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REVIEW

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Potential of liquid chromatography/time-of-flight mass spectrometry for the determination of pesticides and transformation products in water

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Abstract Until now, time-of-flight (TOF) mass analysers have only been very rarely used in pesticide residue analysis (PRA) of water samples. However, the inherent characteristics of TOF MS make these analysers well-suited to this field, mainly for qualitative purposes. Thus, the high sensitivity obtained from full-scan acquisition in comparison to other MS analysers and the high resolution of TOF MS suggest its suitability for screening purposes; it also increases the multiresidue capabilities of methods based on it and decreases the chance of recording false positives. Although these characteristics can also be helpful for quantification, confirmation and elucidation, some limitations on the use of TOF for these purposes have been observed. These limitations are more noticeable when dealing with samples containing very low analyte concentrations, which is the normal situation for PRA in water. The use of hybrid quadrupole–time-of-flight instruments (QTOF) minimises the limitations of TOF, facilitating the simultaneous detection and unequivocal confirmation of pesticides found in the sample. Additionally, the acquisition of accurate product ion full-scan mass spectra can help to elucidate the structures of unknown compounds. In this paper, the potential of TOF and QTOF hyphenated to liquid chromatography for PRA in water is explored, emphasizing both the advantages and limitations of this approach for screening, quantification, confirmation and elucidation purposes. Emphasis is placed on the determination of polar pesticides and transformation products—the analytes that fit well with LC–API–(Q)TOF MS technology.

Keywords Time of flight · Hybrid quadrupole–time-of-flight · Mass spectrometry · Pesticide residue analysis · Transformation products · Water

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Introduction

In recent years, liquid chromatography–mass spectrometry (LC–MS) using an atmospheric pressure ionization interface (API) has become an increasingly popular analytical method for determining polar organic pollutants in water. This is proved by, for example, the growing number of papers published in the last five years concerning the determination of polar pesticides in water by LC–API–MS. Interesting and detailed discussions on the use of LC–MS for the determination of organic pollutants, including pesticides, in water can be found in recent articles [1–7]. One of the main obstacles to the development of multiresidue methods for determining pesticides comes from the wide range of polarities of these compounds—including both nonionic and ionic compounds—that can potentially reach the water. This problem is increased by the current interest in monitoring transformation products (TPs), considered to be emerging contaminants by some authors [8], which are normally more polar than the parent pesticide.

Different strategies can be applied when monitoring pesticides and TPs in water, depending on the objectives pursued. The method requirements will differ depending on whether it is intended to simply detect, to quantify, to confirm the presence of a detected target analyte, or to elucidate a possible residue corresponding to a non-target analyte [5]. Thus, analytical methods can be classified into different categories: (i) screening methods, able to (quickly) detect the presence of one or more compounds based on one or more common characteristics of a class of pesticides in a qualitative or semiquantitative manner at a specified concentration limit; (ii) quantitative (determinative) methods, which should provide precise information concerning the amount of an analyte that may be present, but may only provide indirect information about the identity of the analyte; (iii) confirmatory methods, which should confirm the identity of the suspected analyte, but may or may not have a quantitative or semiquantitative component, and; (iv) elucidation methods, which should discover the identity of a suspected or unknown analyte

that was previously detected by a screening method but not confirmed afterwards.

Screening methods are very useful because they allow samples with no detectable residues (negative samples) to be distinguished from those with evident pesticide residues, ideally in a rapid manner and with little sample manipulation. This allows us to focus analytical efforts on the accurate quantitation and reliable confirmation of samples presumed to be positive.

When determining pesticide residues in water by LC–API–MS, most efforts so far have focused on the first two type of methods, mainly due to the mass analyzers used—single (Q) and triple (QqQ) quadrupole instruments [9–11]—which are adequate for the simultaneous screening and quantification of a number of preselected pesticides, especially when tandem MS with QqQ instruments are used. However, one must also ensure that the detected and quantified signal truly belongs to the suspected target analyte. Therefore, reliable confirmatory methods should be applied in order to avoid false positive findings. Potentially QqQ is well-suited to use for confirmatory purposes, although it has not been investigated much in this regard up to now in real-world samples. However, special care must be taken when selecting the number and specificity of the selected reaction monitored (SRM) transitions chosen [5, 12].

The risk of false-positive findings is significantly reduced with TOF analysers due to their increased mass resolution, mass accuracy and sensitivity in full-scan mode. Also, this analyzer is particularly suited to both non-target and post-target screening [5], as no preselection is required before data acquisition.

Even more useful in terms of confirmatory analysis is the hybrid analyser quadrupole-TOF (QTOF), as it permits the preselection of a precursor ion in the quadrupole filter and the recording of the full-scan product ion spectra with high mass accuracy, which is one of the most valuable tools for confirmatory analysis nowadays. These capabilities have led to QTOF being investigated as a technique for elucidating unknowns in environmental waters [13]. The accurate masses of both the precursor and product ions obtained using this hybrid analyser has facilitated the elucidation of pesticide metabolites and TPs in degradation studies under controlled laboratory conditions [14–19].

All of these characteristics make TOF and, particularly, QTOF analyzers very attractive for organic pollutant analysis in water. Their increasing popularity is demonstrated by the growing number of papers that have appeared in the literature since the pioneering work of Hogenboom et al. [20] in 1999, and by the trends observed in scientific meetings and specialized workshops within this field.

In spite of its enormous analytical potential, not much has been published as yet on pesticide residue analysis (PRA) in water by LC–(Q)TOFMS. Only five papers were found in our search and a few more on the degradation of pesticides under controlled laboratory conditions (Table 1). Thus, in this paper we will give an overview of the scarce scientific literature that exists in this field, focusing on the potential, advantages and drawbacks of TOF MS technology in LC-based methods for the screening, quantification, confirmation and elucidation of pesticide residues in water. Due to the scarcity of the literature, some examples from other fields, such as pharmaceuticals in water or pesticides in food, have been used to illustrate the potential of TOF for residue analysis, as they can easily be extrapolated to PRA in water.

