BUILDING AN EMPIRICAL MASS SPECTRA LIBRARY FOR SCREENING OF ORGANIC POLLUTANTS BY UHPLC-QTOF MS

Ramón Díaz, María Ibáñez, Juan V. Sancho, Félix Hernández*

Research Institute for Pesticides and Water, University Jaume I, E-12071 Castellón, Spain

Tel: +34 964 387366

FAX: +34 964 387368

E-mail: felix.hernandez@qfa.uji.es

ABSTRACT

Hybrid quadrupole time-of-flight mass spectrometry (QTOF MS) has gained wide acceptance in many fields of chemistry, for example proteomics, metabolomics and small molecule analysis. This has been due to the numerous technological advances made to this mass analyser in recent years. In the environmental field, the instrument has proven to be one of the most powerful approaches for the screening of organic pollutants in different matrices due to its high sensitivity in full acquisition mode and mass accuracy measurements.

In the work presented here, the optimum experimental conditions for the creation of an empirical TOF MS spectra library have been evaluated. For this model we have used a QTOF Premier mass spectrometer and investigated its functionalities to obtain the best MS data, mainly in term of mass accuracy, dynamic range and sensitivity. Different parameters that can affect mass accuracy, such as lock mass, ion abundance, spectral resolution, instrument calibration or matrix effect, have also been carefully evaluated using test compounds (mainly pesticides and antibiotics). The role of ultra-high pressure liquid chromatography (UHPLC), especially when dealing with complex matrices, has also been tested. Finally, an in-house empirical spectral library was built for approximately 120 organic pollutants making use of QTOF MS. In addition to the mass accuracy measurements, this analyzer allows the simultaneous acquisition of low and high-collision energy spectra. This acquisition mode greatly enhances the reliable identification of detected compounds due to the useful (de)protonated molecule and fragment ion accurate mass information obtained when working in this mode.

Keywords: hybrid UHPLC-QTOF MS, performance optimisation, empirical mass spectra library, screening, organic contaminants.

1. INTRODUCTION

Hybrid quadrupole orthogonal-acceleration time-of-flight (Q-oaTOF) mass spectrometers have been valuable tools in many fields of chemistry including impurity profiling of pharmaceutical-drug substances[1], metabolomics[2,3] and food safety[4-7]. Recently, they have also gained wide acceptance in the field of environmental analysis for the rapid screening of organic pollutants due to their high sensitivity in full acquisition mode and accurate mass measurements[8]. QTOF MS has also been applied for the elucidation of unknowns because of its capacity to establish reliable elemental composition[9-11]. However, although QTOF mass spectrometers have been commercially available for over ten years, they have had drawbacks which have reduced their analytical application. These include the narrow ion abundance range over which accurate mass measurements could be made with a high degree of certainty, and the low dynamic range[12]. However thanks to continuous development of the technology, the latest TOF analysers show considerable advancement over earlier models.

The QTOF Premier, for example, employs new technology, which offers significant enhancements in mass measurements accuracy, resolution, dynamic range, sensitivity and speed. One of the major problems with TOF MS analysis is the saturation of the time-to-digital converter (TDC) detectors due to their inherent dead time, which affects not only the linearity but also the mass accuracy[13]. To circumvent this problem, this mass analyzer, using a travelling wave ion guide (TWIG) produces an attenuated ion beam, reducing the number of ions that reach the detector, avoiding the detector saturation. This results in a substantially increase in dynamic range for accurate mass measurement[14], known as pDRE. Wide dynamic ranges are necessary for the analysis of unknown samples or for samples that vary widely in analyte concentrations. Without this feature many samples would require a reanalysis after dilution, generating additional work and increasing the time required to obtain reliable data[15]. TWIG also permits binning the ion beam into packets of ions. Thus, by synchronizing the TWIG with the orthogonal pusher operation, the instrument

duty cycle can be significantly increased (Enhanced Duty Cycle, EDC), hereby achieving higher sensitivity for a limited and preselected range of the mass spectra[16].

Another feature implemented in TOF MS analysers is the dedicated reference sprayer in the mass spectrometer source. Early instruments introduced the reference compound/s together with the mobile phase in a post-column T. This approach presented a significant drawback as it favored the matrix ionization suppression, decreasing the reference compound sensitivity and increasing mass errors. There have been different proposals to improve the robustness of the exact mass measurements. One, using an additional electrospray source that orthogonally generates lock mass ions separately allows good mass accuracies to be obtained independently of the sample conditions[17]. Frequency and scan time for lock mass acquisition can be chosen by the user. When using UHPLC in combination with TOF MS, the narrow chromatographic peaks achieved make the overlapping between lock mass acquisition and analyte peak lower. However, when this occurs, the impact on peak shape may be significant.

