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The Power of Hyphenated Chromatography/Time-of-Flight Mass Spectrometry in Public Health Laboratories

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Supporting Information

ABSTRACT: Laboratories devoted to the public health field have to face the analysis of a large number of organic contaminants/residues in many different types of samples. Analytical techniques applied in this field are normally focused on quantification of a limited number of analytes. At present, most of these techniques are based on gas chromatography (GC) or liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS). Using these techniques only analyte-specific information is acquired, and many other compounds that might be present in the samples would be ignored. In this paper, we explore the potential of time-of-flight (TOF) MS hyphenated to GC or LC to provide additional information, highly useful in this field. Thus, all positives reported by standard reference targeted LC−MS/MS methods were unequivocally confirmed by LC−QTOF MS. Only 61% of positives reported by targeted GC−MS/MS could be confirmed by GC−TOF MS, which was due to its lower sensitivity as nonconfirmations corresponded to analytes that were present at very low concentrations. In addition, the use of TOF MS allowed searching for additional compounds in large-scope screening methodologies. In this way, different contaminants/residues not included in either LC or GC tandem MS analyses were detected. This was the case of the insecticide thiacloprid, the plant growth regulator paclobutrazol, the fungicide prochloraz, or the UV filter benzophenone, among others. Finally, elucidation of unknowns was another of the possibilities offered by TOF MS thanks to the accurate-mass full-acquisition data available when using this technique.

KEYWORDS: *gas chromatography, liquid chromatography, time-of-flight mass spectrometry, wide-scope screening, public health, food-safety, water, confirmation, target and nontarget analysis*

1. INTRODUCTION

Over the past decade, food safety, always an important issue, has gained a higher profile following a number of highly publicized incidents all around the world, including dioxins in pork and milk products, contamination of foods or drinks with pesticides, or melamine in dairy products.[1] The use of mass spectrometry (MS) in combination with liquid chromatography (LC−MS) or gas chromatography (GC−MS) has played, and is still playing, a vital role to solve many problems related to food safety. Thus, a wide range of organic residues and contaminants (from pesticides in fruits and vegetables to veterinary drugs in meat, as representative examples) have been determined in many different sample matrices. These methods are normally based on the use of MS analyzers like single quadrupole, ion trap, and, in the past decade, triple quadrupole and are limited to a list of selected analytes that rarely includes more than 100−200 compounds. Using target methods can lead to ignoring other relevant contaminants that might be present in the samples. In addition, this approach increases the analysis time and costs, as a battery of target methods needs to be applied separately to the same sample as a consequence of the different chemical characteristics of the analytes and the intrinsic limitations of the MS analyzer. Nowadays, there is a need of developing wide-scope "universal" screening methods for contaminants in the public health field able to detect and identify a large list of contaminants. Time of flight (TOF) MS analyzers are among the most powerful analytical tools for this purpose.

In recent years, both hybrid quadrupole TOF (QTOF) and single TOF analyzers have been used for screening of pesticides,[2−7] pharmaceuticals,[8,9] antibiotics,[10] drugs of abuse,[11,12] and veterinary drugs[13−15] in different matrices. Most of these methods rarely exceed 100 analytes in their scope despite that TOF MS might be applied to a much larger number of compounds of different chemical families. Recently, Díaz et al.[16] developed a rapid wide-scope screening and identification UHPLC−QTOF MS method for more than 1000 organic pollutants (including pesticides, pharmaceuticals, drugs of abuse, mycotoxins, personal care products, etc.) in water, food, and urine samples taking advantage of the full-spectrum acquisition at accurate mass of this analyzer. Additionally, LC−QTOF MS has also been successfully used to identify nontarget compounds[17−19]

Regarding GC−TOF MS, most applications deal with high speed analyzers in quantitative GCxGC methods[20,21] rather than high resolution (HR) TOF MS. The high sensitivity in full-spectrum acquisition mode of TOF instruments is

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complemented, in the case of GC-HRTOF MS, with mass accuracy, which gives it extraordinary potential for qualitative purposes. Despite the excellent features of GC−HRTOF MS, it has seldom been explored for the investigation of organic contaminants and residues, until recently.[22,23] Almost all applications reported deal with the determination of persistent and other priority GC-amenable pollutants in environmental[24−26] and biological[27−29] samples. Recently, Portolés et al.[30] developed a multiclass screening method for 150 organic contaminants in natural water and wastewater, including PAHs, octyl/nonyl phenols, PCBs, PBDEs, and a notable number of pesticides and several relevant metabolites. In the food safety field, several quantitative applications have also been reported in the screening of pesticides, polybrominated diphenyl ethers (PBDEs), and polycyclic biphenyls (PCBs).[31−33]

The use of GC−TOF and LC−(Q)TOF seems one of the best ways nowadays to investigate the presence of a large number of contaminants in samples due to their complementary characteristics to determine from nonpolar/volatile to polar/nonvolatile compounds. The reasonable sensitivity in full-spectrum acquisition and accurate-mass data provided by TOF MS allow notably increasing the number of compounds to be investigated, with the possibility of the subsequent searching of additional compounds in a retrospective analysis without the need of new sample injections. The combined use of these two techniques has been preliminarily explored in some particular cases, as the investigation of poisoning compounds in honey bees[28] or analysis of wastewater samples.[34] However, no extensive search has been made in the public health field yet.

The aim of this work was to evaluate the added value of two powerful complementary techniques, as GC−(EI)TOF MS and UHPLC(ESI)QTOF MS, in the routine analysis performed at the Public Health Laboratory of Barcelona, commonly based on the use of GC−MS/MS and LC−MS/MS with triple quadrupole analyzers. To this aim, a variety of sample extracts obtained after application of the lab standard operation procedures (SOPs), previously analyzed by GC or LC tandem MS, were reanalyzed by TOF MS pursuing different objectives: (a) to confirm the presence of the organic contaminants previously detected using the target GC−MS/MS and/or LC−MS/MS methodologies, (b) to apply a "post-target" screening trying to find other selected organic contaminants, not included in the target methods routinely applied by the Laboratory of Public Health, (c) to help in the elucidation of suspected compounds that could not be identified/confirmed by tandem MS, and (d) to investigate the presence of nontarget compounds that might be relevant from a public health point of view.

2. MATERIALS AND METHODS

2.1. Instrumentation. *2.1.1. UHPLC−QTOF MS.* A Waters Acquity UPLC system (Waters, Milford, MA, USA) was interfaced to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (Q-oaTOF Premier, Waters Micromass, Manchester, U.K.), using an orthogonal *Z*-spray-ESI interface operating in positive ion mode. Chromatographic UPLC separation was performed using an Acquity UPLC BEH C18 1.7 μm particle size analytical column 100 × 2.1 mm (Waters) at a flow rate of 300 μL min^{-1}. The mobile phases used were (A) formic acid 0.01% in water and (B) formic acid 0.01% in methanol. The following gradient profile was used (time in minutes, % A): (0, 90), (14, 10), (16, 10), (18, 90). Nitrogen (from a nitrogen generator) was used as the drying gas and nebulizing gas. The gas flow was set at 600 L/h. TOF MS resolution was approximately 10,000 at full width half-maximum (fwhm) at m/z 556. MS data were acquired over an m/z range of 50−1000. The microchannel plate (MCP) detector potential was set to 1850 V. A capillary voltage of 3.5 kV and cone voltage of 25 V were used. Collision gas was argon 99.995% (Praxair, Valencia, Spain). The interface temperature was set to 350 °C and the source temperature to 120 °C. The column temperature was set to 40 °C.

For MS^E experiments, two overlapping acquisition functions with different collision energies were created: the low energy function (LE), selecting a collision energy of 4 eV, and the high energy (HE) function, with a collision energy ramp ranging from 15 to 40 eV, in order to obtain a greater range of fragment ions. The LE and HE functions settings were for both a scan time of 0.2 s and an interscan delay of 0.05 s. The automated attenuated function was also selected to correct for possible peak saturations (extended mode).

Calibrations were conducted from m/z 50 to 1000 with a 1:1 mixture of 0.05 M NaOH:5% HCOOH diluted (1:25) with acetonitrile:water (80:20). For automated accurate mass measurement, the lock-spray probe was used, using as lock mass a solution of leucine enkephalin (2 μg/mL) in acetonitrile:water (50:50) at 0.1% HCOOH pumped at 30 μL/min through the lock-spray needle. A cone voltage of 65 V was selected to obtain adequate signal intensity for this compound (~500 counts/s). The protonated molecule of leucine enkephalin at m/z 556.2771 was used for recalibrating the mass axis and ensuring a robust accurate mass measurement along time.

