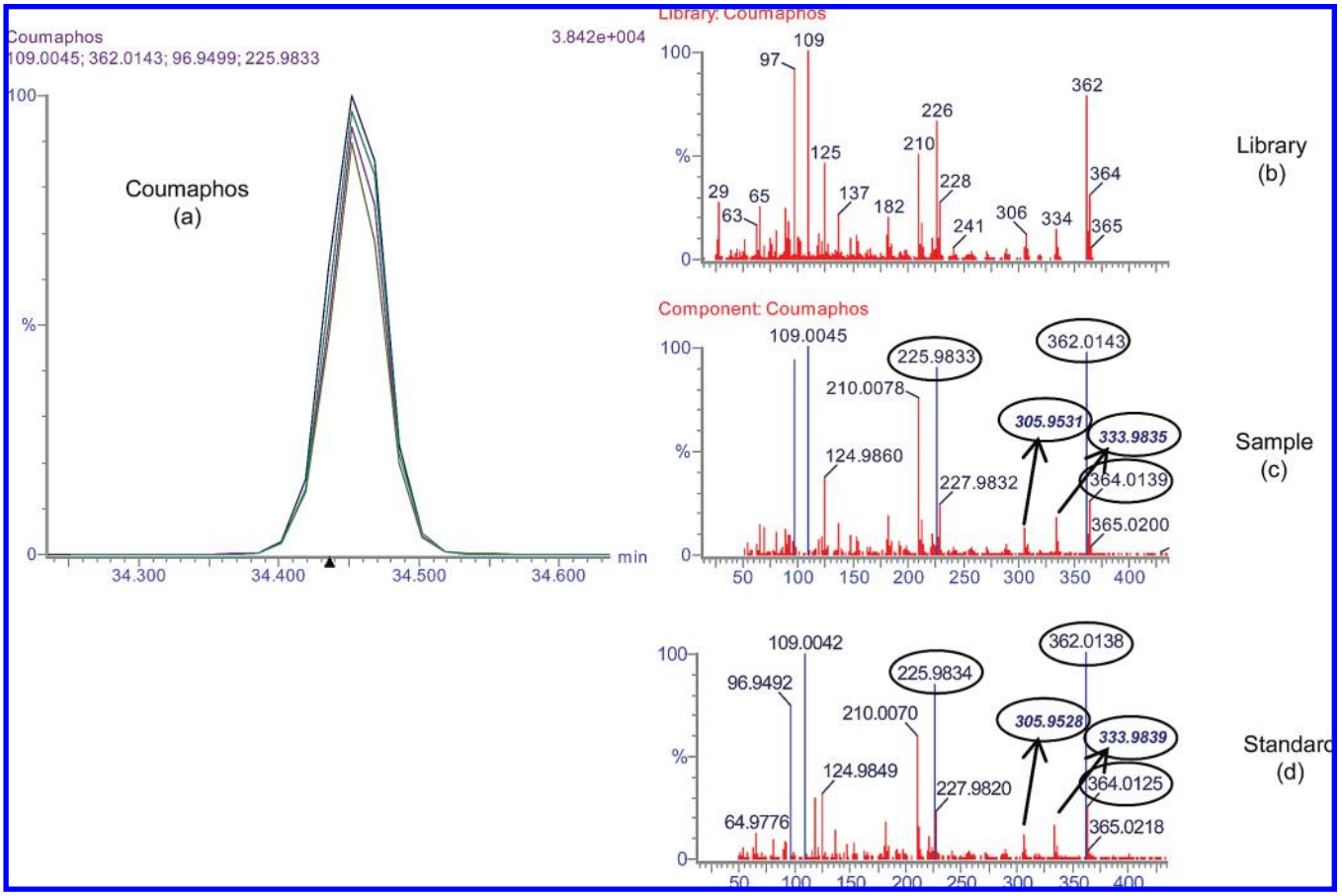

Figure 1. Confirmation of coumaphos in honeybee sample 1 by GC-TOF MS: (**a**) extracted ion chromatograms for four ions used for deconvolution; (**b**) library mass spectrum at nominal masses; (**c**) deconvoluted accurate mass spectrum for the compound detected; (**d**) accurate mass spectrum of a coumaphos reference standard in solvent.

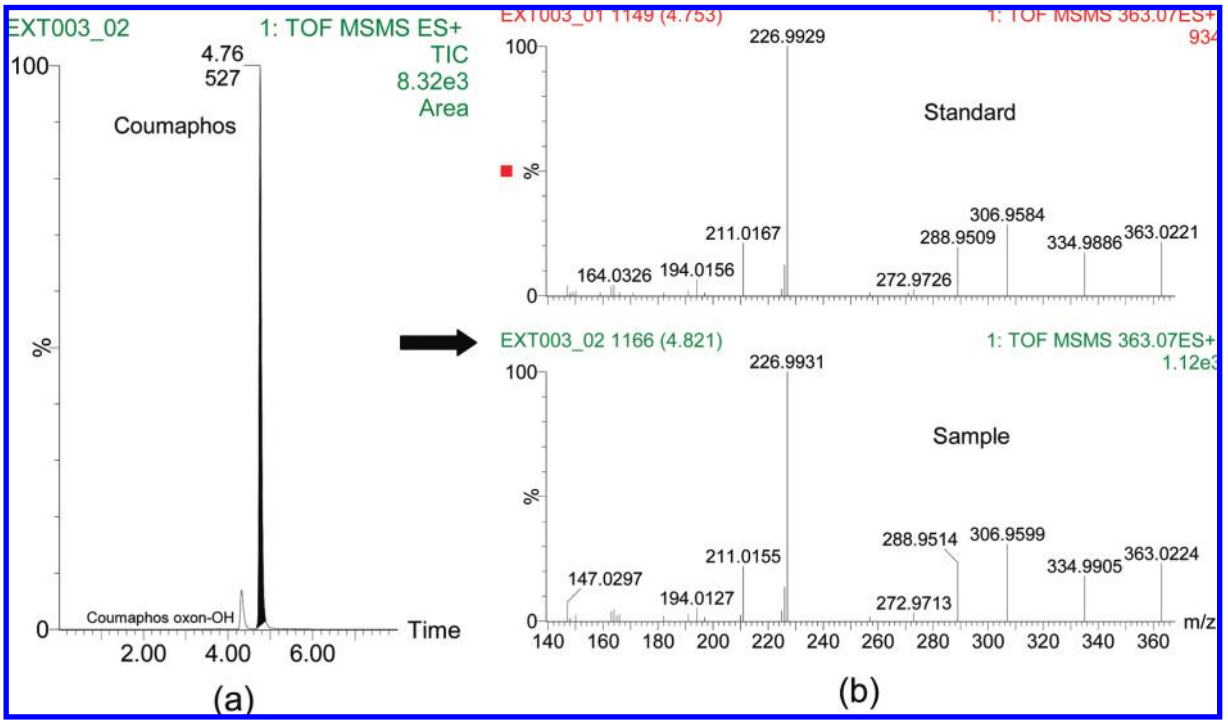

Figure 2. Confirmation of coumaphos in honeybee sample 1 by UHPLC-QTOF MS: (**a**) UHPLC-QTOF MS/MS chromatogram from the sample; (**b**) product ion spectrum (precursor ion *m*/*z* 363) from the sample and from the reference standard.