TOF

Recent advances in mass spectrometry have meant that a new analyzer has become available, the orthogonal-accelerated time-of-flight (oa-TOF) mass spectrometer. Due to inherent advantages associated with its ion separation and detection principles compared to other mass analysers (quadrupoles, ion traps), this type of instrument is often used to identify either small or big molecules. Its high mass resolving power (>5000 FWHM) provides better

Table 1 Selected bibliography dealing with the screening, quantification, confirmation or elucidation of pesticides in water samples using TOF or QTOF analysers

Analytes	Matrix[a]	Instrument	Use	Year	Ref
10 Pesticides	SW	TOF	screening and quantification	1999	[20]
Alachlor	SW	TOF	elucidation (photodegradation)	2000	[14]
Acetolachlor, alachlor, 2 TPs	GW	TOF	screening	2002	[39]
Diuron	STD	TOF	elucidation (photodegradation)	2003	[15]
Diazinon	STD	QTOF	elucidation (photodegradation)	2003	[16]
Rotenone	SW	TOF	quantification	2003	[23]
47 Pesticides and TPs	SW GW	QTOF	confirmation	2004	[27]
1 Insect repellent	WW	TOF	elucidation	2004	[40]
Triazines	SW	QTOF	elucidation (photodegradation)	2004	[17]
Quaternary ammonium herbicides	DW	TOF	quantification	2004	[25]
18 Pesticides, 9 TPs	GW SW	QTOF	screening, quantification and confirmation	2005	[5]
Carbofuran	STD	QTOF	elucidation (photodegradation)	2005	[18]
Diazinon	SW	QTOF	elucidation (photodegradation)	2006	[19]

[a]SW: Surface water, GW: groundwater, STD: standards, WW: waste water, DW: drinking water

confirmatory ability and signal-to-noise ratios than single quadrupole analysers, especially when dealing with complex matrix samples. A unique feature of accurate mass determinations (<5 ppm) performed using TOF is the useful information obtained about elemental compositions, which can confirm or rule out potential molecular formulae. Additionally, the inherently high sensitivity of the TOF analysers when used in full-scan mode is useful for detecting organic pollutants at relevant environmental levels.

The characteristics of TOF mass analyzers make them useful when developing analytical methodologies for screening, quantifying, confirming and elucidating pesticide residues and their TPs in water. LC–TOFMS methods are well-suited for polar (and ionic), nonvolatile, thermolabile compounds—a wide range of non-GC amenable analytes—which are often found in water, mainly in groundwater as a consequence of their higher leachability from soil environments.

Screening

Screening methods should detect the presence of target compounds in a qualitative or semiquantitative manner at a specified concentration limit. In MS-based methods, the characteristic mass of an analyte is screened for when monitoring its presence in water.

The benefits of using a TOF analyzer comes from its measuring principle, which allows it to perform full-scan acquisitions with superior sensitivity and high mass accuracy. Therefore, the monitoring of an specific mass of an analyte is not predefined before data acquisition, and post-target screening can also be performed if desired. This fact allows us to detect the presence of an unlimited number of potential contaminants without reanalysis, provided that all of these compounds share both ionization and separation modes, even in cases where no sample is left to be reanalysed. An illustrative example has been reported by our research group [13] when analyzing an urban wastewater sample in the Castellon province, an area with a predominance of citrus crops. The presence of the post-harvest fungicide imazalil led us to suspect that other post-harvest fungicides also used in citrus crops may be present too. The previously acquired dataset was re-evaluated, extracting chromatograms at the specific masses of different fungicides. In this way, the presence of thiabendazole ($[M+H]^+$ *m/z* 202.0439) was detected in a post-target style.

The high multiresiduality of this approach is not easily achieved by quadrupole mass analyzers (both Q and QqQ, working in SIM or SRM mode, respectively) due to the need to predefine the masses to be monitored and because it is difficult to reduce the dwell time below a threshold value while maintaining a suitable sensitivity.

On the other hand, the elevated mass resolution of TOF analyzers allows us to reduce the mass window when extracting a specific mass from the full-scan dataset. A smaller mass window leads to a substantial reduction in the chemical noise, facilitating the detection of the screened compound in the extracted ion chromatogram (XIC, also named EIC or RIC depending on the manufacturer). Some authors call these chromatograms microwindow XICs (mwXICs) and the benefits of reducing the mass window from 1 Da scale (similar to that for quadrupole or ion trap analyzers) down to 10–20 mDa were reported some time ago [20] in the environmental field. However, a drawback of using narrower mass windows, apart from the reliability of the mass accuracy attainable by the TOF analyzer used, is the significant mass errors produced by coeluting isobaric interferents that cannot be resolved by the analyser. The increase in mass errors can be so high that the compound being screened for may fall out of the monitored mass window, leading to a false negative being reported. This situation was observed by Benotti et al. [21] when screening some pharmaceuticals in wastewater effluents. Mass errors as high as 20 mDa were observed in their work for caffeine due to the presence of the ^{13}C isotope peak from a coeluting compound with a mass 1 Da lower than the analyte. Therefore, if the mwXICs would have been reconstructed with a small mass window, typically ±5–10 mDa, the presence of caffeine would have been masked and a false negative sample would have been reported.

Thus, when performing screening in real samples, one should be cautious and avoid using unreasonably narrow mass windows. As a compromise between improving baseline noise and signal-to-noise ratio and preventing reporting false negatives, a 50 mDa mass window is recommended when reconstructing mwXICs. However, this mass window will presumably be narrowed following the expected increase in resolving power achievable by TOF analysers.

Quantitation

When dealing with pesticide residues in water, the sensitivity attainable is possibly the key issue. In this sense, TOF offers high sensitivity under full-scan conditions compared to other analyzers, but triple quadrupole instruments working in SRM mode show their superiority in target pesticide quantitation. Thus, in the determination of pesticides and transformation products in water, TOF was found to be around one order of magnitude less sensitive than a triple quadrupole instrument used in SRM mode [5]. This lower sensitivity hampered the detection of some pesticides by TOF, which were easily detected by triple quadrupole instruments. This gap of around one order of magnitude was also found in the determination of other pesticides (carbamates, organophosphorus and triazines) in water [20] and in other fields such as the determination of pharmaceuticals [21] and cyanobacteria toxins [22] in water. However, in all cases, the sensitivity achieved by TOF was sufficient regarding the required detection limit of each application.