2.1.2. GC−TOF MS. An Agilent 6890N GC system (Palo Alto, CA, USA) equipped with an Agilent 7683 autosampler was coupled to a time-of-flight mass spectrometer, GCT (Waters, Milford), 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, Folson, CA, USA). 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 2700 V. TOF MS resolution was about 8,500 (fwhm) at m/z 614.

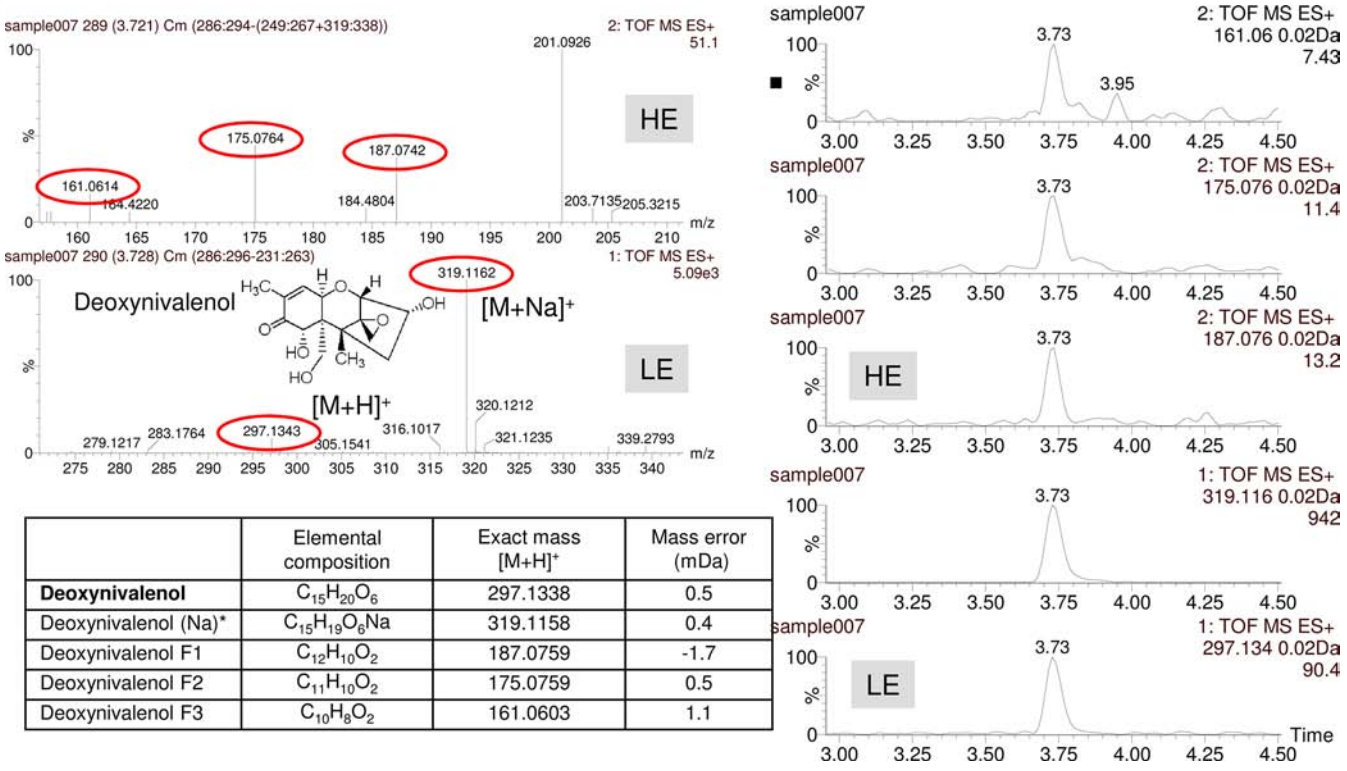
Heptacose, used for the daily mass calibration as well as lock mass, was injected via syringe in the reference reservoir at 30 °C for this purpose. The m/z ion monitored was 218.9856.

2.2. Samples. Eight drinking water samples, one grape sample, and one cucumber sample, together with several fish samples—two tuna, two salmon, one mackerel—previously analyzed by GC−MS/MS were reanalyzed by GC−TOF MS. Regarding LC−TOF MS, 29 vegetable and fruit samples (including lettuce, tomato, pear, orange, and apple samples, among others), which had been analyzed by LC−MS/MS, were reanalyzed by UHPLC−QTOF MS. In addition, 4 rice and 2 flour samples were also reanalyzed. For elucidation of suspect compounds, one bovine muscle sample and one water sample were investigated.

2.3. Sample Preparation. Sample preparation for food commodities was made in this work following the standard operation procedures (SOPs) of the Public Health Laboratory of Barcelona, based on the use of accelerated solvent extraction (ASE) with ethyl acetate as "universal" extraction solvent. Automated gel permeation chromatography (GPC) was applied for cleanup purposes when necessary (fatty matrices). As regards water samples, they were processed by applying a generic solid-phase extraction procedure. Details on sample treatment (extraction and cleanup) are shown in the Supporting Information.

2.4. Reference GC−MS/MS and LC−MS/MS Targeted Methods. Information on reagents, chemicals, and equipment used for extraction of samples and GPC cleanup as well as the triple quadrupole instruments used in this work is shown in the Supporting Information.

2.5. Analytical Strategies. Searching for organic contaminants in food and water samples was carried out following different data



	Elemental composition	Exact mass $[M+H]^+$	Mass error (mDa)
Deoxynivalenol	$C_{15}H_{20}O_6$	297.1338	0.5
Deoxynivalenol (Na)*	$C_{15}H_{19}O_6Na$	319.1158	0.4
Deoxynivalenol F1	$C_{12}H_{10}O_2$	187.0759	-1.7
Deoxynivalenol F2	$C_{11}H_{10}O_2$	175.0759	0.5
Deoxynivalenol F3	$C_{10}H_8O_2$	161.0603	1.1

Figure 1. Detection and identification of mycotoxin deoxynivalenol in a flour sample by UHPLC−QTOF MS (MS^E approach). LE and HE spectra for sample; XICs at 20 mDa mass window for $[M + H]^+$ and $[M + Na]^+$ in LE function and main fragments in HE function; elemental composition and mass errors (in mDa) for protonated and sodiated molecule as well as fragment ions.

processing strategies depending on the origin of the full spectrum acquisition data, i.e. GC−(EI)TOF MS or LC−(ESI)TOF MS.

2.5.1. Analysis by UHPLC−QTOF MS. Full spectrum acquisition data, generated simultaneously at low and high collision energies (MS^E), were processed using specialized software. ChromaLynx XS (Waters) offers the possibility of applying a "post-target" processing method based on monitoring exact masses of the selected analytes using narrow mass windows (commonly 10−20 mDa), that permits rapid and simple reviewing by classifying analytes, as a function of mass error. In addition, this software allows the simultaneous visualization of the complete spectrum of positive findings. In this work, a database containing around 1,100 organic contaminants was used.[16] Compounds searched included pharmaceuticals, pesticides, drugs of abuse, toxins, hormones, UV-filter agents, colorants, preservants, phenols and surfactants, and a notable number of degradation products. Around 250 reference standards were available at our laboratory, and therefore information about retention time, fragmentation, and adduct formation was also included in the target list for those compounds to facilitate and enhance reliability in the identification/elucidation process. The presence of the (de)protonated molecule measured at its accurate mass, at the expected retention time (when available), was evaluated in the samples. Additionally, collision induced dissociation (CID) fragments (in any of the two functions acquired, at low or high collision energy) or characteristic isotopic ions were also evaluated. Calibration curves were included in each sequence of analysis. Following this approach, a notable number of compounds were detected and identified in the samples.

2.5.2. Analysis by GC−TOF MS. The methodological approach previously developed for screening and confirmation of organic compounds in water and adipose tissue[29,30] was applied in this work for searching target and nontarget contaminants in vegetables and fish, as well as in water samples.

For investigation of target compounds up to 5 narrow-window (20 mDa) extracted ion chromatograms (nw-XIC) at selected m/z ions were monitored for each compound. This information was available for around 200 target compounds, including PCBs, PAHs, PBDEs, alkyphenols, and a notable number of pesticides like insecticides (organochlorine, organophosphorous, carbamates, and pyretroids), herbicides (triazines and chloroacetanilides), fungicides, and some metabolites, for which their reference standards were available. The application manager TargetLynx was employed to automatically process data and to confirm the identity of target compounds detected in samples. The presence of, at least, two ions measured at their accurate mass and the compliance of their intensity ratio within specified tolerances were required for a reliable confirmation. This methodology was previously developed and validated for qualitative purposes, i.e. to ensure the reliable and sensitive identification of compounds detected in samples at a certain level of concentration.[30] Additionally, in this work, 5 polychloronaphthalene compounds were added to the list of target compounds. For this purpose, reference standard solutions of these compounds were injected in the GC−TOF MS and the most abundant m/z ions were selected. In order to investigate the selectivity of the fragments, accurate mass measurements of the different ions were obtained and subsequently used for elemental composition calculation. Calibration curves were made with standards in solvent and were included in every sequence of sample analysis.