Table 3. UHPLC-(ESI)-QTOF MS Confirmation of Coumaphos and Metabolites in Honeybee Samples 1 and 2[a]

			deviation (mDa)				deviation (mDa)	
compd	elemental composition precursor ion $[M + H]^+$	theor mass precursor ion $[M + H]^+$	sample 1	sample 2	elemental composition product ion	theor mass product ion	sample 1	sample 2
1 (coumaphos)	$C_{14}H_{17}O_5PSCl$	363.0223	0.1	−0.3	$C_{12}H_{13}O_5PSCl$	334.9910	−0.5	0.8
					$C_{10}H_9O_5PSCl$	306.9597	−0.2	−0.3
					$C_{10}H_7O_4PSCl$	288.9491	−2.3	0.9
					$C_{10}H_8O_2SCl$	226.9934	−0.3	−0.2
					$C_{10}H_8O_3Cl$	211.0162	0.7	1.0
					$C_{10}H_7O_2Cl$	194.0135	0.8	0
2 (CMHC)	$C_{10}H_8O_3Cl$	211.0162	0.8	1.5	$C_9H_8O_2$	148.0524	0.9	
					C_9H_7O	131.0497	0	
					C_8H_7O	119.0497	−1.2	
					C_8H_7	103.0548	0.5	
					C_7H_7	91.0548	−0.1	
3 (coumaphos-OH)	$C_{14}H_{17}O_6PSCl$	379.0172	1.9	0.8	$C_{12}H_{13}O_6PSCl$	350.9862	0.8	
					$C_{10}H_9O_6PSCl$	322.9557	1.0	
					$C_{10}H_7O_5PSCl$	304.9457	0.6	
					$C_{10}H_8O_3SCl$	242.9892	−0.8	
					$C_9H_8O_5P$	227.0096	1.5	
4 (coumaphos oxon-OH)	$C_{14}H_{17}O_7PCl$	363.0400	−1.5	0.9	$C_{12}H_{13}O_7PCl$	335.0087	0.2	
					$C_{10}H_9O_7PCl$	306.9774	1.5	
					$C_{10}H_7O_6PCl$	288.9669	1.9	
					$C_{10}H_8O_4Cl$	227.0111	0.5	
					$C_{10}H_6O_3Cl$	209.0005	0.8	
					$C_9H_6O_2Cl$	181.0051	0.5	
6 (potasan)	$C_{14}H_{18}O_5PS$	329.0613	3.6	nd	$C_{12}H_{14}O_5PS$	301.0300	1.7	
					$C_{14}H_{16}O_5S$	296.0718	1.8	
					$C_{10}H_{10}O_5PS$	272.9987	3.6	
					$C_{10}H_8O_4PS$	254.9881	2.1	

[a] Mass fragments and mass errors for the proposed compounds. nd, not detected.

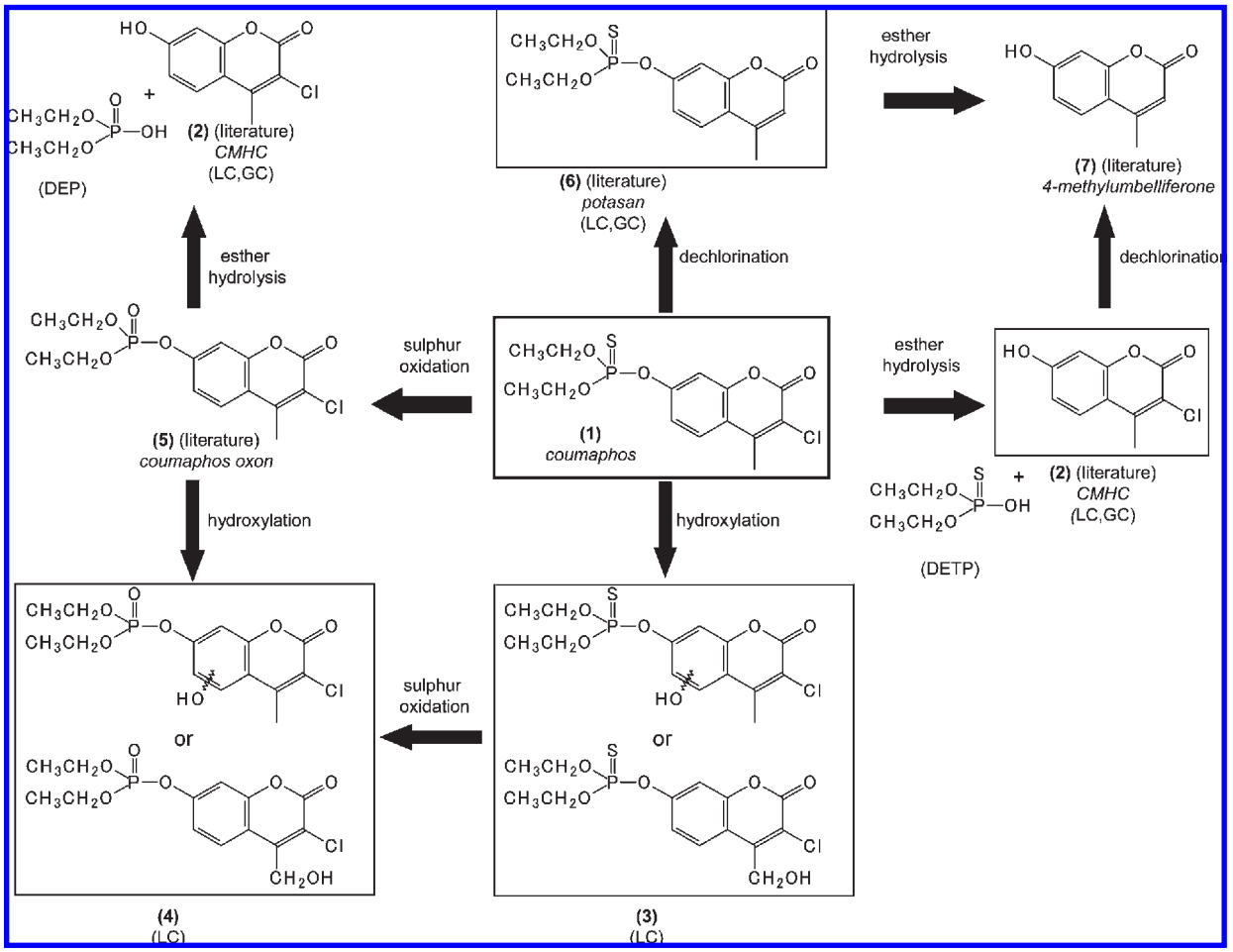

Figure 3. Coumaphos metabolites identified in honeybees. Proposed degradation pathway. "LC, GC" indicates that the metabolite was identified in the honeybee sample in the present work.