In addition to sensitivity, the feasible linear dynamic range of the TOF response is of paramount importance when applied for quantitative purposes. These instruments

usually suffer from narrow dynamic ranges. Thus, linear ranges of a maximum of two orders of magnitude are typically used for quantitative purposes, as in the case of the multiresidual determination of pesticides in water [20, 23] or in vegetables [24]. Although this range can be sufficient for quantitative purposes, there are some applications, such as the quantification of cyanobacteria toxins in water [22], which have been performed by using a single point calibration.

As an illustrative example of the potential of TOF instruments for quantification purposes, the determination of quaternary herbicides in water by on-line SPE was performed [25]. Using this approach, suitable LODs (lower than 0.1 μg/L) were achieved for mineral water samples loaded with 30 mL of sample, although higher values (around 0.5 μg/L) were obtained in tap and groundwater samples. The use of triple quadrupole instruments in SRM mode decreased the LODs, which were always lower than 0.06 μg/L. In terms of the linear dynamic range, a calibration plot covering around three orders of magnitude was feasible in the determination by triple quadrupole, while the calibration range was reduced down to 1.5 orders of magnitude when working with TOF instruments.

Confirmation

Confirmatory methods should verify the identity of the suspected analytes detected during the screening process in order to confirm the finding and avoiding the reporting of false positives.

The confirmation process is typically achieved by acquiring more mass information regarding the suspected compound and applying various criteria in order to ensure data quality. The criterium established in a Commission Decision of the EU is one of the most useful and widely applied [26]. This Decision proposes the use of so-called identification points (IPs), with at least three IPs being required to confirm a positive finding. IPs are earned by detecting mass ions, both precursor or product ions, and the number of IPs earned depends on the technique used. The EU Decision assumes that an ion measured with a high-resolution MS instrument (resolving power of 10,000 at the *m/z* being measured, based on 5% valley or 20,000 resolving power for FWHM) would give twice the number of IPs given by a low-resolution MS. This assignment of 2 IPs versus 1 IP is based on resolution power, rather than on mass accuracy, although the accurate mass measurements provided by these instruments seem to be more relevant within this subject [27]. Thus, although TOF analysers do not normally reach resolving powers of up to 20,000, they have been considered to be high-resolving instruments in the confirmation of pesticides in vegetables, and 2 IPs per ion measured were assigned in this case [28]. Thus, the potential of TOF analysers for confirmation is evidenced by the higher number of IPs assigned in comparison to low-resolution instruments.

To qualify for IPs, at least one ion ratio must be also measured, and it must be within specified tolerances. This means that a minimum of two ions must be measured with TOF instruments, earning 4 IPs and allowing us to confirm both regulated and even banned compounds. However, the measurement of two ions using API interfaces may be troublesome if the suspected analyte does not show a rich characteristic isotopic pattern or abundant in-source fragmentation. The presence of elements with abundant heavier isotopes can help us to achieve the required number of IPs, but no additional structural information is obtained. In this sense, the measurement of in-source fragment ions seems to be more valuable. Thus, Ferrer et al. [28] proposed a confirmatory approach based on the in-source fragmentation of three chloronicotinyl pesticides in vegetable samples. In this methodology, four different ions were obtained for imidacloprid and two ions for acetamiprid and thiacloprid at two different fragmentor voltages. The halogen isotopic pattern must be taken into account in order to obtain a correct confirmation in this case.

However, some drawbacks may be encountered when using the in-source fragmentation approach in the environmental field, where confirmation at low analyte concentrations might be troublesome due to the lower abundance of fragment ions compared to the precursor ones. Besides, the low *m/z* value usually obtained for fragment ions is more prone to interference, and the origin of the fragment ions may not be unequivocal, which can complicate the confirmation process, particularly for complex matrix samples.

Despite the growing understanding in the scientific community about the need to provide reliable confirmations of positive findings, there are still some papers where confirmations have been made using only the mass error obtained on one ion. Thus, some pharmaceuticals [21] and nitrotoluensulfonic acids [29] in water have been confirmed with this approach. The number of compounds sharing the same empirical formula and therefore exact mass can be surprisingly high, which makes information on fragments necessary.

Elucidation

The high resolution and accurate mass measurements obtained by TOF analysers can be a great advantage in the elucidation of unknown compounds. Thus, the combination of the accurate mass together with a detailed study of the isotopic pattern of the unknown allows the number of potential molecular formulae for the compound detected to be reduced. This strategy has been used by different authors in the elucidation of some pesticide metabolites. Thus, Garcia-Reyes et al. [30] successfully elucidated different chlorinated pesticide metabolites using the isotopic pattern as a filter and the measured accurate mass to discriminate between all possible formulae.

In the elucidation of unknowns, the molecular formula obtained can be fed into databases, leading to a reduced number of possible structures for the unknown compound. However, this approach presents several drawbacks which limit its application in the elucidation process. The most important one is the inability to distinguish between

isomeric compounds, as compounds with the same molecular formula can not be distinguished using TOF instruments. Other limitations arise from the absence of a characteristic isotopic pattern for the investigated compound or the lack of unequivocal information about possible candidates with the exact mass obtained, which can also reduce the applicability of TOF for this purpose.

The complementary use of other techniques is normally required to obtain the molecular formula [14–17]. As an example, Hogenboom et al. complement the data obtained by TOF with those obtained with triple quadrupole and GC–MS in order to elucidate alachlor metabolites [14]. In the same way, GC–MS and TOF analysis were used in the elucidation of some diazinon metabolites [16], and diuron metabolites have been elucidated with complementary information obtained from ion trap and TOF analysis [15]. In the elucidation of triazine herbicide metabolites, this complementary information was obtained by QTOF [17], which had the additional advantage that both TOF and QTOF analysis could be performed with the same instrument. The potential of QTOF for elucidation will be discussed in the next section.