GC−TOF also allowed the investigation of nontarget compounds using appropriate processing software (ChromaLynx XS in untargeted mode) able to manage MS data in a more effective way than in LC−TOF, due to the availability of commercial libraries for electron ionization spectra. This software automatically detects peaks with a response over user-defined parameters, displays their deconvoluted mass spectra, searches them against the commercial nominal-mass NIST02 library, and produces a hit list with positive matches (library match >700 was used as criterion). An Elemental Composition Calculator is applied to derive the five most likely chemical formulas of up to five most intense ions in the experimental (EI)TOF MS spectrum. These fragment formulas are tested against the molecular formulas of the top-five library hits in order to test the likelihood that they could be in accordance with the proposed formula depending on

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Table 1. Summary of Positive Findings Reported by LC−MS/MS and UHPLC−QTOF MS

		UHPLC−QTOF MS		
sample	analytes reported by LC−MS/MS (QqQ)	confirmation	post-target	elucidation of suspects
lettuce (21398)	imidacloprid 0.58 mg/kg	yes		
tomato (21399)	imidacloprid 0.14 mg/kg	yes		
pear (13233A)	imazalil 0.02 mg/kg	yes	azinphos methyl[a]	
			flusilazole[a]	
			pyrimethanil[a]	
flour (14810)	deoxynivalenol 1.45 mg/kg	yes	pirimiphos methyl[a]	
flour (14811)	deoxynivalenol 0.24 mg/kg	yes	pirimiphos methyl[a]	
tomato (3295)	azoxystrobin 0.25 mg/kg	yes	buprofezin[a]	
			chlorpyriphos methyl[a]	
			procymidone[a]	
apple (15851)	acetamiprid 0.06 mg/kg	yes	phosmet[a]	
	thiabendazole 0.13 mg/kg	yes		
orange (13030)	imazalil 0.80 mg/kg	yes		
grape (9083)	cyprodinil 0.17 mg/kg	yes		
	imidacloprid 0.05 mg/kg	yes		
cherry (8760)	omethoate 0.09 mg/kg	yes		
	dimethoate <0.01 mg/kg	yes		
tomato (11017A)	fenhexamid 0.15 mg/kg	yes	tebuconazole[a]	
pear (11015)	thiabendazole 0.05 mg/kg	yes	bupirimate[a]	
rice (8726)	thiabendazole <0.01 mg/kg	yes		
pear (9385)	acetamiprid 0.03 mg/kg	yes	azinphos methyl[a]	
	thiabendazole 0.31 mg/kg	yes	thiacloprid[b]	
	carbendazim <0.01 mg/kg	yes		
banana (2141)	cyprodinil 0.02 mg/kg	yes		
	imazalil 0.015 mg/kg	yes		
lettuce (2146)	boscalid 0.02 mg/kg	yes		
	imidacloprid 0.09 mg/kg	yes		
	triadimenol 0.05 mg/kg	yes		
peach (8761)	fenhexamid 0.04 mg/kg	yes		
banana (4025)	imazalil >400 mg/kg	yes		
pear (14128)			thiacloprid[b]	
rice (8723)			tebuconazole[c]	
pear (4027)	imazalil >400 mg/kg	yes	phosmet[a]	
	imidacloprid <0.01 mg/kg	yes	iprodione[a]	
	thiabendazole <0.01 mg/kg	yes	paclobutrazol[b]	
	carbendazim <0.01 mg/kg	yes	terbuthylazine[b]	
			tebuconazole[a]	
rice (8724)			tebuconazole[c]	
apple (4026)	imazalil 0.20 mg/kg	yes	phosmet[a]	
	thiabendazole <0.01 mg/kg	yes	tebuconazole[a]	
rice (2777)	carbaryl 0.18 mg/kg	yes	malathion[a]	
	azoxystrobin 0.26 mg/kg	yes	pirimiphos-methyl[a]	
cucumber (2957)	carbofuran 0.1 mg/kg	yes	prochloraz[b]	
			pirimiphos-methyl[a]	
			pirimicarb[a]	
pear (3011)	cyprodinil 0.23 mg/kg	yes		
apple (3012)	boscalid 0.03 mg/kg	yes		
tomato (3013)	dimethomorph 0.02 mg/kg	yes	tebuconazole[a]	
	fenhexamid <0.01 mg/kg		flutriazole[a]	
grape (2143)	fenhexamid 0.17 mg/kg	yes		
	azoxystrobin 0.02 mg/kg	yes	cyprodinil	
pepper (3015)	azoxystrobin 0.04 mg/kg	yes	bupirimate[a]	
	triadimenol 0.02 mg/kg	yes	flutriazole[a]	
			pyrimethanil[a]	
tomato (4029)	cyprodinil 0.02 mg/kg	yes		
grape (2955A)	imidacloprid 0.13 mg/kg	yes	quinoxyfen[a]	
	monocrotophos 0.19 mg/kg	yes	quinalphos[a]	
	kresoxim methyl 0.11 mg/kg	yes		
grape juice (29574A)	methiocarb 0.18 mg/kg	yes	ethion[a]	

Table 1. continued

		UHPLC−QTOF MS		
sample	analytes reported by LC−MS/MS (QqQ)	confirmation	post-target	elucidation of suspects
	methiocarb sulfoxide <0.01 mg/kg	yes	pyriproxyfen[a]	
bovine muscle (22223)				same transitions as flumequine (262 > 244 and 262 > 202) but different ion ratio and retention time
water (2346)				same transitions as norfloxacin (320 > 302 and 320 > 276.2) but different ion ratio
orange (2584)				same transitions as fluazifop (328 > 254 and 328 > 282) but different ion ratio

[a]These compounds are routinely analyzed by GC−MS/MS only. [b]These compounds are not routinely included in either GC−MS/MS or LC−MS/MS. [c]These compounds are routinely analyzed by GC−MS/MS, although in other sample matrices.