In this paper, we are particularly interested in assessing the parameters that contribute to the accuracy and precision of mass measurements using this new QTOF with UHPLC as the separation technique. Resolution, dynamic range and sensitivity are also important parameters to be considered. The main parameters studied are those that can affect the mass accuracy, such as the acquisition interval and the scan time for lock mass or DRE. The sensitivity improvement when increasing the duty cycle was also investigated as well as its influence on mass accuracy. We have also evaluated the influence of the UHPLC technique on matrix effects when dealing with the analysis of complex matrices, as this is a key parameter in accurate mass measurements[18]. The results have been compared with those obtained in W acquisition mode. This is particularly relevant when isobaric compounds are coeluting (more likely in highly complex matrices). To date only a few papers have been reported on testing capabilities of MS instruments[12,15,19,20], and these have mostly been based on a few model compounds. In order to obtain more complete knowledge of QTOF MS capabilities in

environmental analysis, it would be desirable to increase the number of compounds evaluated as well as the range of their physico-chemical characteristics.

Full spectrum MS data is obtained using TOF MS. Therefore, when performing screening analysis, the contaminants presence in samples must be extracted from the Total Ion Chromatogram (TIC). There are two main ways that can be considered when screening by LC-high resolution MS in environmental analysis[8,11,21]: target screening searching for a list of suspected compounds of interest (with or without reference standards), and non-target screening that would ideally require a mass spectral library to rapidly and automatically identify those components found by the algorithm applied. Otherwise an extensive, tedious investigation would be required in non-target screening with the aim of assigning reliable empirical formulae to each peak extracted from the TIC chromatogram, and searching afterwards for potential chemical structures in compound databases. Unlike GC-EI-MS, commercial mass spectral libraries are not available in LC-API-MS yet, mainly due to the non-availability of a standardized interface. Currently the differences in the ionization process between the existing interfaces, together with the variability in the results depending on the mobile phase composition or in-source voltages applied, make it difficult to use standardized libraries[22]. Therefore, building home-made mass spectral libraries would facilitate searching, with particular focus on relevant compounds. Obviously, the higher the number of compounds included in the library, the greater the possibility of detecting and identifying more contaminants in samples.

After the optimization of the above mentioned instrumental parameters, the optimum conditions for building an empirical TOFMS spectral library have been studied in this paper, trying to establish the most appropriate fragmentation approach to improve compound identification. When using soft-ionisation sources (e.g. electrospray) relatively poor spectrum information is obtained compared with GC-EI-MS, because only the (de)protonated molecule and/or some adduct ions are normally present. Promoting the analyte fragmentation becomes advantageous to greatly increase the number of characteristic ions that can then be matched against the empirical spectra library. With the work

performed in this paper, extended knowledge of the QTOF instrument and its capabilities is pursued, with the objective of enhancing the efficiency and reliability when performing screening analysis in real-world samples.

2. EXPERIMENTAL

2.1 Reagents and chemicals

In this work, around 40 analytes were studied for performance optimisation and a total of 115 compounds were included in the empirical mass spectral library (see Table 1). Reference compounds were purchased from Across Organics (Geel, Belgium), Bayer Hispania (Barcelona, Spain), Fort Dodge Veterinaria (Gerona, Spain), Vetoquinol Industrial (Madrid, Spain), Aventis Pharma (Madrid, Spain), Sigma Aldrich (St Louis, MO, USA), Cerilliant (Round Rock, TX, USA), Dr. Ehrenstorfer (Augsburg, Germany), Riedel–de Haën (Seelze, Germany), the National measurement Institute (Pymble, Australia) and Fluka (Buchs, Switzerland). All reference materials presented purity higher than 93%.

HPLC-grade water was obtained from deionized water passed through a Milli-Q water purification system (Millipore, Bedford, MA, USA). HPLC-grade methanol (MeOH) and acetonitrile (ACN) were purchased from ScharLab (Barcelona, Spain). Formic acid (HCOOH) (> 98%) was obtained from Fluka. Sodium hydroxide (>99%) was obtained from ScharLab. Leucine enkephalin was purchased from Sigma Aldrich.

Working standards of 2.5, 10, 25, 50, 100 and 250 µg/L were prepared from 50 mg/L-mixed standards solutions in acetone, by diluting with water.