As an example of the potential and limitations of TOF for elucidation, Fig. 1 shows a XIC chromatogram obtained when investigating a compound that seemed to be the herbicide diuron. Although the retention time and the nominal mass were similar to those for this compound, both the accurate mass (*m/z* 233.1121) and the isotopic pattern differed significantly from the expected ones (*m/z* 233.0248); therefore, the water sample was reported as negative for this herbicide. In an attempt to gain a wider knowledge about the sample composition, TOF was used for the elucidation of this isobaric compound. A search for the molecular formula was attempted without any restriction, neither in the number of atoms (C 0–100, H 0–200, N 0–20, S 0–3, P 0–3, F 0–20, O 0–20) nor in the double bond equivalent (DBE −0.5–50). Under these conditions, 66 possible formulae were obtained for this compound. The restriction imposed by the isotopic pattern reduced the number of possible formulae down to nine. The plausible molecular formulae, together with isotopic abundance pattern, are shown in Table 2. Despite the great potential of TOF to reduce the number of possible molecular formula, the absence of a characteristic isotopic pattern (presence of chlorine or bromine atoms) hampered the elucidation. Additionally, Table 2 also shows the results obtained when searching for plausible formulae in different databases. Thus, only three molecular formulae presented entries in databases, with a total of 14 possible structures. The use of TOF could not be used to distinguish between these 14 structures and additional experiments with other instruments, such as GC–MS, IT or QTOF, would be required in order to unequivocally elucidate this unknown.

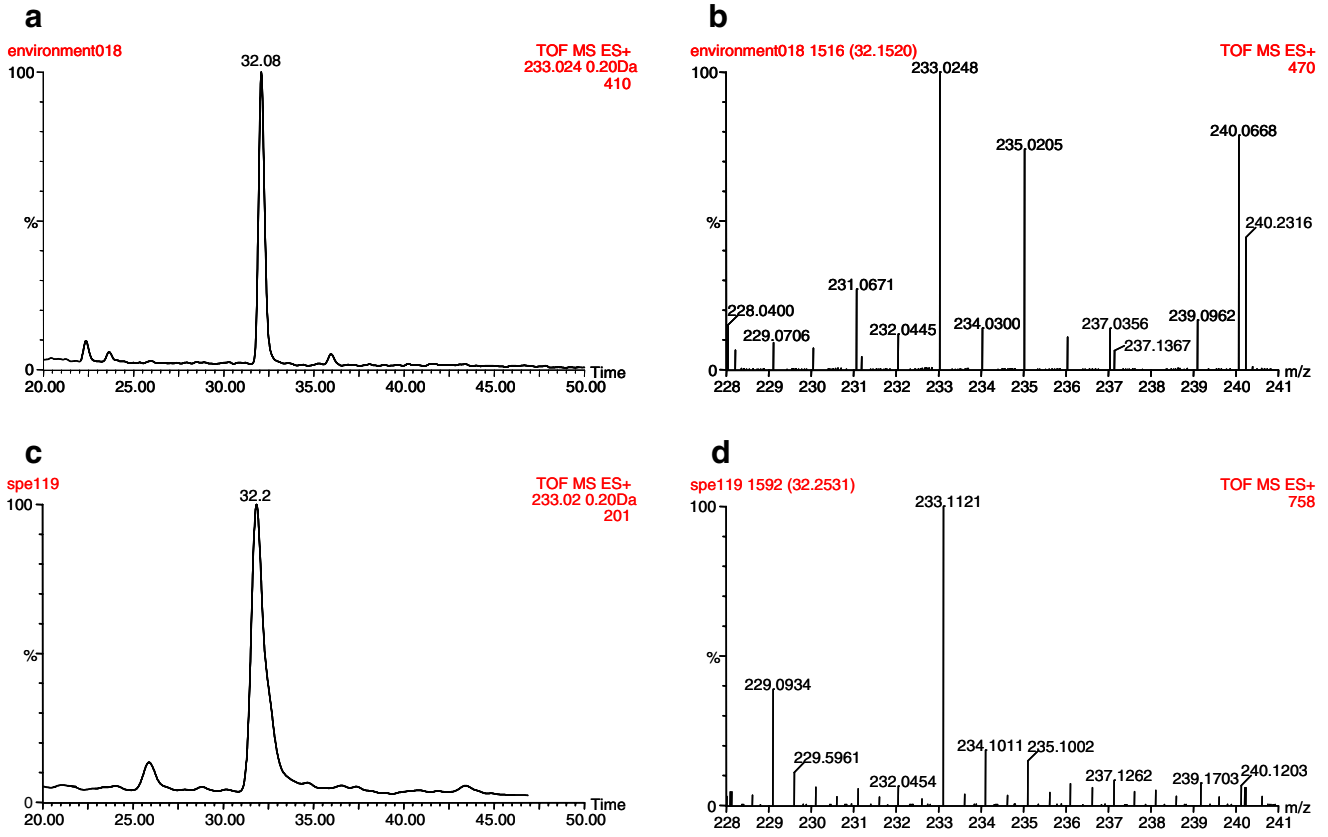

Fig. 1a–d Use of TOF for diuron screening. **a** Chromatogram for a diuron standard (cone 25 V), **b** TOF spectrum of the standard at the diuron retention time, **c** chromatogram for a water sample suspected to be positive (cone 25 V) and **d** TOF spectrum of the water sample at the diuron retention time

Application to water samples

Although TOF instruments are a valuable tool in environmental analysis, their potential can be substantially limited in the determination of organic micropollutants—like pesticides and transformation products—in water samples due to the high sensitivity normally required. This fact can complicate the detection of some analytes at regulatory low levels (e.g., 0.1 μg/L in drinking water). The application of higher preconcentration factors during sample treatment is an obvious and efficient alternative that can be used to improve the sensitivity of the method, but it may also lead to undesirable effects such as analyte breakthrough in SPE procedures or the preconcentration of matrix interferences, which can produce ionization suppression or enhancement. Controlling these adverse effects is crucial in quantitative methods, although it seems less important for qualitative purposes (i.e., screening, confirmation and elucidation). As an example, Nuñez at al. [25] preconcentrated 30 mL of water as a compromise between sensitivity and breakthrough. When using this sample volume, although some analytes such as difenzoquat present satisfactory recoveries (90%) that achieve the required detection limits, for other compounds such as chlormequat significant breakthrough starts to occur, resulting in recoveries of around 50 %. In any case, this volume was not enough to obtain LODs below the required levels (0.1 μg/L) in the matrices tested.

The low concentrations normally present in the environment may make some of the TOF limitations previously stated more noticeable. Thus, the analyte mass deviation produced by the presence of an isobaric coeluting interference increases with the relative abundance of the interfering compound. Therefore, the lower the analyte concentration, the higher the mass deviation observed when this type of interference is present. Even some of the mass ions produced by the mobile phase can interfere in the analyte mass measurement when present at residue concentration levels.