(molecular ion, if available, and fragment ions) were chosen from available nominal library spectrum. A possible elemental composition of those selected m/z ions was deduced, and their exact mass calculated and introduced in a target processing method (see **Table 2**). Experimental GC-TOF MS data were then submitted to the developed post-target processing method, and the presence of the selected ions (nw-XIC of 0.02 Da) in the sample extract was tested. Analyte confirmation was performed by comparison of the experimental intensity ratios in samples with the theoretical ones, calculated from the library spectrum. Additionally, mass accuracy for the most characteristic ions was evaluated.

This approach was applied for the investigation of CMHC, 4-methylumbelliferone, and coumaphos oxon, as their EI spectra were available in the NIST library. The CMHC metabolite was found in sample 1, which showed a chromatographic peak for the five preselected ions at the same retention time with the ion intensity ratios within specified tolerance. Besides, mass errors for these ions were always below 2 mDa (see **Table 2**). However, no signal was observed for the other two described analytes. Investigation of metabolite potasan (compound **6**) was more difficult as no previous information about its EI spectrum was available in the library. Although no data about the abundance of the molecular ion in potasan EI spectrum were known, a nw-XIC at its theoretical exact m/z (328.0543) was performed. As a notable chromatographic peak appeared at 12.65 min, a background-subtracted combined spectrum for this peak was performed. Accurate masses from this spectrum were submitted to an elemental composition calculation program to obtain elemental compositions, which were compared to the theoretical ones. The resulting elemental compositions fit well with possible fragments of potasan, with low mass errors, as **Figure 4** shows, leading to the conclusion that the compound detected in honeybee sample 1 was the metabolite potasan.

UHPLC-(Q)TOF MS Studies. Regarding LC-MS, data were processed using MetaboLynx software (Micromass v 4.1), which has been proved to be useful in previous pesticide degradation/metabolism studies (*34*). Two LC-MS data files (one corresponding to the sample and the other one to a blank sample) are compared, and the differences resulting from the presence of new compounds, which could be in principle attributed to transformation processes in the sample, are highlighted. Following the approach applied in previous works for compounds detected by MetaboLynx, the accurate mass of protonated/deprotonated molecules was determined on the basis of averaged spectra obtained in the TOF MS survey scan. On the basis of their accurate mass, possible elemental compositions of the peaks of interest were calculated using the elemental composition calculator with a maximum deviation of 2 mDa from the measured mass. Maximum and minimum parameter settings for all compounds were restricted as a function of the structure of coumaphos: C, 0−14; H, 0−18; O, 0−10; P, 0−1; and S, 0−1. The appropriate number of Cl was determined from the observed isotopic pattern and added if required. The possibility of performing MS/MS experiments helped us to elucidate the structure of several metabolites thanks to the information given by the product ion spectrum with the exact mass of the fragments.

According to the metabolites detected in honeybee samples 1 and 2 by UHPLC, four important processes were found to occur

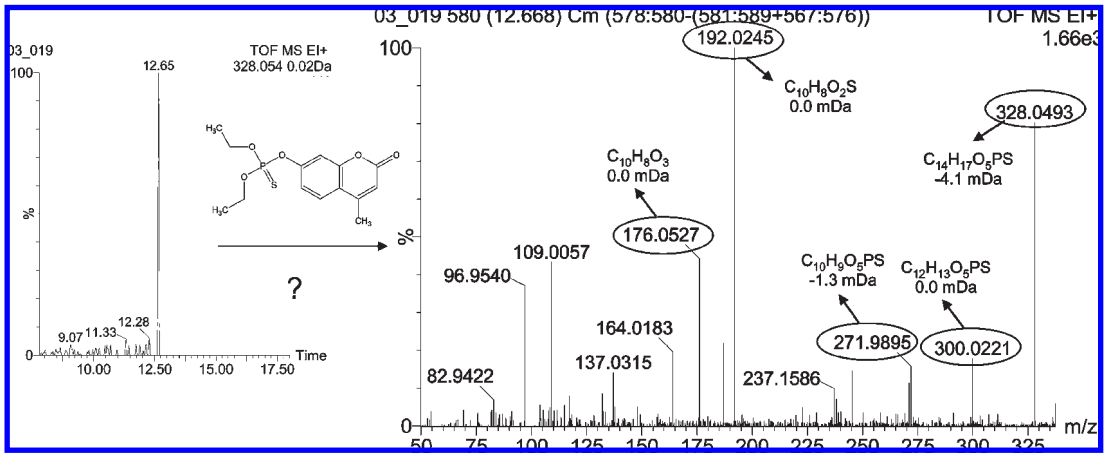

Figure 4. Positive finding of the coumaphos metabolite potasan in honeybee sample 1.

in the metabolism of coumaphos in honeybees, as can be seen in **Figure 3**: *hydrolysis* of the ester moiety, *hydroxylation*, *oxidation* of the sulfur atom, and *dechlorination*. A combination of these processes was also observed.

The *hydrolysis* of the ester moiety originated CMHC (compound **2**). However, the metabolite diethyl thiophosphate (DETP) was not found probably due to the low sensitivity for alkylphosphates in negative electrospray interfaces and the need to add an ion-pairing reagent to obtain a good chromatographic separation (*35*). As shown in **Figure 3**, *hydroxylation* was observed in the aromatic or in methyl group (compound **3**), as explained in more detail in following paragraphs. A combination of *oxidation* of the sulfur atom on the P=S functional group plus *hydroxylation* was also observed (compound **4**). Finally, our data suggested a loss of the chlorine atom (potasan, compound **6**).