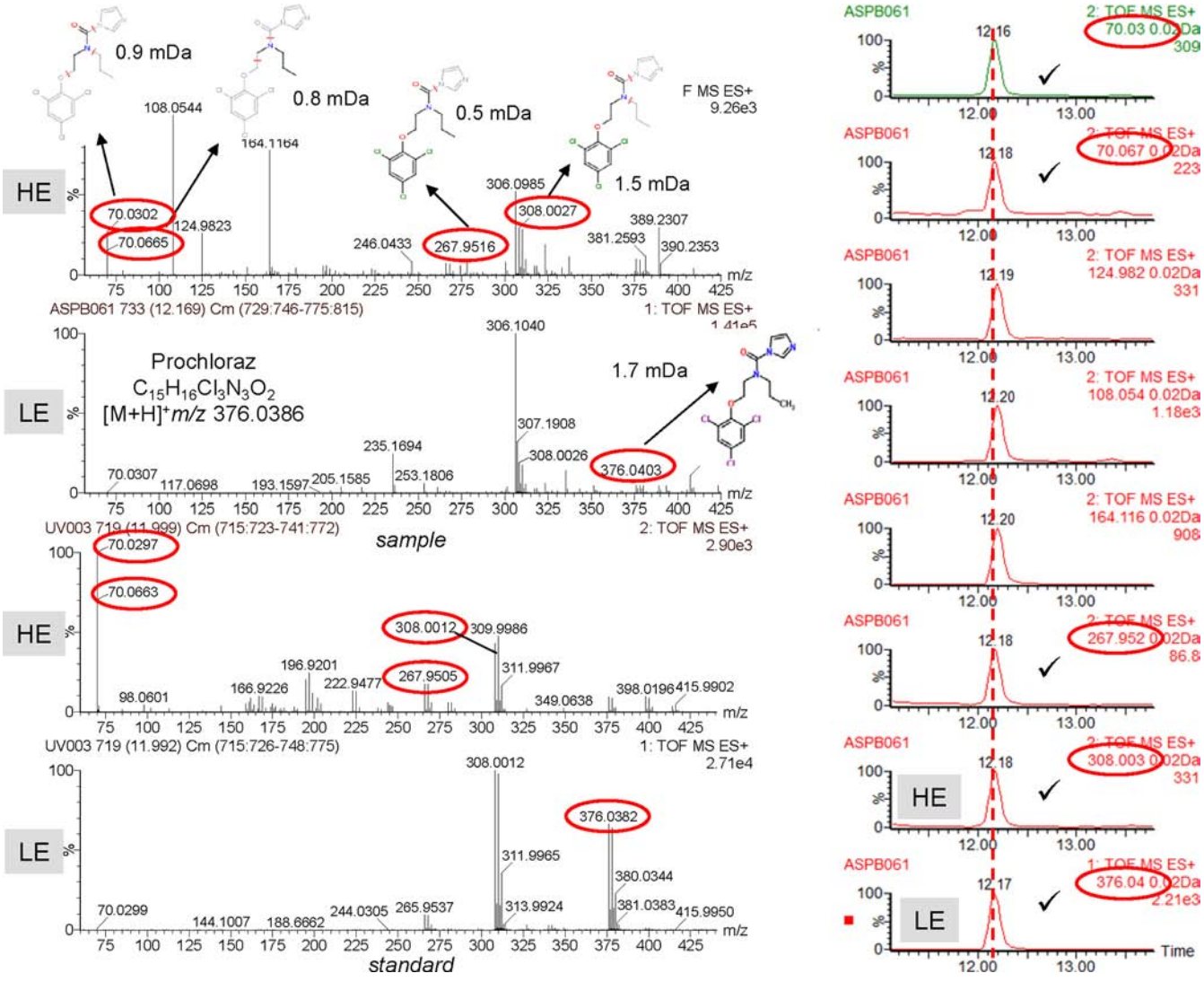

Figure 2. Detection and identification of fungicide prochloraz in a cucumber sample by UHPLC−QTOF MS (MS^E approach). LE and HE spectra for sample (with justified fragments using MassFragment software) and for reference standard; XICs at 20 mDa mass window for $[M + H]^+$ in LE function and different ions observed in HE function. Those corresponding to $[M + H]^+$ as well as to main fragments are marked with a check mark.

the mass error observed. So, accurate mass confirmation of the library search was automatically performed in this step.

3. RESULTS AND DISCUSSION

3.1. Analysis by UHPLC−QTOF MS. *3.1.1. Confirmation of Organic Contaminants Reported by LC−MS/MS.* All findings reported by LC−MS/MS QqQ were unequivocally confirmed with the data provided by LC−QTOF MS on experimental accurate mass spectra and retention times, as reference standards were available for all of them. For example, Figure 1 shows the LE and HE TOF MS spectra for a flour sample, where the mycotoxin deoxynivalenol was detected. As reference standard was available at our laboratory, information on retention time, fragment ions, and adduct formation had

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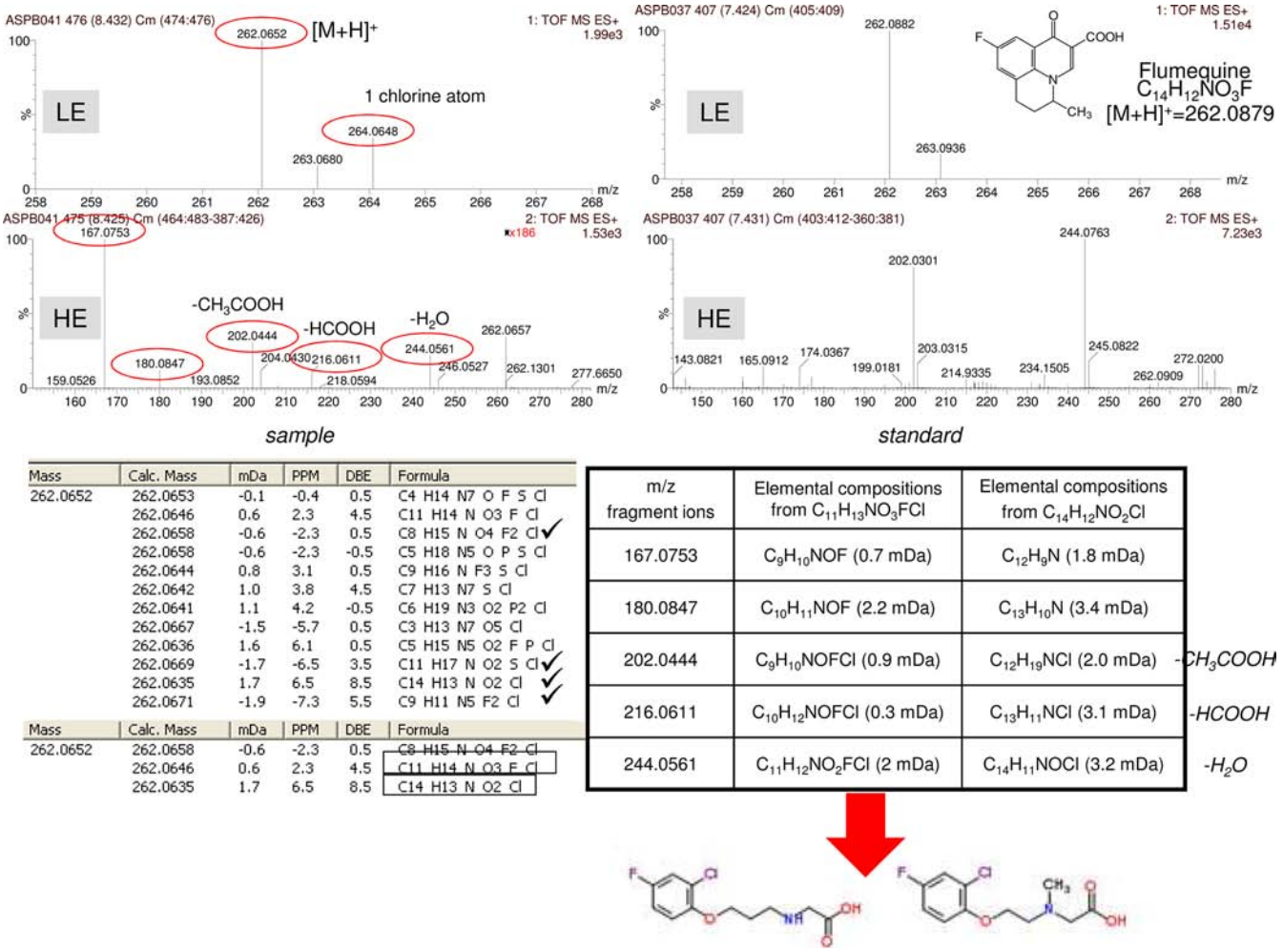

Mass	Calc. Mass	mDa	PPM	DBE	Formula
262.0652	262.0653	-0.1	-0.4	0.5	C4 H14 N7 O F S Cl
	262.0646	0.6	2.3	4.5	C11 H14 N O3 F Cl
	262.0658	-0.6	-2.3	0.5	C8 H15 N O4 F2 Cl ✓
	262.0658	-0.6	-2.3	-0.5	C5 H18 N5 O P S Cl
	262.0644	0.8	3.1	0.5	C9 H16 N F3 S Cl
	262.0642	1.0	3.8	4.5	C7 H13 N7 S Cl
	262.0641	1.1	4.2	-0.5	C6 H19 N3 O2 P2 Cl
	262.0667	-1.5	-5.7	0.5	C3 H13 N7 O5 Cl
	262.0636	1.6	6.1	0.5	C5 H15 N5 O2 F P Cl
	262.0669	-1.7	-6.5	3.5	C11 H17 N O2 S Cl ✓
	262.0635	1.7	6.5	8.5	C14 H13 N O2 Cl ✓
	262.0671	-1.9	-7.3	5.5	C9 H11 N5 F2 Cl ✓

Mass	Calc. Mass	mDa	PPM	DBE	Formula
262.0652	262.0658	-0.6	-2.3	0.5	C8 H15 N O4 F2 Cl
	262.0646	0.6	2.3	4.5	C11 H14 N O3 F Cl
	262.0635	1.7	6.5	8.5	C14 H13 N O2 Cl

m/z fragment ions	Elemental compositions from $C_{11}H_{13}NO_3FCl$	Elemental compositions from $C_{14}H_{12}NO_2Cl$	
167.0753	$C_9H_{10}NOF$ (0.7 mDa)	$C_{12}H_9N$ (1.8 mDa)	
180.0847	$C_{10}H_{11}NOF$ (2.2 mDa)	$C_{13}H_{10}N$ (3.4 mDa)	
202.0444	$C_9H_{10}NOFCl$ (0.9 mDa)	$C_{12}H_{19}NCl$ (2.0 mDa)	$-CH_3COOH$
216.0611	$C_{10}H_{12}NOFCl$ (0.3 mDa)	$C_{13}H_{11}NCl$ (3.1 mDa)	-HCOOH
244.0561	$C_{11}H_{12}NO_2FCl$ (2 mDa)	$C_{14}H_{11}NOCl$ (3.2 mDa)	$-H_2O$

Figure 3. Elucidation of a suspect compound which shares two transitions with flumequine in a bovine muscle sample. LE and HE spectra for sample and for flumequine reference standard; possible elemental compositions obtained for m/z 262.0652, before and after applying the carbon and the sulfur filter. Elemental compositions and mass errors for main fragment ions; structures returned by the database by the elemental composition $C_{11}H_{13}NO_3FCl$.

been previously included in the target list. As shown in Figure 1, both the sodium adduct and the protonated molecule were detected in the LE function, with mass errors of 0.4 and 0.5 mDa, respectively, with a chromatographic peak at the expected retention time (3.7 min). In the HE function, 3 fragments were also detected at the same retention time, with mass errors lower than 2 mDa. In this way, an ultimate confirmation of the presence of the compound was achieved. It must be noted that the $[M + H]^+$ ion had a relative intensity lower than 10%, $[M + Na]^+$ becoming the most abundant ion in the mass spectrum. This example shows the need of including sodium adducts in the target list in those cases where it is the most abundant ion to avoid potential false negatives.[16] In the same way, other positives such as omethoate in cherry, acetamiprid in pear, and azoxystrobin in tomato, rice, and grape, among others, were confirmed.