Another aspect should also be emphasized when confirming positive findings at low concentrations. In-source fragments are required for a safe confirmation in many cases. The ions obtained from this type of fragmentation are frequently less abundant than $[M+H]^+$ or $[M-H]^-$ ions, and this fact can hamper confirmation at low concentration levels. Besides, in complex matrices it can be difficult to obtain information about the origin of the ion origin some interferences may share the same mass as the in-source fragment. Some nonisobaric interferences may even produce isobaric fragments that would interfere with the confirmation. This situation is more problematic when low *m/z* fragments are selected, as this region of the spectrum tends to be noisier when the cone voltage is increased. The use of QTOF drastically minimizes these limitations, increasing confidence about the origin of the ion and also reducing the isobaric interferences, as discussed in the next section.

QTOF

The development of hybrid quadrupole–time-of-flight (QTOF) instruments has presented the analyst with an attractive new tool for the determination of pesticides in the environment. Although its use is still very limited in this field, mainly due to its high cost, QTOF has undoubted potential due to its inherent characteristics. QTOF presents all of the advantages indicated above for TOF, as it can be used in TOF mode, with the first quadrupole acting as an ion guide. However, the biggest advantage of QTOF is not obtained when it is used in TOF mode.

The main advantage of QTOF is its ability to perform accurate product ion mass scans. Thus, while the accurate

Table 2 Feasible molecular formulae for an unknown (m/z 233.1112) found in a wastewater sample

Unknown compound						
	Absolute abundance			Relative abundance (%)		
	M	M+1	M+2	M+1	M+2	
Intensity in combined spectrum	532	76	42	14.2	7,9	
+Acceptable error[a]				17.0	11,8	
−Acceptable error[a]				11.4	3,9	
Feasible molecular formulae						
	Absolute abundance			Relative abundance (%)		
	M	M+1	M+2	%M+1	%M+2	NIST database entries
$C_{10}H_{17}N_2OFS$	8402	1088	454	12.9	5.4	–
$C_{11}H_{21}OPS$	8366	1120	456	13.4	5.5	–
$C_{13}H_{16}N_2S$	8148	1323	461	16.2	5.7	9
$C_{10}H_{20}N_2S_2$	7999	1099	779	13.7	9.7	–
$C_8H_{16}N_4O_2S$	8508	977	462	11.5	5.4	–
$C_{12}H_{18}F_2S$	8297	1196	447	14.4	5.4	–
$C_7H_{16}N_6OS$	8560	947	444	11.1	5.2	–
$C_{11}H_{20}OS_2$	7950	1126	794	14.2	10.0	4
$C_{11}H_{20}O_3S$	8327	1120	488	13.5	5.9	1

[a]Tolerance range extracted from [11]: ±20% for M+1 and ±50% for M+2

mass obtained from TOF allows us to establish the elemental composition of a compound, QTOF allows us to establish the elemental compositions of all of the product ions obtained, which is very helpful when attempting to elucidate unknowns. The accurate mass, when combined with the acquisition of the full-scan spectra for the product ions, also provides a powerful tool for the unequivocal confirmation of positives (target analytes).

The emergence of QTOF opens up new possibilities for the determination of pesticides in the environment regarding screening, quantification, confirmation and elucidation, although some limitations should be taken into account in order to properly evaluate the potential of this powerful analysis technique in this field.

Screening

The use of QTOF in MS/MS mode implies the preselection of the analytes, which makes it necessary to know the analyte mass in order to filter it in the quadrupole. This is a limitation on its use for screening purposes as it would impede the performance of post-target screening. Thus, the great benefit of QTOF for screening purposes is actually found in pre-target applications. Obviously, to avoid preselection of the analytes, the QTOF instrument could be used in TOF mode, but the main advantages of this hybrid analyser are not apparent when working in MS/MS mode.

The screening by QTOF also presents a drawback related to the limited number of compounds that can be monitored simultaneously. The sequential mode aquisition performed by the quadrupole and the relatively long time needed by the TOF to get an adequate response for the measured product ion spectrum (around a second) significantly reduces the number of analytes that can be selected in a short time period. Under these conditions, efficient chromatographic separation is essential for reliable screening.

For these reasons, the use of QTOF for screening purposes has normally been limited to a few pre-target compounds. Thus, QTOF was investigated for the multiresidue screening of around 30 pesticides and transformation products, but due to the limitations stated above, QTOF was considered to be more valuable for confirmative analysis [5]. Another example of the use of QTOF for screening purposes can be found in the determination of pharmaceuticals in water [31]. In this case, a multiresidue method that included 13 pharmaceuticals was developed for simultaneous screening and confirmation at the low μg/L level.

The number of analytes that can be included in the method could be increased by using automated MS to MS/MS switching, as demonstrated by Bobeldijk et al. [32]. This approach is based on the possibility of automatically changing from MS to MS/MS mode when the compound of interest is eluting from the analytical column. The instrument is initially set-up as TOF acquiring in full-scan mode; when a specific mass exceeds a predefined number of counts, the instrument automatically changes to MS/MS mode, recording the product ion spectrum of this mass, and returning to TOF mode when the spectrum is acquired. This approach was tested for six pesticides used as model compounds, which showed its suitability for screening and identifying them. Subsequently, the developed methodology was extended to four unknowns, which demonstrated one of the most important advantages of this approach: it avoids the need to preselect the analytes before screening. The most noticeable limitation is the need to predefine a threshold value above which a compound is considered relevant.

Despite some limitations, the use of QTOF for screening purposes presents important advantages derived from the inherent characteristics of this analyser: the acquisition of the complete and accurate product ion mass spectrum allows the simultaneous screening and confirmation of the selected analyte. Thus, when using QTOF in screening applications, an additional injection for confirmation is not necessary. Recent QTOF instruments, that have acquire spectra faster, will surely allow us to increase the number of compounds screened in the near future.

Quantitation

The application of QTOF to the quantification of organic pollutants in environmental samples has been quite limited so far [31,33–35], and to our knowledge no study quantifying pesticides in environmental samples by QTOF has been performed. The main reasons are almost certainly its lower sensitivity and linear dynamic range compared to triple quadrupole in SRM mode, as well as its high price. The sensitivity achieved by a QTOF is of the same order as that achieved by TOF, and normally around tenfold lower than QqQ in SRM mode [5, 33]. The linear range achieved by QTOF is also limited, tending to be around one order of magnitude [33, 35] or even lower [31, 34].