In this paper, the potential of TOF MS was useful to distinguish between compounds **1** and **4**. Both have the same nominal mass (*m*/*z* 363), and therefore they would be indistinguishable by quadrupole instruments. However, their accurate masses (*m*/*z* 363.0219 compound **1**, *m*/*z* 363.0385 compound **4**) showed a difference of 16.6 mDa, which was sufficient for an appropriate identification. After the application of elemental composition calculator with the selected parameters (maximum deviation = 5 mDa), only one hit appeared for each compound. Thus, it was easy to assign their correct elemental composition.

The results obtained in LC-(Q)TOF experiments are summarized in **Table 3**. This table illustrates the identification of the parent compound and four metabolites detected. As can be seen, most of deviations were ≤1 mDa, with the highest values observed for metabolite **6** (potasan). With all of these data, with a minimum of four ions for each compound, one can be confident about the elemental composition given for each analyte.

Despite the capability of TOF analyzers to distinguish between isobaric compounds (mass differences of < 1 Da), its usefulness is limited when dealing with isomers, as they present the same molecular formula and, consequently, the same mass. However, hybrid QTOF instruments give the possibility of performing tandem MS acquisitions obtaining product ion spectra with accurate mass, which in some cases can help to differentiate between isomeric analytes in a more confident way than when using nominal mass instruments.

We performed MS/MS experiments with the QTOF to investigate the chemical structure of compound **3**. Comparing the elemental composition of coumpahos ($[M + H]^+$ $C_{14}H_{17}O_5PSCl$) with the calculated composition for compound **3** ($[M + H]^+$ $C_{14}H_{17}O_6PSCl$, *m*/*z* 379.0172), one can predict this compound is a monohydroxylated product of coumaphos. However, there was no information on where the hydroxylation occurred: in the aromatic methyl group, in the ethyl group of the thiophosphoric esther, or in the aromatic group. To elucidate this metabolite, MS/MS experiments on the precursor ion $C_{14}H_{16}O_6PS^{35}Cl$ (*m*/*z* 379) were carried out. In addition, MS/MS experiments on the isotopic peak ($C_{14}H_{16}O_6PS^{37}Cl$, *m*/*z* 381) were also performed to learn the product ions that maintained the chlorine atom. These experiments were useful and allowed some candidates to be discarded, as in several cases two plausible elemental compositions (one with a chlorine atom and the other without) were feasible. In a similar way, MS/MS experiments for all metabolites were carried out.

Product ion spectrum of compound **3** ($[M + H]^+$ $C_{14}H_{17}O_6PSCl$, *m*/*z* 379.0191) (see **Figure 5**) showed fragment ions at *m*/*z* 350.9870 (ΔmDa = 0.8, with regard to the theoretical exact mass), 322.9567 (ΔmDa = 1.0), and 304.9463 (ΔmDa = 0.6), which resulted from losses of one ethyl group, two ethyl groups, and two ethyl groups plus water from the precursor ion *m*/*z* 379.0191, respectively, showing that the hydroxylation could not occur in the ethyl radicals. Performing MS/MS experiments of both precursor ions (corresponding to ^{35}Cl and ^{37}Cl) led to useful information. Thus, the fragment ion *m*/*z* 242.9884 was initially assigned to $C_9H_8O_4PS$, which would have resulted from a hydroxylation in the aromatic ring. However, after performing MS/MS experiments (precursor ion *m*/*z* 381, ^{37}Cl), we observed that this fragment maintained the chlorine atom, being therefore assigned to $C_{10}H_8O_3SCl$ instead of $C_9H_8O_4PS$. Then two possibilities (hydroxylation in the methyl group and hydroxylation in the aromatic ring) were still feasible. Something similar occurred with compound **4** ($C_{14}H_{17}O_6PCl$). Data obtained in the MS/MS spectra were not sufficient to discover if the hydroxylation had occurred in the aromatic ring or in the methyl group.

In a similar way, MS/MS experiments were carried out for all metabolites. Although between 8 and 12 product ions were justified for each compound, only the most abundant ones are shown in **Table 3**.

Other Cases of Poisoning. Three additional honeybee samples (samples 3−5) also suspected to be intoxicated by insecticide applications, together with one sample of nectarine flowers and leaves (sample 6) (supposedly responsible for the sample 3 honeybee intoxication), were received at our laboratory a few months after the first poisoning case. These samples were investigated following the above-mentioned methodology.

Regarding GC-TOF MS analysis, positive findings of coumaphos, fenitrothion, chlorfenvinphos, and methiocarb were found in the honeybee samples. In the nectarine flower and leaf sample, pyriproxyfen and thiamethoxam were found (**Table 1**). As an

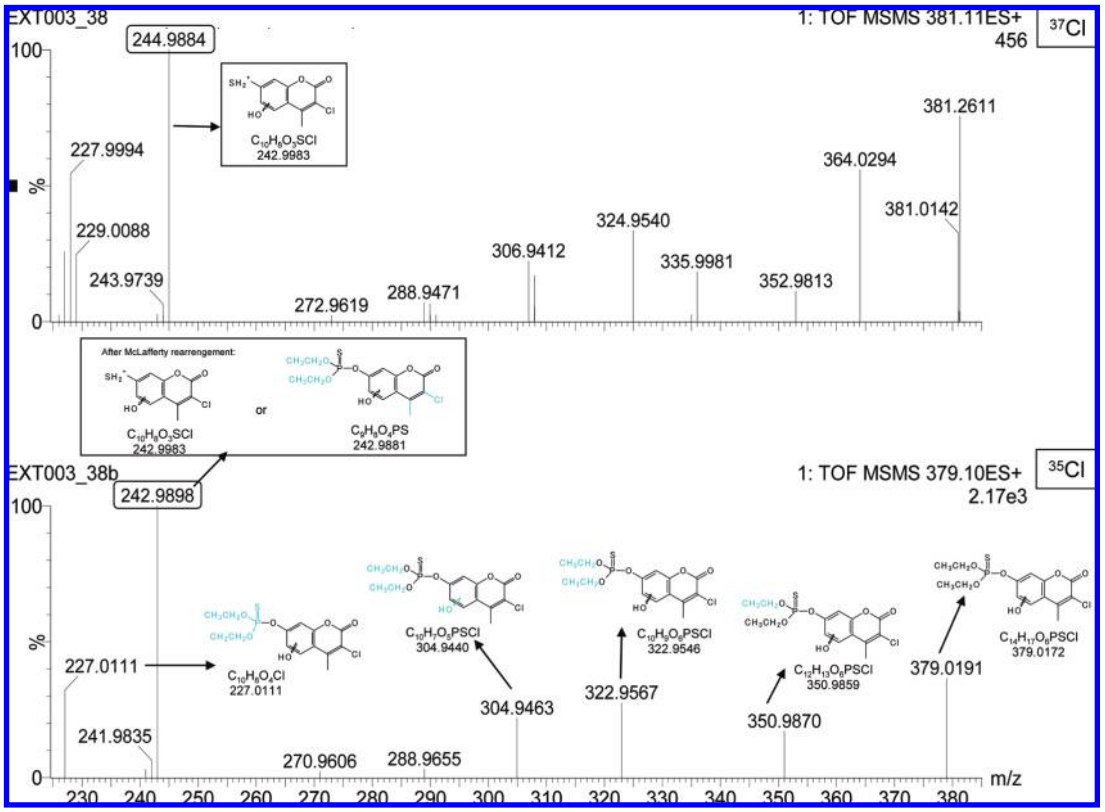

Figure 5. Product ion spectra of the coumaphos precursor ions (**a**) *m/z* 379 (^{35}Cl) and (**b**) *m/z* 381 (^{37}Cl) from sample 1.