Another case was the confirmation of the insecticide imidacloprid in a lettuce sample (see Figure 1S in the Supporting Information). The LE spectrum of imidacloprid in the sample showed the m/z corresponding to the protonated molecule (256.0621) with a mass error of 2 mDa (Figure 1S in the Supporting Information). The HE spectrum also showed the remaining protonated molecule and five fragment ions mostly with mass errors below 1 mDa. In this figure, XICs for the six m/z ions are also depicted, with a chromatographic peak at exactly the same retention time (4.06 min). This example illustrates that an unequivocal confirmation of imidacloprid in the lettuce could be made using the MS^E approach.

A summary of the positive findings confirmed by UHPLC−QTOF MS in the samples analyzed is shown in Table 1. As it can be seen, 100% of compounds previously detected by LC−MS/MS (QqQ) were confirmed by QTOF MS, even those found at concentration levels below 0.05 mg/kg.

3.1.2. Post-Target Analysis. The wide-scope QTOF MS screening applied also allowed detecting other compounds not investigated by LC−MS/MS. Some of these compounds, as the insecticide buprofezin in tomato or the fungicides bupirimate and pyrimethanil in pepper, had been already detected by GC−MS/MS. Several of them, as the insecticides phosmet in apple or ethion in grape juice, could be detected at concentrations even lower than 0.01 mg/kg. In all these cases, the reference standards were available.

It is important to remark the detection of the insecticide thiacloprid, the plant growth regulator paclobutrazol, or the fungicide prochloraz, as these compounds were not included in either LC or GC tandem MS analysis. Figure 2 illustrates the detection and identification of prochloraz in cucumber by UHPLC−QTOF MS. The protonated molecule of prochloraz

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was detected in the LE function, with a mass error of 1.7 mDa. Moreover, the combined spectra of this chromatographic peak showed a typical three-chlorine atom isotopic pattern, being therefore in accordance with the chemical structure of prochloraz ($C_{15}H_{16}Cl_3N_3O_2$). However, in this case the reference standard was not available at our laboratory. Then, the accurate mass of the fragment ions was justified using the MassFragment software (Waters). This software applies a bond-disconnecting methodology to obtain possible structures for the fragment ions from a given molecule. In the HE function, up to 4 fragments of this compound were detected with chromatographic peaks at the same retention time, and mass errors lower than 2 mDa. In order to avoid spectrum interference that would complicate the identification process, recognizing which ions are fragments, and which are not, becomes mandatory. In this sense, UHPLC turned valuable for choosing perfectly coeluting ions (see chromatographic peak at 12.17 min). The elemental composition for all fragments (m/z 308.0027, 267.9516, 70.0665 and 70.0302) was calculated, obtaining errors below 2 mDa in relation to the theoretical exact masses predicted. In the same cucumber extract, a compound practically coeluting with prochloraz was observed, which was identified as the insecticide pirimiphos methyl (m/z 306.1040 in LE spectrum corresponding to the protonated molecule (−0.1 mDa), and fragment ions at m/z 124.9823 (−0.3 mDa) and 164.1164 (−2.4 mDa) in HE function).

The tentative identification of prochloraz was supported by the MS/MS product ions reported in the literature. Two fragments (m/z 308.0027 and 267.9516) observed in the HE spectrum had been previously reported (in nominal mass) for the determination of this compound by LC−MS/MS QqQ.[35] After this careful evaluation process, the reference standard was finally acquired and injected, allowing the ultimate confirmation of this compound in the sample.

In all cases of positive findings, the experimental information obtained on fragment ions was added to our target list in order to facilitate future screenings.

Other interesting examples were the detection of the fungicide tebuconazole in rice, as this compound is monitored by GC−MS/MS although not in cereal samples, or the herbicide terbuthylazine in pear, monitored by LC−MS/MS but only in water samples. These findings confirm the possibilities of reporting false negatives due to the limited selection of the analytes when applying pretarget QqQ methods to different types of samples.

3.1.3. Elucidation of Unknowns. In some particular cases, the potential of UHPLC-(Q)TOF was used in the elucidation process of suspect compounds. Below, two illustrative examples are shown.

An Unknown Compound Sharing the Same Selected SRM Transitions as Flumequine. Figure 3 illustrates the elucidation process of an unknown compound detected in a bovine muscle sample. This compound shared the two transitions selected for the antibiotic flumequine (262 > 244 and 262 > 202), but it had different ion ratio and close retention time.

The accurate mass of the protonated molecule of this unknown (retention time 8.4 min) in the LE MS spectrum was m/z 262.0652, which differs 22.7 mDa from the exact mass of flumequine ($C_{14}H_{12}NO_3F$). Moreover, the combined spectra of this chromatographic peak showed a typical one chlorine atom isotopic pattern. Possible elemental compositions, with a maximum deviation of 2 mDa from the measured mass were calculated, using the Elemental Composition program within the MassLynx software. Parameter settings for all calculations were as follows: C 0−30, H 0−50, N 0−10, O 0−10, F 0−5, S 0−2, P 0−2, and Cl 1−1. The double bond equivalent (DBE) parameter was set from −0.5 to 50, giving information about aromaticity of the calculated elemental composition. Additionally, the option "even electrons ions only" was selected for precursor and "odd and even electrons ions" for product ions. Within the search limits outlined above, calculation of the possible elemental compositions resulted in 12 formulas. When applying the carbon and the sulfur filter, 4 formulas remained (marked as a check mark in Figure 3).

Trying to reduce the number of possible molecular formulas, HE TOF MS spectrum was investigated. Figure 3 shows the HE spectra of the suspect compound and of flumequine standard. Both compounds shared fragment ions at nominal m/z 244 and 202, which would explain that both shared the same transitions in the LC−MS/MS method. However, accurate masses were quite different. For the suspect compound, the fragments at m/z 244.0561, 216.0611, and 202.0444 corresponded to losses of water, formic acid, and acetic acid, respectively. With this information, all compositions without, at least, two atoms of oxygen were discarded, reducing the number of plausible elemental compositions to three, $C_8H_{15}NO_4F_2Cl$, $C_{11}H_{14}NO_3FCl$, and $C_{14}H_{13}NO_2Cl$ (expressed as protonated molecules).

The three remaining elemental compositions were searched for potential structures in chemical databases. We choose Reaxys (Elsevier), a web-based search and retrieval system for chemical compounds, bibliographic data, and chemical reactions that contains more than 18,000,000 substances, as well as ChemSpider, which links together compound information across the web, providing free text and structure search access of millions of chemical structures. No hits were found for $C_8H_{14}NO_4F_2Cl$. After searching the formula $C_{14}H_{12}NO_2Cl$, 639 hits were found in ChemSpider and 711 hits in Reaxys. Reaxys allows limiting the search of a formula taking into account a substructure which notably reduces the number of possible chemical structures for a given formula. Limiting the above search to those structures with a CH_3COOH group, a total of 186 structures were returned by the database. A final manual filtering of those structures containing a terminal −COOH group with a nonaromatic carbon atom in α position resulted in 17 plausible structures. Regarding $C_{11}H_{13}NO_3FCl$, 17 hits were found in ChemSpider and 10 hits in Reaxys. The manual filtering limited the plausible structures to 2.