However, the use of QTOF for quantification may have some advantages over TOF. For example, mw-XIC is not normally necessary for a correct quantification as most of the interferences are filtered in the quadrupole. Only the isobaric interferences isolated in the quadrupole that produces isobaric product ions would affect the quantification of the analyte, which is only happens very rarely in real samples. Therefore, correct quantification, without noticeable interferences and with an extremely low background, can be obtained without the need to achieve an extremely accurate mass. Thus, some deviations in accurate mass measurements, such as those produced by high concentrations of analyte or by the presence of quasi-isobaric compounds [21] which can produce poor quantification when using TOF with mw-XIC, are less important when using QTOF for quantification purposes.

In our opinion, the most important advantage of QTOF is its inherent ability to confirm analyte identity at the same time as performing quantification, without the need for an additional confirmatory analysis.

Confirmation

As stated before, one of the main advantages of QTOF instruments is the inherent confirmation provided by the acquisition of the accurate full product ion mass spectrum. When confirming positive findings by QTOF, both the exact masses and the relative intensities of all of the available product ions of a sample can be compared with those of the reference standard. The number of IPs reached when using QTOF is much higher than the minimum required, and the confirmation achieved by QTOF can be considered to be the ultimate confirmation of analyte identity [5]. In this way, although some authors propose the use of LC–TOF to confirm the analyte identity, based on the measurement of the accurate mass of one ion [21], this confirmation may still not be sufficient, and confirmation by QTOF would be better [5, 27, 31, 33].

Another important advantage of QTOF in confirmatory applications is its ability to obtain abundant fragmentation without any significant interference. The use of QTOF minimises the limitations of TOF instruments when working with in-source fragment ions, as the selection of a precursor ion in the first quadrupole increases confidence about the origin of the product ion and decreases the chemical noise. Additionally, the low chemical noise and/or the efficient fragmentation produced in the collision cell increase the number and relative intensities of product ions when using QTOF, improving the quality of the confirmation quality and also enabling us to confirm positive samples at concentration levels close to the limit of detection.

This approach has been successfully applied by Stolker et al. [33] to the confirmation of different drugs in several matrices, including environmental waters, showing the suitability of QTOF compared to other analysers such as triple quadrupole. Going back to pesticide examples, Fig. 2 and Table 3 show the ultimate confirmation achieved in a groundwater sample suspected of being positive for terbumeton. The deviations obtained in the measured masses of all of the product ions were lower than 2 mDa. Additionally, when comparing the relative abundances observed in the sample suspected to be positive with the relative abundances obtained for a reference standard, all of the deviations were within the limits proposed by the European Decision 2002/657/EC [26] except for the less sensitive product ion (relative abundance 2.3%). Therefore, this sample was confirmed by QTOF to be positive for terbumeton, obtaining 13.5 IPs.