illustrative example, **Table 4** shows the confirmation of pesticides detected in honeybee sample 5 and in the nectarine sample (sample 6). Mass errors for every ion were typically below 2 mDa, except for a few low-abundant ions. Additionally, when the ion intensities of findings in samples were compared with the theoretical ones from reference standards, all deviations were within maximum tolerances (*31*). **Figure 6** shows the positive findings of methiocarb and fenitrothion in honeybee sample 5 when using the deconvolution process.

With regard to UHPLC-TOF MS analysis, among the three samples of honeybee, previous positive GC-TOF MS findings of coumaphos and chlorfenvinphos were confirmed. In the nectarine sample, the presence of pyriproxyfen and thiamethoxam was also confirmed (see **Table 1**). As an example, **Figure 7** and **Table 4** show the safe confirmation achieved in the nectarine sample suspected to be positive for thiamethoxam and pyriproxyfen. Deviations in the measured masses of all product ions were lower than 2 mDa, except for one product ion of thiamethoxam. Additionally, all relative ion abundances observed in the positive sample were within the maximum values allowed (*31*). In sample 5, no MS/MS experiments were possible a priori for chlorfenvinphos due to the low level found. To confirm the presence of this compound, MS/MS experiments were carried out but with the raw extract in 100% methanol to avoid the 10-fold dilution. Regarding fenitrothion and methiocarb, these compounds were not detected by LC-MS.

Validation of the Confirmation Process. To validate the applicability of the procedure used—confirmation of nontarget detected compounds—six honeybee blank samples were spiked at two concentration levels: 1 μg/g (n = 3) and 10 μg/g (n = 3). The "blank" sample was previously analyzed, and no presence of the analytes was found. The spiked samples were extracted and analyzed as previously described and injected in GC-TOF MS and UHPLC-QTOF MS. Six insecticides were studied by GC-TOF MS and four by UHPLC-QTOF MS (see **Table 1**).

The presence of up to five ions measured at their accurate mass (nw-XIC of 0.02 Da) was evaluated for the six replicates at the two levels tested. Additionally, their intensity ratios were compared to the average ratios calculated from reference standards in solvent: six injections of a 1.5 mg/L standard in GC-TOF MS with RSD < 20% and two injections of standards at four concentration levels (0.15, 0.3, 1.5, and 3.0 mg/L) in UHPLC-QTOF with RSD < 16%. Although European Comission Decision 2002/657/EC (*31*) requires the attainment of at least one ion ratio deviation, in this study up to four ratio deviations were measured. In all cases, experimental ion ratios in spiked samples, at the two concentration levels, were in agreement with those obtained for reference standards in solvent. All deviations were within the specified tolerances accepted by European guidelines. Data obtained showed that the correct identification and confirmation of analytes could be successfully performed at the concentration range assayed.

In summary, the combination of GC-TOF MS and UHPLC-(Q)TOF MS has been shown as an advanced tool for the screening and confirmation of nontarget analytes in honeybee samples. Without previous selection of the analytes to be searched, the methodology employed (based on a peak deconvolution process followed by a library search and accurate mass scoring) allowed the discovery of the presence of some pesticides, such as pyriproxyfen, chlorfenvinphos, or coumaphos, among others. In addition, the potential of these techniques has been proved by the fact that several pesticide metabolites were also discovered in poisoned honeybee samples. The availability of commercial libraries with more than 150,000 EI spectra normally makes easier the identification of nontarget analytes when using GC-TOF MS instruments without injecting reference standards. Many detected compounds are normally found in the library, and the accurate mass measurements generated by TOF MS help the confirmation of the identified analyte. The possibility of performing a safe identification and confirmation in a unique

Table 4. Confirmation of Pesticides Identified in Honeybee Sample 5 and Nectarine Sample (Sample 6)[a]

GC-TOF MS

	molecular peak		ion 1		ion 2		ion 3		ion 4		ion 5	
compd	mol formula	mol mass	elemental composition	theor *m*/*z* (error in mDa)	elemental composition	theor *m*/*z* (error in mDa)	elemental composition	theor *m*/*z* (error in mDa)	elemental composition	theor *m*/*z* (error in mDa)	elemental composition	theor *m*/*z* (error in mDa)
fenitrothion (sample 5)	$C_9H_{12}NO_5PS$	277.0174	$C_2H_6O_2PS$	124.9826 (1.1)	$C_2H_6O_3P$	109.0055 (0.8)	$C_9H_{12}NO_5PS$	277.0174 (2.4)	$C_9H_{11}NO_4PS$	260.0146 (2.8)	CH_4O_2P	78.9949 (0.9)
methiocarb (sample 5)	$C_{11}H_{15}NO_2S$	225.0824	$C_9H_{12}OS$	168.0609 (0.8)	C_8H_9OS	153.0374 (1.8)	C_7H_9O	109.0653 (1.4)	$C_{11}H_{15}NO_2S$	225.0824 (4.1)		
chlorfenvinphos (sample 5)	$C_{12}H_{14}Cl_3O_4P$	357.9695	$C_8H_6Cl_2O_4P$	266.9381 (0.4)	$C_8H_6{}^{35}Cl^{37}ClO_4P$	268.9353 (−0.2)	$C_{12}H_{14}Cl_2O_4P$	323.0007 (−0.7)	$C_{10}H_{10}Cl_2O_4P$	294.9694 (−1.4)	$C_{12}H_{14}{}^{35}Cl^{37}ClO_4P$	324.9980 (−1.7)
thiamethoxam (sample 6)	$C_8H_{10}ClN_5O_3S$	291.0193	$C_8H_{10}N_3O_2S$	212.0494 (−1.2)	C_4H_3NSCl	131.9675 (0.3)	$C_7H_8N_3OS$	182.0388 (−1.0)	$C_8H_{10}N_3O_2SCl$	247.0182 (−0.1)	$C_8H_{10}N_3O_2S^{37}Cl$	249.0153 (1.1)
pyriproxyfen (sample 6)	$C_{20}H_{19}NO_3$	321.1365	$C_8H_{10}NO$	136.0762 (1.0)	$C_{12}H_{10}O_2$	186.0681 (−0.3)	$C_{15}H_{14}O_2$	226.0994 (0.9)				