The 19 structures finally suggested were evaluated based on the fragmentation patterns observed in the HE spectra, as fragment ions should be compatible with the chemical structures assigned to the unknown. In this case, it was not possible to discard any additional structure. However, considering the average mass errors obtained for all product ions (mean values of 1.2 and 2.7 mDa for $C_{11}H_{13}NO_3FCl$ and $C_{14}H_{12}NO_2Cl$, respectively), the most plausible composition would correspond to $C_{11}H_{13}NO_3FCl$, therefore leaving two possible structures (see Figure 3). This process of elucidation takes lot of time and effort and illustrates the difficulties associated with discovering unknowns in the samples by using LC−QTOF MS.[36] The following step would be the acquisition of reference standards, if available, and subsequent injection to test retention time and experimentally confirm the presence of fragment ions generated. This would allow the unequivocal confirmation of the unknown.

Table 2. Summary of Positive Findings Reported by GC−MS/MS (QqQ) and GC−TOF MS

		GC−TOF MS			
sample	analytes reported by GC−MS/MS (QqQ)	confirmation	post-target		nontarget
grape (3139)			*p,p*-DDE	penconazole	
cucumber (3137)			chlorpyriphos		pentachloroaniline
water 01 (10-14977)			phenanthrene[b]		1,3-cyclopentadiene
					BHT
					BHT-CHO
					benzophenone
water 02 (10-13298)	lindane <0.02 μg/L	no	phenanthrene[b]		
			desethyl-atrazine[a]		
water 03 (10-13329)	octachloroestyrene <0.02 μg/L	yes	phenanthrene[b]		BHT
					BHT-CHO
					benzophenone
water 04 (10-14959)	lindane <0.02 μg/L	yes	phenanthrene[b]	metolachlor[c]	BHT
	alachlor 0.21 μg/L	yes	terbuthylazine[a]	chlorpyriphos[c]	D-verbenone
			malathion[c]	desethyl-terbuthylazine[b]	BHT-CHO
					benzophenone
water 05 (10-23632)			phenanthrene[b]		BHT
				pyrene[b]	BHT-CHO
				desethyl-atrazine[a]	benzophenone
water 06 (10-16176)	alachlor 0.06 μg/L	yes	phenanthrene[b]	fenitrothion[b]	BHT
			pyrene[b]	malathion[c]	BHT-CHO
			simazine[a]	chlopyriphos[c]	propazine[c]
			atrazine[a]	chlorfenvinphos[c]	caffeine
				diazinon[b]	benzophenone
				fenthion[b]	
water 07 (10-16114)			phenanthrene[b]		BHT
					BHT-CHO
					benzophenone
water 08 (10-19014)	PCB 28 0.10 μg/L	yes	phenanthrene[b]		BHT
	PCB 52 0.03 μg/L	yes			BHT-CHO
	PCB 101 < 0.02 μg/L	yes			benzophenone
	PCB 118 < 0.02 μg/L	yes			PCB 3Cl (× 3)
					PCB 4Cl (× 5)
fish 01 (10-10295)	*p,p*′-DDE 0.03 mg/kg	yes	HCB	PCB 118[c]	
			p,p′-DDD	PCB 153[c]	
			mirex[b]	PCB 123[c]	BHT
			PCB 28[c]	PCB 138[c]	benzophenone
			PCB 52[c]	PCB 180[c]	
			PCB 101[c]	phenanthrene[b]	
tuna 01 (5700)	pentachloronaphthalene <0.005 mg/kg	yes			
	hexachloronaphthalene <0.005 mg/kg	yes			
	heptachloronaphthalene <0.005 mg/kg	yes			
	octachloronaphthalene <0.005 mg/kg	yes			
tuna 02 (4229)	hexachloronaphthalene <0.005 mg/kg	yes			
	PBDE-47 < 0.005 mg/kg	no			
salmon 01 (9304)	pentachloronaphthalene <0.005 mg/kg	no	PCB 52[c]	naphthalene[b]	
	PBDE-47 < 0.005 mg/kg	no	PCB 101[c]	fluorene[b]	
			PCB 153[c]	phenanthrene[b]	
salmon 02 (5706)	PBDE-47 < 0.005 mg/kg	no	PCB 28[c]	PCB 153[c]	
	PBDE-99 < 0.005 mg/kg	no	PCB 52[c]	naphthalene[b]	
	PBDE-100 < 0.005 mg/kg	no	PCB 101[c]	fluorene[b]	
			PCB 118[c]	phenanthrene[b]	
mackerel 01 (4907)	pentachloronaphthalene <0.005 mg/kg	yes			
	hexachloronaphthalene <0.005 mg/kg	yes	PCB 52[c]		
	heptachloronaphthalene <0.005 mg/kg	no	PCB 153[c]		
	PBDE-47 < 0.005 mg/kg	no	PCB 138[c]		
	PBDE-99 < 0.005 mg/kg	no			

[a]These compounds are routinely analyzed by LC−MS/MS only. [b]These compounds are not routinely included in either GC−MS/MS or LC−MS/MS. [c]These compounds are routinely analyzed by GC−MS/MS, although in other sample matrices.

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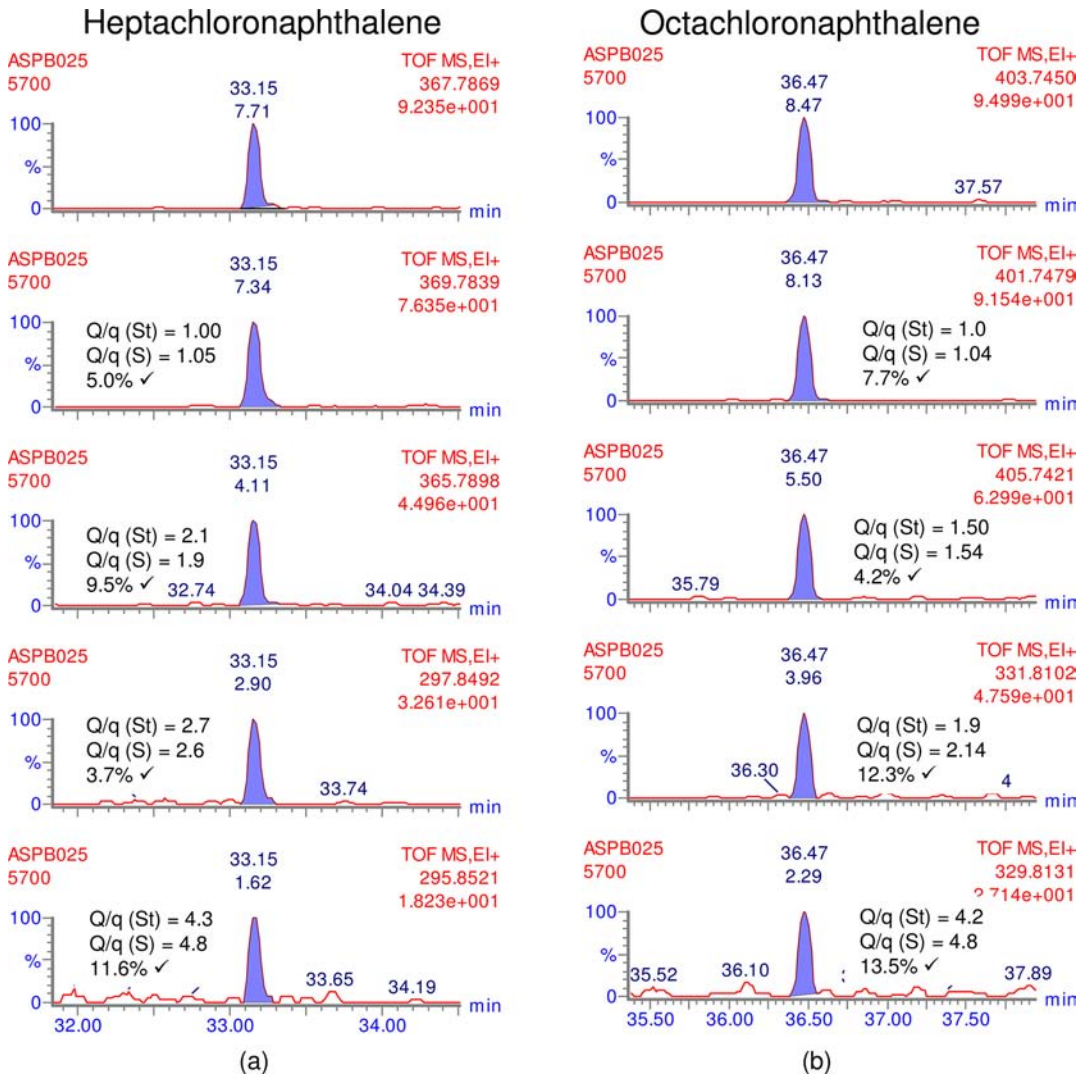

Figure 4. GC−TOF MS extracted-ion chromatograms at different m/z (mass window 20 mDa) for heptachloronaphthalene (a) and octachloronapthalene (b) in a tuna sample. Q, quantitative ion; q, confirmative ion; St, reference standard; S, sample; check mark, Q/q ratio within tolerance limits.