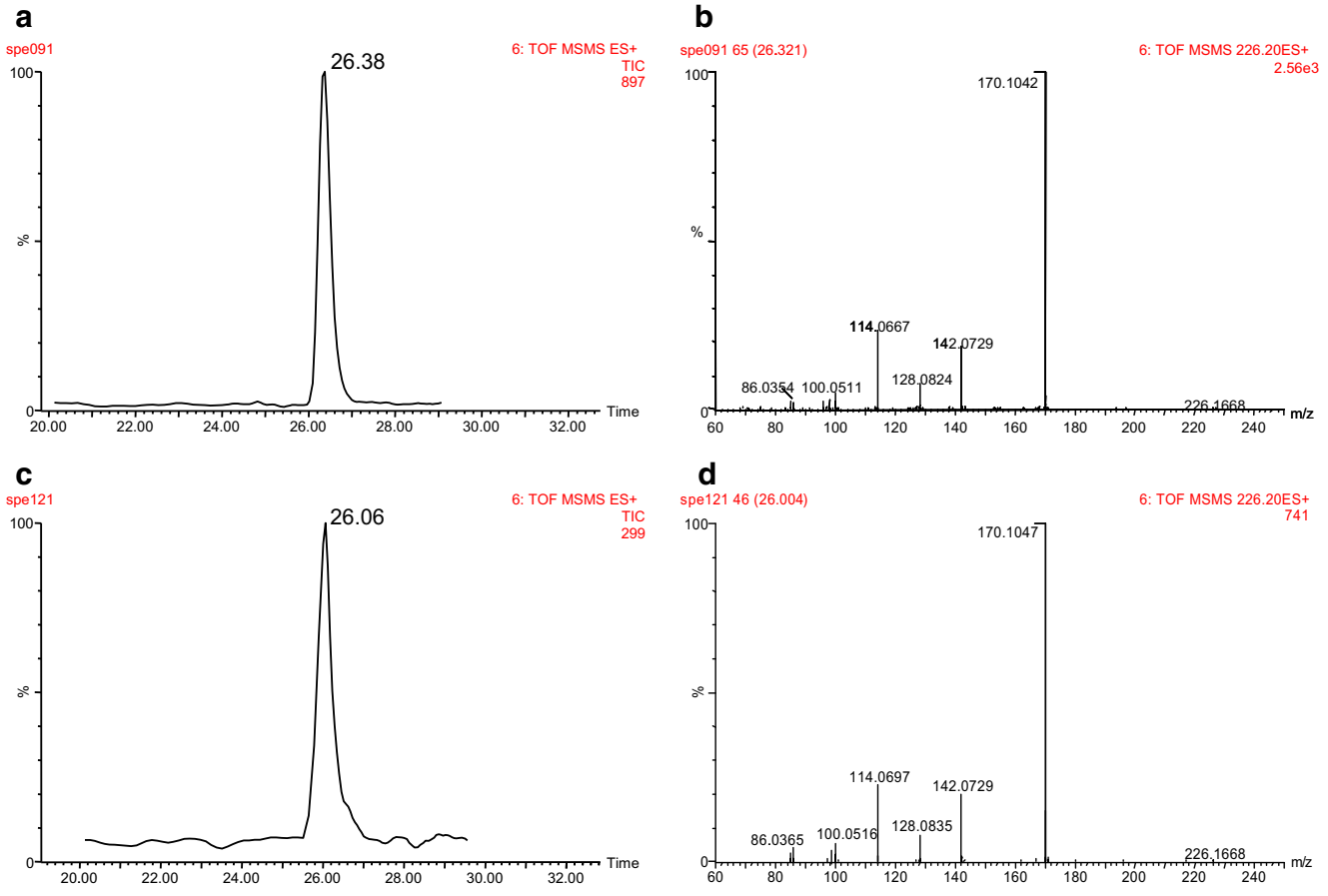

Fig. 2a–d Confirmation of a positive water sample by QTOF. **a** QTOF chromatogram and **b** product ion spectrum of *m/z* 226 (cone 25 V, collision energy 25 eV) from a terbumeton standard, **c** QTOF chromatogram and **d** product ion spectrum of *m/z* 226 (cone 25 V, collision energy 25 eV) from a water sample suspected of being positive for terbumeton (0.056 μg/L). See Table 3

Elucidation

Apart from providing the ultimate confirmation of positive findings, the valuable information yielded by the accurate product ion mass spectra aids attempts to elucidate the structures of unknown compounds. In some cases, when the experiment is carried out under controlled conditions, the elucidation achieved via QTOF can be considered to be definitive, even without the use of any additional technique.

Thus, QTOF has been used for the elucidation of pesticide metabolites in human fluids [19] and in photodegradation studies [16–18]. The elucidation of metabolites under controlled conditions can be performed by comparing a sample spiked with the pesticide to a blank sample subjected to the same experimental process. The occurrence of a peak in the sample chromatogram which is not present in the control sample reveals the existence of a metabolite/photodegradation product, which can be identified by means of the product ions obtained. As an example, triazine photodegradation products have been elucidated in detail using LC–QTOF [17]. In a first step, the fragmentation pathways of the parent herbicides were proposed based on the accurate masses of the product ions in order to obtain detailed knowledge of the MS/MS behaviour of this family of analytes. Then, the molecular formulae of the observed photodegradation products were obtained from their accurate masses. In order to elucidate the final structure of each photodegradation product, its MS/MS spectrum was compared with the parent compound assuming a similar fragmentation pathway. Using this approach, most of the photodegradation products of triazines were unequivocally elucidated.

The potential of QTOF to elucidate unknowns is more limited when dealing with samples where no previous knowledge is available about the possible structures of the investigated compounds. In these cases, the most common approach is to obtain the molecular formula and to then search in a database. The accurate product ion mass spectrum provides additional structural information which is useful for discriminating between possible isomeric structures, making elucidation feasible in some cases. Comparison of the retention time and the MS/MS spectrum with a reference standard, if available, tends to be the final way to unequivocally identify the unknown compound [13].

In the previous example related to an isobaric compound of diuron, which could not be solved by TOF, the use of QTOF permitted discrimination between most of the 14 chemical structures found in NIST database. As Fig. 3 shows, after acquiring the product ion spectrum of the parent ion *m/z* 233, only one intense product ion was observed at *m/z* 151.0325, and this was obtained after the loss of cyclohexene (C_6H_{10}, theoretical *m/z* 82.0783, experimental error 0.4 mDa). Only two out of the 14 potential compounds presented a cyclohexane moiety, and therefore the number of plausible structures was reduced down to these two candidates. In order to get the ultimate confirmation of the unknown, a comparison between the product ion spectra and the retention times for both the sample and the standard, if available, would have been necessary.

The application of this approach is helpful in the elucidation of unknowns, but unsuccessful attempts frequently occur. The most common drawback is the absence of the predicted molecular formula in the database, as many compounds, including most pesticide metabolites/transformation products, are not included in commercial databases. Thus, although analysis by QTOF can provide the molecular formula and some specific fragments, the assignment of a concrete structure is virtually impossible in many cases. This means that the elucidation of organic pollutants in environmental samples is troublesome [13, 36]. The elucidation by QTOF is more problematic when there is no specific fragmentation in the analyte molecule. This is illustrated in the work of These et al. [37], where the elucidation of fulvic acids was hampered because the only losses observed corresponded to water and carbon dioxide, which are not specific and are in fact common to all compounds of this family.

Application to water samples

To our knowledge, there are almost no works on the application of LC–QTOF to pesticide residue analysis in the environment, despite its strong potential in this field. This hybrid analyser circumvents most of the limitations of TOF regarding quantification and confirmation, just as tandem MS is far more powerful than single MS in terms of its analytical characteristics. The most significant limitation when applied in environmental fields is probably its relatively low sensitivity, mainly when compared to triple quadrupole instruments, which can hamper the detection of

Table 3 Results obtained in the confirmation of the herbicide terbumeton ($C_{10}H_{19}N_5O$) detected in a groundwater sample from the Spanish Mediterranean area (0.056 μg/L). See also Fig. 2

Product ion	Accurate mass			Relative abundance (%)		
	Theoretical	Experimental	Deviation (mDa)	Theoretical	Experimental	Deviation
$C_6H_{12}N_5O$	170.1042	170.1047	0.5	100	100	–
$C_4H_8N_5O$	142.0729	142.0729	0	18.6	20.5	10.2
$C_5H_{10}N_3O$	128.0824	128.0835	1.1	7.8	8.5	9.7
$C_4H_8N_3O$	114.0667	114.0697	3	24.4	22.9	6.3
$C_3H_6N_3O$	100.0511	100.0516	0.4	5.0	5.8	15.3
$C_2H_4N_3O$	86.0354	86.0365	1.1	2.3	3.9	68.2