UHPLC-QTOF MS

	parent ion		product ion 1		product ion 2		product ion 3		product ion 4		product ion 5	
compd	mol formula	theor *m*/*z* (error mDa)	elemental composition	theor *m*/*z* (error in mDa)	elemental composition	theor *m*/*z* (error in mDa)	elemental composition	theor *m*/*z* (error in mDa)	elemental composition	theor *m*/*z* (error in mDa)	elemental composition	theor *m*/*z* (error in mDa)
chlorfenvinphos (sample 5)	$C_{12}H_{16}O_4PCl_3$	358.9774 (−1.5)	$C_8H_4Cl_3$	204.9379 (1.0)	$C_4H_{12}O_4P$	155.0473 (1.0)	$C_2H_8O_4P$	127.0160 (−0.4)	PO_4H_4	98.9847 (−0.8)	$C_8H_4Cl_2$	169.9690 (−1.8)
thiametoxam (sample 6)	$C_8H_{11}N_5O_3SCl$	292.0271 (0.8)	$C_8H_{11}N_4OS$	211.0654 (0.1)	$C_7H_9N_4S$	181.0548 (0)	C_4H_3NSCl	131.9661 (−0.9)	$C_6H_8N_3$	122.0718 (−0.1)	$C_6H_6N_3S$	152.0282 (2.3)
pyriproxyfen (sample 6)	$C_{20}H_{20}NO_3$	322.1444 (0.1)	C_5H_6NO	96.0449 (−1.6)	$C_{15}H_{15}O_2$	227.1072 (−0.5)	$C_{20}H_{20}NO_3$	322.1444 (0.1)	$C_{12}H_9O_2$	185.0603 (−0.5)	$C_9H_{10}O$	134.0732 (−1.0)

[a] Mass fragments and mass errors for the compounds obtained by GC-TOF MS and UHPLC-ESI-QTOF MS.

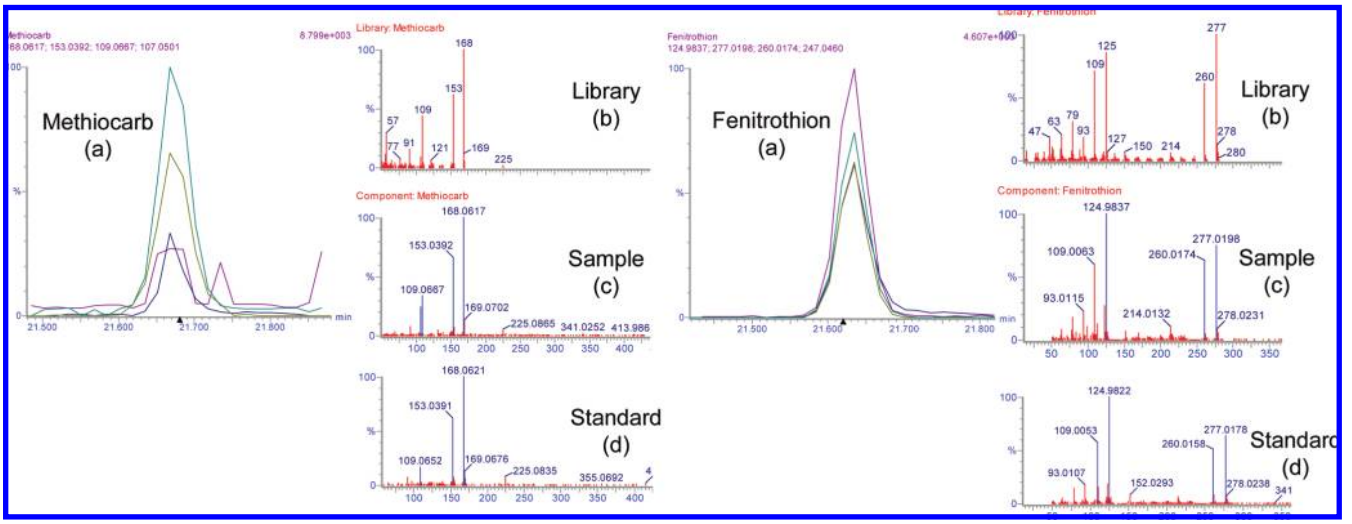

Figure 6. GC-TOF MS confirmation of positive findings of methiocarb and fenitrothion in honeybee sample 5: (**a**) extracted ion chromatograms for four ions used for deconvolution; (**b**) library mass spectrum at nominal masses; (**c**) deconvoluted accurate mass spectrum for compounds detected in the sample; (**d**) accurate mass spectrum of a reference standard in solvent.