An Unknown Compound Sharing the Same Selected SRM Transitions as Norfloxacin. Figure 2S in the Supporting Information shows the elucidation process of an unknown compound previously detected by triple quadrupole in a water sample used for animal feeding. The compound presented the same two transitions (320 > 302 and 320 > 276) as the antibiotic norfloxacin, but different ion ratio and retention time. It was found relevant to discover the identity of this compound as it might be an unknown antibiotic used in farms as an illicit veterinary drug.

The accurate mass of the protonated molecule was measured to be m/z 320.1401. Based on this accurate mass, all possible elemental compositions with a maximum deviation of 2 mDa were calculated, using the Elemental Composition program. In this case, the combined spectra of the chromatographic peak did not show any abundant characteristic isotopic pattern. Following a strategy similar to the previous example shown, possible elemental compositions resulted in 28 formulas. When applying the carbon and sulfur filters, 6 formulas still remained.

Trying to reduce the number of possible molecular formulas, the TOF HE spectrum was then evaluated. Figure 2S in the Supporting Information shows the TOF HE spectra of the unknown compound and norfloxacin standard. As it can be seen, both compounds shared 3 product ions at m/z 302.1321 (corresponding to a water loss, 1.6 mDa), 276.1516 (CO_2 loss, 0.4 mDa), and 205.0790 ($C_{11}H_{10}N_2OF$, 1.3 mDa). With this information, new parameter settings can be used (C 11−30, H 10−50, N 2−10, O 2−10, F 1−4, S 0−2, P 0−2), reducing the number of plausible elemental compositions to only one, $C_{16}H_{19}N_3O_3F$ (as protonated molecule), exactly the same as norfloxacin.

The elemental composition $C_{16}H_{18}N_3O_3F$ resulted in 86 structures when searching in Reaxys database. The observed loss of CO_2 could imply the presence of a carboxylic acid near a carbonyl group. This fact allowed discarding 69 structures. Three among the 16 remaining structures (marked with 2 check marks in Figure 2S in the Supporting Information) presented the same functional groups as norfloxacin, but located at different positions. Among these, possibly the one marked with two check marks would be the most plausible, as the carbons next to the fluorine atom have no hydrogens. This would explain the lack of HF loss in contrast to norfloxacin. It

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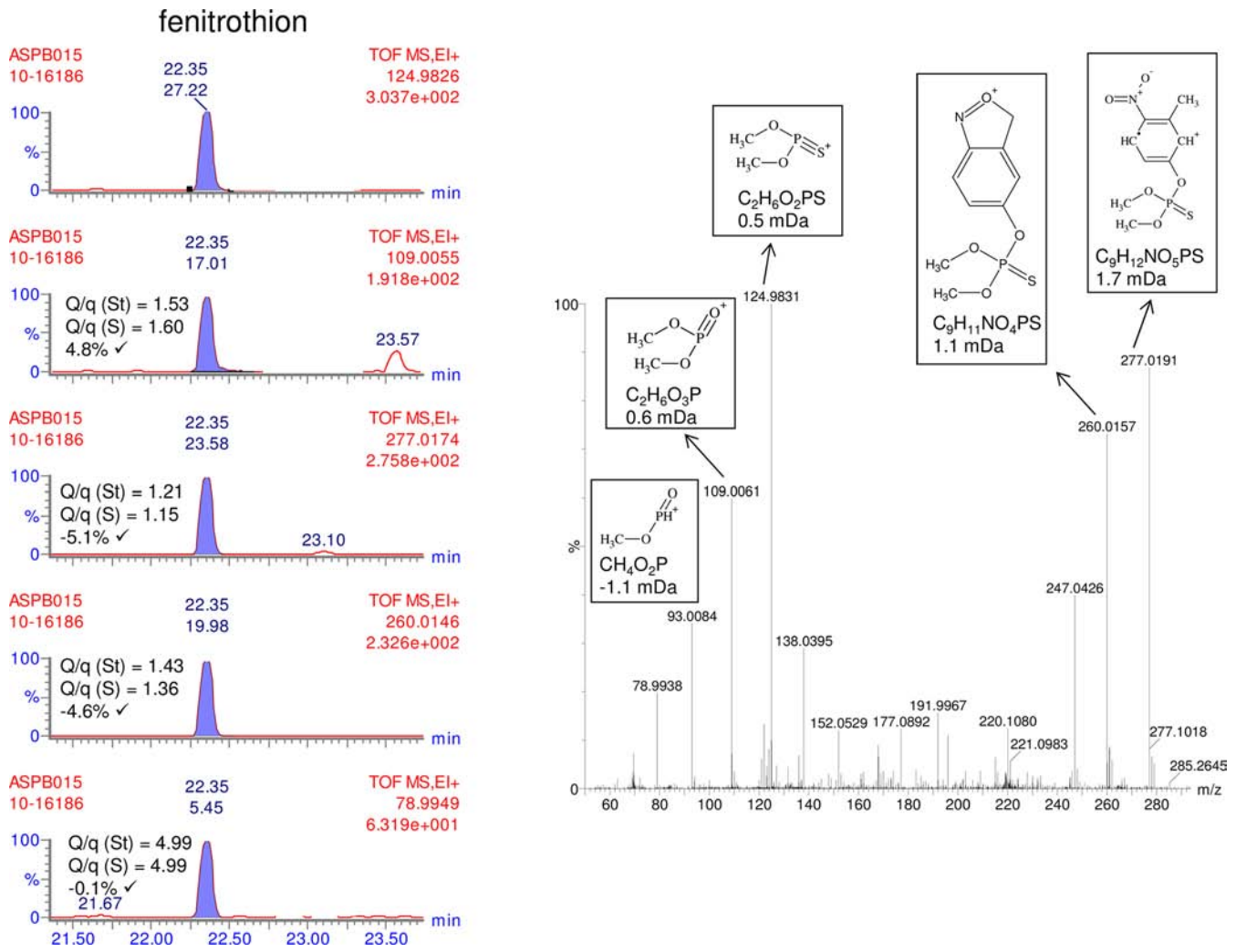

Figure 5. GC−TOF MS extracted-ion chromatograms at different m/z (mass window 20 mDa) for insecticide fenitrothion in a water extract. Experimental EI accurate-mass spectrum and chemical structures proposed for the most abundant EI fragment ions and mass errors. Q, quantitative ion; q, confirmative ion; St, reference standard; S, sample; check mark, Q/q ratio within tolerance limits.

seems that a compound closely related to the antibiotic norfloxacin was present in this water sample.

These two examples show the importance of selecting transitions as specific as possible and the need for efficient chromatographic separation, to decrease the possibilities of reporting false positives in tandem MS/MS methods.[37] Our experience has shown that unknown sample components can share the same transitions as the analytes, and this may lead to misinterpretations of the analytical results. TOF MS is a useful tool that provides additional helpful information in these cases.

3.2. Analysis by GC−TOF MS. The use of GC−TOF MS allowed us to confirm 61% of target compounds previously reported by GC−MS/MS (water and fish samples). Additionally, it was possible to detect and identify other compounds, not included in the target GC−MS/MS methods, thanks to the accurate-mass full-spectrum acquisition data provided by GC−TOF MS. The elucidation of several unknown compounds (nontarget analysis) was also carried out. The results obtained are summarized in Table 2.

3.2.1. Confirmation of Organic Contaminants. Table 2 shows the compounds previously detected by GC−MS and GC−MS/MS target methods in water, vegetable, and fish samples. Regarding water analysis, the GC−TOF MS target approach allowed confirmation of the presence of lindane, alachlor, PCB 28, 52, 101, and 118, and octachlorostyrene in the samples analyzed. Only one analyte (lindane) could not be confirmed in a water sample as no chromatographic peak was found at its accurate mass at the expected retention time. This might be explained by the low concentration reported for this compound in the sample (<0.02 μg/L), which was below the detection capabilities of TOF MS. Although TOF sensitivity in full acquisition is rather satisfactory, it is lower than QqQ working under selected reaction monitoring (SRM) mode. Surely, new instrument generations will improve sensitivity making it closer to that of GC−tandem MS.