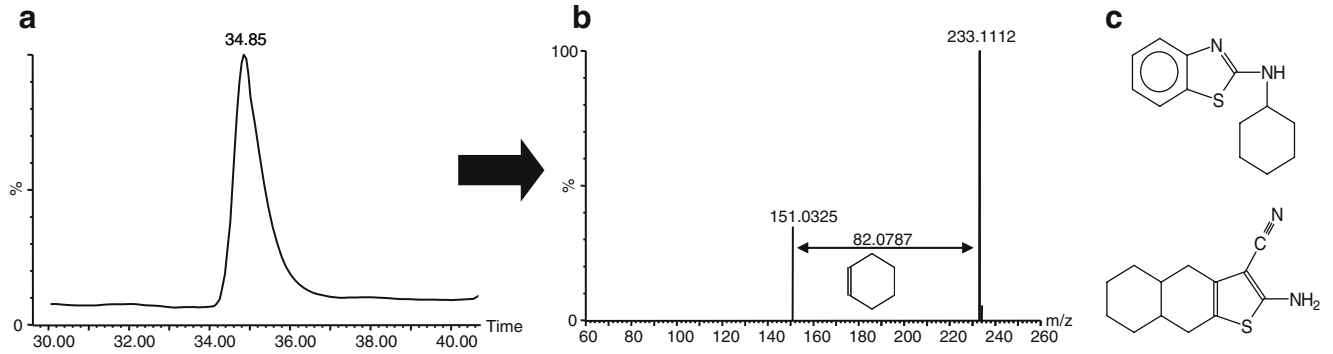

Fig. 3a, b Elucidation of unknowns by LC-QTOF. **a** Chromatogram and **b** product ion spectrum of *m/z* 233 from a sample containing a diuron isobaric compound. **c** Feasible structures for this unknown

some analytes at sub-ppb levels. As an example, the detection of several antibiotics in water by QTOF was not feasible at the concentrations found in water (normally between 10 and 30 ng/L, determined by QqQ) even after increasing the preconcentration factor used in off-line SPE procedures sixfold [38].

The unequivocal confirmation of the analyte identity is automatically achieved by QTOF when used in both screening and quantitative methods. Theoretically, this ultimate confirmation would only be hampered by the unusual presence of an analyte coeluting with isobaric interference that also presents several common product ions. Due to the low probability of encountering this scenario, one can conclude that QTOF is the ideal tool for confirmation purposes. However, one should be aware that coeluting isobaric interferences may hamper the confirmation, even in the case that they do not share any isobaric product ion, a situation that is more likely to occur in complex matrix samples. As the confirmation of the analyte is usually performed by comparing the product ion spectra for both the standard and the sample, the presence of coeluting isobaric interferences may lead to a complex composite spectrum containing product ions from the analyte and the interferent, which would make a comparison of both spectra troublesome. This situation is favoured at low analyte concentrations, such as those that occur in environmental samples. Thus, the lower the required concentration level, the higher the number of potential compounds that can interfere with the analysis [12]. This effect is more problematic when elucidating unknowns, as a composite spectrum can result in misinterpretation of the observed fragmentation, causing either an impossible or a false elucidation.

The easiest way to reduce this limitation is to improve the chromatographic separation between the analyte and the interference. This solution is relatively easily applied when confirming positive findings, but its application to the elucidation of unknowns is more limited because the analyst does not have any evidence of the occurrence of a coeluting interferent.

More than 100 water samples from the Valencian region, a Spanish Mediterranean area with a long agricultural tradition and a predominance of citric crops, were investigated, and the most relevant positive findings were confirmed by LC–QTOF [5]. Most of the confirmed findings (reaching typically a minimum of 7 IPs) were herbicides, mainly triazines and their TPs, normally present at concentrations between 0.1 and 0.5 μg/L. Some samples with lower analyte concentrations could also be confirmed with a high number of IPs. As an example, Fig. 2 and Table 3 show the confirmation of terbumeton at 0.056 μg/L.

Conclusions

The inherent characteristics of TOF MS (mass accuracy and high resolution) make this analyser very attractive in the pesticide residue analysis of water samples. Its potential is more evident when used for screening purposes because the acquisition of full-scan spectra with high sensitivity increases the multiresidue capability of the method and facilitates the application of LC–TOF methods to the screening of, in principle, an unlimited number of compounds. Additionally, the use of mw-XIC also allows the number of interferents to be reduced, making the screening more efficient. On the other hand, the use of hybrid QTOF for screening limits the number of analytes that can be included in the method, although an unequivocal confirmation of the analyte identity is achieved at the same time.

In terms of quantification, both TOF and QTOF present a common limitation, which derives from the low linear dynamic ranges of TOF analysers. This limitation is expected to be minimised in the upcoming generation of instruments equipped with ADC digitizers, which will surely be able to increase the linear range, improving the applicability of these instruments to quantification.

The advantages of using TOF for screening, quantification, confirmation and elucidation purposes can decrease when a (quasi)isobaric interferent coelutes with the analyte. The presence of this interferent can affect the accurate mass obtained by TOF, generating an erroneous quantification, even after using mw-XICs, and it also makes the assignment of the correct molecular formula more difficult. In such a case, the confirmation of the analyte identity and, obviously, the elucidation of unknown (non-target) compounds is hampered. Under these circumstances, the applicability of TOF instruments is quite limited, making it necessary to perform an efficient chromatographic

separation in order to minimise the risk of obtaining coeluting interferents.

This problem is minimised when using QTOF because the chances of finding a coeluting isobaric interferent which also presents an isobaric fragment are much less. Thus, the quantification can be correctly performed by selecting a product ion that does not encounter interference in the MS/MS mode. However, the presence of a coeluting isobaric interferent might affect the confirmation and elucidation processes, even if no isobaric fragments are shared with the analyte. In this situation, a composite product ion spectrum would be obtained, causing difficulties when attempting confirmation by comparison with a reference standard and making it particularly difficult when elucidating an unknown structure based on the fragments obtained.

In summary, when a finding is confirmed by QTOF, this confirmation can be taken as being unequivocal. However, a finding not confirmed by QTOF may in some cases actually be a positive hampered by coeluting isobaric interferences. The possibility of reporting false negatives is certainly quite low, but it increases when the analyte is present at very low concentrations, such as sub-ppb levels. More research would be necessary for samples not confirmed by QTOF, including a detailed study of the product ion mass spectra and the chromatographic separation, in order to obtain reliable results.

The potential of QTOF for elucidating unknowns comes from the accurate mass measurements it can take in the product ion spectrum. Although the utility of this hybrid analyser has been demonstrated in recent scientific articles, the elucidation of non-target compounds in environmental samples is still a challenge for analytical chemists due to understandable difficulties associated with this subject. A considerable increase in the number of publications dealing with TOF MS applications in pesticide residue analysis is expected in the very near future, as expected from the great potential of this technique.

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