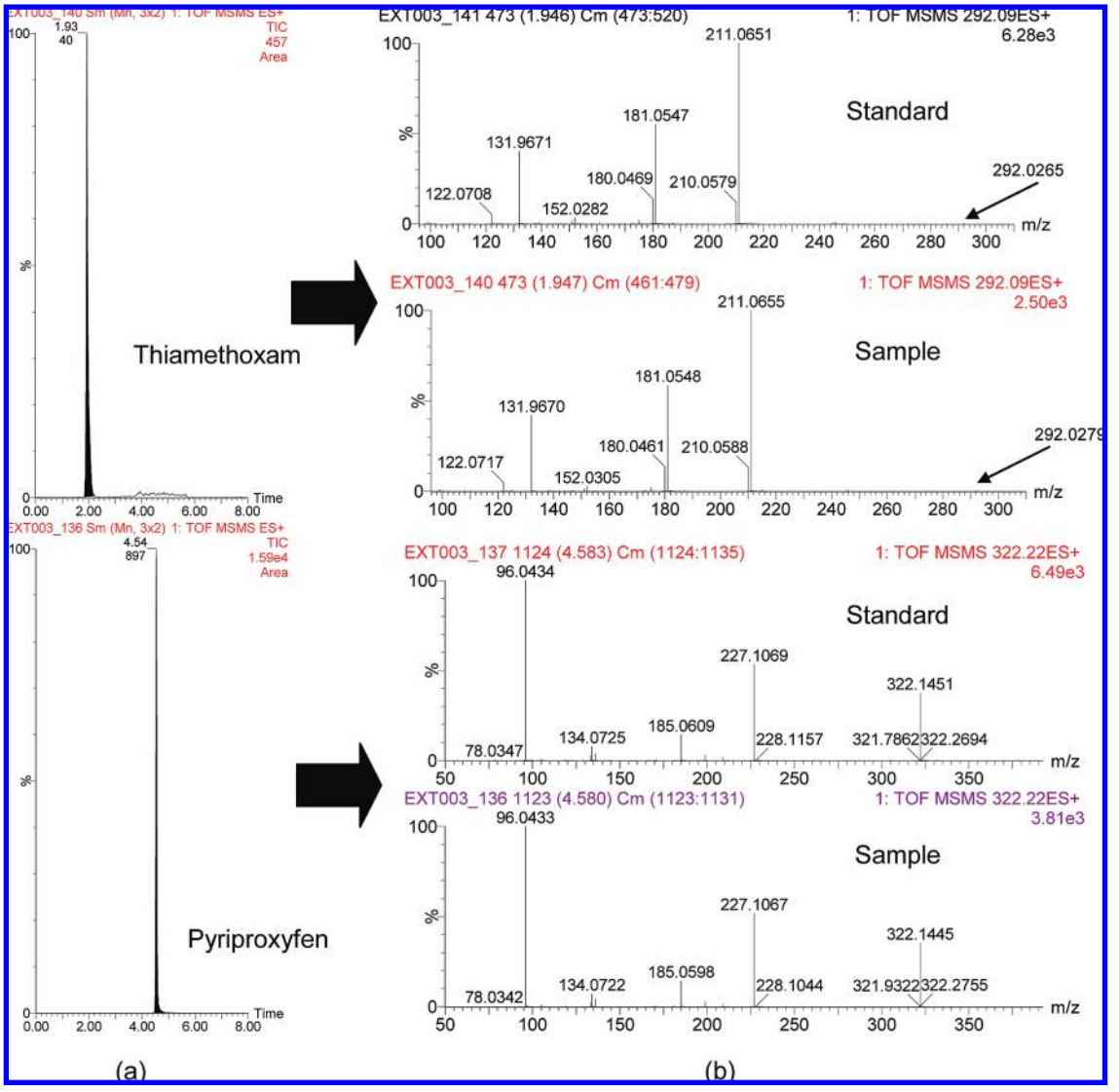

Figure 7. Confirmation of the two compounds detected in the nectarine sample (sample 6) by UHPLC-QTOF MS: (**a**) UHPLC-QTOF MS chromatograms from the sample; (**b**) product ion spectrum of the precursor ion (*m*/*z* 292 for thiamethoxam and *m*/*z* 322 for pyriproxyfen) from the sample and from the standard.

analysis is an advantage when using EI spectra, as the number of fragment ions available is normally enough for confirmation purposes. However, the elucidation of a compound that is not present in a library (as normally occurs for most metabolites) is more difficult, as no security in the presence of the molecular ion in the spectrum exists. However, the presence of the molecular ion in the LC-ESI-TOF MS spectrum is one of the main advantages of this technique, which facilitates the obtaining of the elemental composition of an "unknown" compound, both organic pollutants or their metabolites. Furthermore, the possibility of performing MS/MS experiments in QTOF instruments helps to elucidate and/or confirm the structure of the compound detected, as the product ion spectra with the exact mass of fragments is obtained, information that is very useful in the elucidation process.

In this work, making use of a nontarget approach, the insecticides fenitrothion, chlorfenvinphos, coumaphos, and methiocarb were found in the honeybee samples suspected to be poisoned by insecticide applications. Moreover, thiamethoxam and pyriproxyfen were identified in nectarine flowers and leaves, which were supposedly responsible for a honeybee intoxication case. Additionally, up to four metabolites of coumaphos were detected in one honeybee sample that contained high levels of parent coumaphos. To our knowledge, two of these metabolites had not been previously described in the available scientific literature.

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LITERATURE CITED

(1) Rancan, M.; Rossi, S.; Sabatini, A. G. Determination of thiamethoxam residues in honeybees by high performance liquid chromatography with an electrochemical detector and post-column photochemical reactor. *J. Chromatogr., A* **2006**, *1123*, 60–65.

(2) Morzycka, B. Simple method for the determination of trace levels of pesticides in honeybees using matrix solid-phase dispersion and gas chromatography. *J. Chromatogr., A* **2002**, *982*, 267–273.

(3) Fernández, M.; Picó, Y.; Girotti, S.; Manes, J. Analysis of organophosphorus pesticides in honeybee by liquid chromatography−atmospheric pressure chemical ionization−mass spectrometry. *J. Agric. Food Chem*. **2001**, *49*, 3540–3547.

(4) Lewis, G.; Thompson, H.; Smagghe, G. In focus: pesticides and honeybees—the work of the ICP-BR bee protection group editorial. *Pest Manag. Sci*. **2007**, *63*, 1047–1050.

(5) Fernández, M.; Padrón, C.; Marconi, L.; Ghini, S.; Colombo, R.; Sabatini, A. G.; Girotti, S. Determination of organophosphorus pesticides in honeybees after solid-phase microextraction. *J. Chromatogr., A* **2001**, *922*, 257–265.

(6) Hernández, F.; Portolés, T.; Pitarch, E.; López, F. J.; Beltrán, J.; Vázquez, C. Potential of gas chromatography coupled to triple quadrupole mass spectrometry for quantification and confirmation of organohalogen xenoestrogen compounds in human breast tissues. *Anal. Chem*. **2005**, *77*, 7662–7672.

(7) Sancho, J. V.; Pozo, O. J.; Hernández, F. Liquid chromatography and tandem mass spectrometry: a powerful approach for the sensitive and rapid multiclass determination of pesticides and transformation products in water. *Analyst* **2004**, *129*, 38–44.

(8) Frenich, A. G.; González-Rodríguez, M. J.; Arrebola, F. J.; Martínez Vidal, J. L. Potentiality of gas chromatography−triple quadrupole mass spectrometry in vanguard and rearguard methods of pesticide residues in vegetables. *Anal. Chem*. **2005**, *77*, 4640–4648.

(9) Pihlström, T.; Blomkvist, G.; Friman, P.; Pagard, U.; Österdahl, B.-. Analysis of pesticide residues in fruit and vegetables with ethyl acetate extraction using gas and liquid chromatography with tandem mass spectrometric detection. *Anal. Bioanal. Chem*. **2007**, *389*, 1773–1789.

(10) Petrović, M.; Hernando, M. D.; Díaz-Cruz, M. S.; Barceló, D. Liquid chromatography−tandem mass spectrometry for the analysis of pharmaceutical residues in environmental samples: s review. *J. Chromatogr., A* **2005**, *1067*, 1–14.