In the case of fish samples, the presence of most PCNs previously reported by GC−MS/MS was confirmed. On the contrary, PBDEs could not be detected by GC−TOF MS, again probably due to their low concentration levels. As an example, Figure 4 shows the nw-XICs for two positive findings of heptachloronaphthalene and octachloronaphthalene in tuna that were confirmed by GC−TOF MS. In both cases, the presence of chromatographic peaks at expected retention time and the agreement of all Q/q ratios when comparing with the reference standard allowed the unequivocal confirmation of these findings in the samples. Moreover, mass errors (below 2.6

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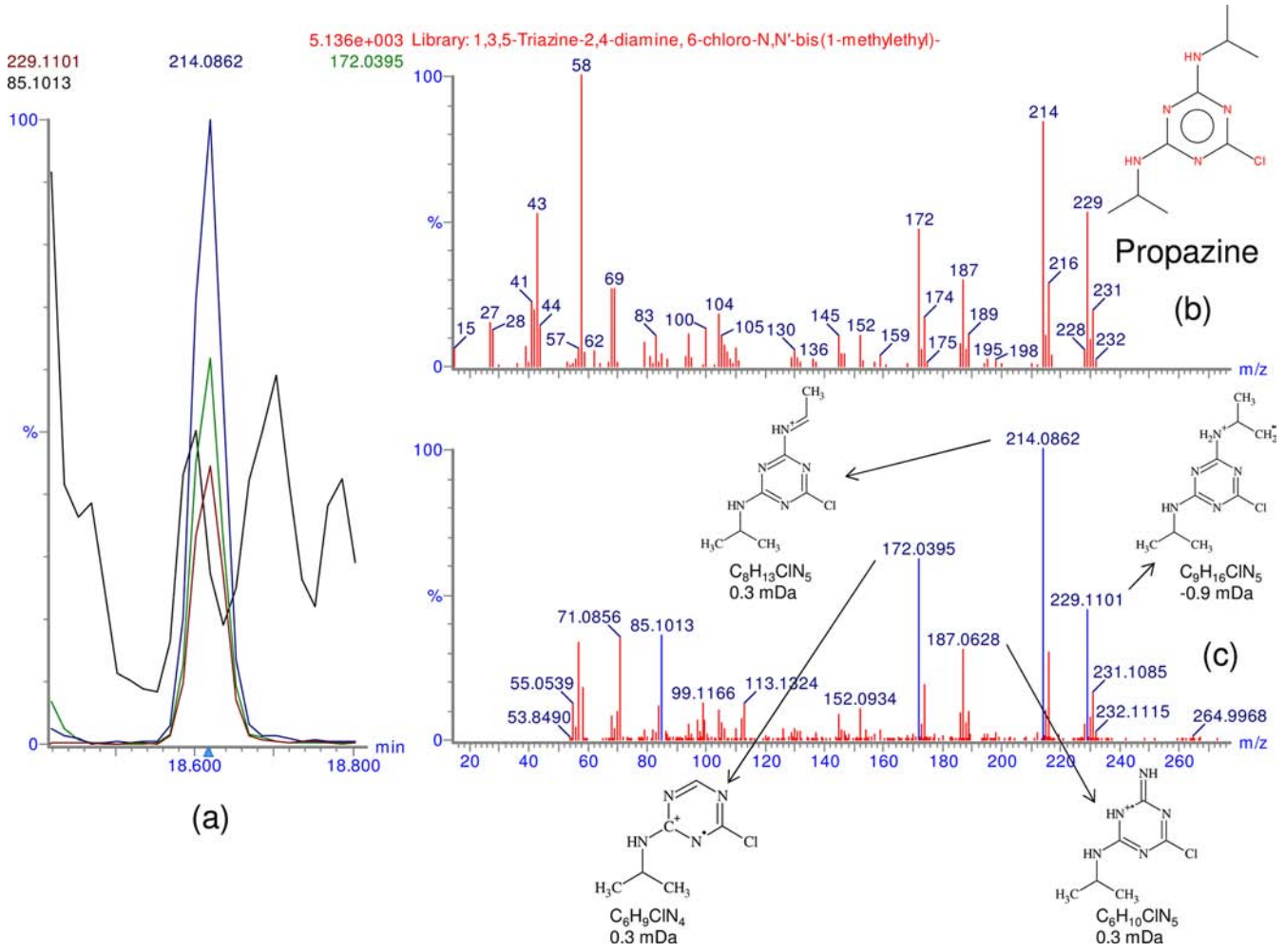

Figure 6. Identification of nontarget propazine by GC−TOF MS in a water extract. (a) Extracted-ion chromatogram for four *m*/*z* ions detected during deconvolution. (b) Library mass spectrum of propazine at nominal mass. (c) Deconvoluted accurate mass spectrum of propazine in the water sample and chemical structures proposed for the most abundant EI fragment ions and mass errors.

mDa) for molecular ion and most abundant fragments supported this confirmation.

3.2.2. Post-Target Analysis. The complete spectral information acquired by GC−TOF MS allowed us to perform a post-target analysis for around 200 compounds in the samples under study. In addition, searching of selected compounds in a retrospective way is also feasible at any time, once the samples have been injected into the GC−TOF MS system. The detection of two PAHs (phenanthrene and pyrene), six organophosphate insecticides (fenitrothion, diazinon, fenthion, chlorpyriphos, chlorfenvinphos, and malathion), a chloroacetanilide herbicide (metolachlor), and an herbicide metabolite (desethyl terbuthylazine) in water extracts is noteworthy. These compounds had not been included in either GC or LC tandem MS target methods for water samples. As an example, Figure 5 shows illustrative nw-XICs for fenitrothion detected in water. In addition to the accurate mass measurements, the reliable confirmation was feasible as all Q/q ratios were within specified tolerances. Experimental EI accurate mass spectra generated by TOF MS led to mass errors for five representative ions generally below 1.1 mDa.

Similarly, in the case of fish samples (tuna, salmon, and mackerel), several PAHs (naphthalene, fluorene, and phenanthrene) that had not been investigated by either GC or LC tandem MS were detected in the two salmon samples analyzed, and also some PCB congeners in salmon and mackerel samples.

Other compounds, like the herbicides simazine, atrazine, and terbuthylazine, and one metabolite, desethyl atrazine, had been previously reported in the LC−MS/MS routine analysis in several water samples. As these compounds are GC-amenable, they could be investigated by GC−TOF as well, and, in fact, they were confirmed to be present in the samples.

3.2.3. Nontarget Analysis. A nontarget screening in vegetable, water and fish extracts was also carried out by applying the ChromaLynx Application Manager. Nontarget screening is highly favored in GC−MS due to the availability of commercial spectral libraries, oppositely to LC−MS. The match of an unknown compound detected against spectral libraries is highly useful as a first step of the identification process. However, as commercial libraries are available in nominal mass, an accurate mass confirmation of the molecular ion (if present) and/or the main fragment ions should be done in a subsequent step. Undoubtedly, spectral libraries in accurate mass would be very valuable in this step. Using this approach, several compounds, not included in the target lists, were tentatively identified as potential contaminants (Table 2). Benzophenone, a UV filter used primarily as photoinitiator and fragrance enhancer, but also used in the manufacture of

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agricultural chemicals and pharmaceuticals, was identified in most of the water samples. Other compounds of interest were caffeine and the widely used antioxidant 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) and its metabolite 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (BHT-CHO). It is worth noticing that a water sample (water 08) presented several PCB congeners, different from those detected by our target GC−TOF MS method, especially with low number of chlorine (a total of three trichloro-PCB congeners and five tetrachloro-PCB congeners). This sample also had a high concentration of PCB 28 (0.1 μg/L), as revealed by GC−MS/MS analysis.

As an illustrative example, Figure 6 shows a positive finding of the herbicide propazine in water using the GC−TOF MS nontarget approach. Accurate mass confirmation automatically performed by the software for four representative ions led to the confirmation of the identity of propazine with mass errors below 1 mDa for all of them. This compound had not been previously included in the GC−MS/MS routine methods, and did not form a part of the (post)target list of the 200 compounds investigated by GC−TOF MS either. However, it could be successfully identified by GC−TOF MS screening, showing the complementariness of target and nontarget analysis when using GC−TOF MS.

In summary, the potential of TOF MS for screening and confirmation of organic contaminants in different types of samples of interest for public health laboratories has been illustrated in this work, as well as the complementariness of LC−TOF and GC−TOF to cover a wider range of compounds to be investigated. The research has been focused on the qualitative field, where TOF MS has strong potential, thanks to the accurate-mass full-spectrum acquisition data provided. The combined use of LC−(Q)TOF MS and GC−(Q)TOF MS appears nowadays as one of the most powerful approaches to investigate a huge number of contaminants/residues in environmental, food, or biological samples. An enhancement in sensitivity is desired and expected in the new TOF instruments launched. Although at present the triple quadrupole analyzer is the workhorse for quantitative analysis, in the near future we will surely see also interesting quantitative applications of TOF MS in the public health field.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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