(11) Wang, J.; Leung, D. Analyses of macrolide antibiotic residues in eggs, raw milk, and honey using both ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry and high-performance liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom*. **2007**, *21*, 3213–3222.

(12) Alder, L.; Greulich, K.; Kempe, G.; Vieth, B. Residue analysis of 500 high priority pesticides: better by GC-MS or LC-MS/MS?. *Mass Spectrom. Rev*. **2006**, *25*, 838–865.

(13) Picó, Y.; Font, G.; Ruiz, M. J.; Fernández, M. Control of pesticide residues by liquid chromatography−mass spectrometry to ensure food safety. *Mass Spectrom. Rev*. **2006**, *25*, 917–960.

(14) Hernández, F.; Pozo, O. J.; Sancho, J. V.; López, F. J.; Marín, J. M.; Ibáñez, M. Strategies for quantification and confirmation of multiclass polar pesticides and transformation products in water by LC-MS^2 using triple quadrupole and hybrid quadrupole time-of-flight analyzers. *Trends Anal. Chem*. **2005**, *24*, 596–612.

(15) Sancho, J. V.; Pozo, O. J.; Ibáñez, M.; Hernández, F. Potential of liquid chromatography/time-of-flight mass spectrometry for the determination of pesticides and transformation products in water. *Anal. Bioanal. Chem*. **2006**, *386*, 987–997.

(16) Hernández, F.; Portolés, T.; Pitarch, E.; López, F. J. Target and nontarget screening of organic micropollutants in water by solid-phase microextraction combined with gas chromatography/high-resolution time-of-flight mass spectrometry. *Anal. Chem*. **2007**, *79*, 9494–9504.

(17) Portolés, T.; Pitarch, E.; López, F. J.; Sancho, J. V.; Hernández, F. Methodical approach for the use of GC-TOF MS for screening and confirmation of organic pollutants in environmental water. *J. Mass Spectrom*. **2007**, *42*, 1175–1185.

(18) Grange, A. H.; Sovocool, G. W. Identification of compounds in water above a pollutant plume by high-resolution mass spectrometry. *Environ. Forensics* **2007**, *8*, 391–404.

(19) Grange, A. H.; Genicola, F. A.; Sovocool, G. W. Utility of three types of mass spectrometers for determining elemental compositions of ions formed from chromatographically separated compounds. *Rapid Commun. Mass Spectrom*. **2002**, *16*, 2356–2369.

(20) Hernández, F.; Portolés, T.; Pitarch, E.; López, F. J. Searching for anthropogenic contaminants in human breast adipose tissues using gas chromatography-time-of-flight mass spectrometry. *J. Mass. Spectrom*. **2008**, *43*, 1–11.

(21) Fay, L. B.; Newton, A.; Simian, H.; Robert, F.; Douce, D.; Hancock, P.; Green, M.; Blank, I. Potential of gas chromatography−orthogonal acceleration time-of-flight mass spectrometry (GC-oa-TOFMS) in flavor research. *J. Agric. Food Chem*. **2003**, *51*, 2708–2713.

(22) Ibáñez, M.; Sancho, J. V.; Hernández, F.; McMillan, D.; Rao, R. Rapid non-target screening of organic pollutants in water by ultra-performance liquid chromatography coupled to time-of-light mass spectrometry. *Trends Anal. Chem*. **2008**, *27*, 481–489.

(23) Zhao, X.; Wang, W.; Wang, J.; Yang, J.; Xu, G. Urinary profiling investigation of metabolites with cis-diol structure from cancer patients based on UPLC-MS and HPLC-MS as well as multivariate statistical analysis. *J. Sep. Sci*. **2006**, *29*, 2444–2451.

(24) Jones, M. D.; Plumb, R. S. The application of sub-2-μm particle liquid chromatography-operated high mobile linear velocities coupled to orthogonal accelerated time-of-flight mass spectrometry for the analysis of ranitidine and its impurities. *J. Sep. Sci*. **2006**, *29*, 2409–2420.

(25) Yin, P.; Zhao, X.; Li, Q.; Wang, J.; Li, J.; Xu, G. Metabonomics study of intestinal fistulas based on ultraperformance liquid

chromatography coupled with Q-TOF mass spectrometry (UPLC/Q-TOF MS). *J. Proteome Res*. **2006**, *5*, 2135–2143.
(26) Picó, Y.; Farré, M. L.; Soler, C.; Barceló, D. Identification of unknown pesticides in fruits using ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry. Imazalil as a case study of quantification. *J. Chromatogr., A* **2007**, *1176*, 123–134.
(27) Fernández, M.; Picó, Y.; Mañes, J. Simultaneous determination of carbamate and organophosphorus pesticides in honeybees by liquid chromatography−mass spectrometry. *Chromatographia* **2003**, *58*, 151–158.
(28) Cabras, P.; Martini, M. G.; Floris, I.; Spanedda, L. Residues of cymiazole in honey and honey-bees. *J. Apic. Res*. **1994**, *33*, 83–86.
(29) Bogdanov, S.; Kilchenmann, V.; Imdorf, A. Acaricide residues in some bee products. *J. Apicult. Res*. **1998**, *37*, 57–67.
(30) Fernández, M.; Picó, Y.; Mañes, J. Rapid screening of organophosphorus pesticides in honey and bees by liquid chromatography−mass spectrometry. *Chromatographia* **2002**, *56*, 577–583.
(31) European Commission Decision 2002/657/EC, implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Off. J. Eur. Communities* 2002, *L221*, 8.
(32) Olsson, A. O.; Nguyen, J. V.; Sadowski, M. A.; Barr, D. B. A liquid chromatography/electrospray ionization-tandem mass spectrometry method for quantification of specific organophosphorus pesticide biomarkers in human urine. *Anal. Bioanal. Chem*. **2003**, *376*, 808–815.
(33) Jindal, T.; Singh, D. K.; Agarwal, H. C. Persistence, degradation and leaching of coumaphos in soil. *J. Environ. Sci. Health B* **2000**, *35*, 309–320.
(34) Ibáñez, M.; Sancho, J. V.; Pozo, O. J.; Hernández, F. Use of quadrupole time-of-flight mass spectrometry in environmental analysis: elucidation of transformation products of triazine herbicides in water after UV exposure. *Anal. Chem*. **2004**, *76*, 1328–1335.
(35) Hernández, F.; Sancho, J. V.; Pozo, O. J. Direct determination of alkyl phosphates in human urine by liquid chromatography/electrospray tandem mass spectrometry. *Rapid Commun. Mass Spectrom*. **2002**, *16*, 1766–1773.

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