2.2 Instrumentation

An ultra-performance liquid chromatography (UPLC) system Waters Acquity (Waters, Milford, MA, USA) was interfaced to a QTOF mass spectrometer (QTOF Premier, Waters Micromass, Manchester, UK) using an orthogonal Z-spray-electrospray interface. The LC separation was performed using two Acquity UPLC BEH C_{18} 1.7 µm particle size analytical columns of 50 x 2.1 mm, and, 150 x 2.1 mm (both of Waters), at a flow rate of 300 µL/min. The mobile phases used

were A = H_2O and B = MeOH, both with 0.01% HCOOH. The percentage of MeOH was linearly increased from 10% to 90% in 5 min (14 min for the 150 mm column), followed by a 1 min isocratic period (2 min for the 150 mm Column) and returned to initial conditions with a total run time of 8 min (18 min for the 150 mm column). The injection volumes were 20 and 50 µL, for the 50 and 150 mm columns, respectively. Drying gas as well as nebulising gas was nitrogen (Praxair, Valencia, Spain). The gas flow was set at 600 L/h. TOF-MS resolution was ~10 000 at full width half maximum (FWHM) in V-mode and 17 500 FWHM in W-mode, at *m/z* 556. MS data were acquired over an *m/z* range of 50-1000 Da at a scan time of 0.3 s. The microchannel plate (MCP) detector potential was set to 1750 V. A capillary voltage of 3.5 kV and a cone voltage of 25V in positive ionization mode were used. Collision gas was argon 99.995% (Praxair, Valencia, Spain). The interface temperature was set to 350ºC and the source temperature to 120ºC. Temperature column was set to 40ºC for 50 mm column and 60ºC for the 150 mm one (in order to minimize back-pressure).

For automated accurate mass measurement, the lock-spray probe was used, using as lockmass a solution of leucine enkephalin (2 µg/mL) in ACN:water (50:50) at 0.1% HCOOH pumped at 30 µL/min through the lock-spray needle. A cone voltage of 60-70 V was selected and checked daily to obtain adequate signal intensity for this compound (~ 500 counts). 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.

Calibration of the mass-axis was performed weekly using the built-in single-syringe pump, directly connected to the interface. Calibration from 50 to 1000 Da was conducted with a 1:1 mixture of 0.05 M NaOH: 5% HCOOH diluted (1:25) with water/ACN (20:80 v/v) plus imazalil (*m/z* 297.0561) at a final concentration of 500 µg/L.

Data station operating software was Masslynx v 4.1.

3. RESULTS AND DISCUSSION

As stated in the introduction, LC-MS still does not have commercially available mass spectral libraries due to the non-availability of a standardized interface. This implies the building of home-made libraries to facilitate the searching, with a focus on particularly relevant compounds.

3.1 Building an empirical library database

Several experiments were carried out to evaluate and optimize the main parameters that can affect sensitivity, mass accuracy and fragmentation provided by UHPLC coupled to TOF MS. The objective of our work was to establish the optimum working conditions in order to obtain reliable spectra to be included in the library for further non-target analysis. To this aim, a significant number of compounds (concretely 43, including pesticides and antibiotics), with *m/z* ranging from 192 ($[M+H]^+$ corresponding to carbendazim) to 837 ($[M+H]^+$ corresponding to roxythromycin), were selected to provide a more complete and realistic knowledge of the instrument potential.

In all these experiments, 20 mDa narrow-window extracted ion chromatograms (nwXIC) were generated for each analyte at $[M+H]^+$ ion, and the mass spectra were combined over the corresponding chromatographic peaks. Mass accuracy was calculated as the difference between the theoretical exact mass and the experimental one, and expressed in mDa units.

Effect of the lockspray parameters on the mass accuracy

The QTOF Premier mass spectrometer comes with a lock spray interface, independent of the sample interface, used for the introduction of the reference substance. This system avoids matrix suppression of the lock mass but it can affect the chromatographic peak shape if sampled when the top of the peak is eluting. The effect on mass accuracy of the acquisition interval and the scan time acquisition of the lock mass was evaluated, trying to minimize the possible interference of the

lock mass in the analyte's peak. Default settings given by the software were 1 s scan time and 10 s acquisition interval. To perform this experiment, a 50 μg/L mix standard was analysed at different lock spray scan times (0.25, 0.5 and 1 s) and acquisition intervals (10, 20, 30 and 50 s).

As shown in Table 2, despite increasing the acquisition interval of lock mass as well as decreasing the scan time, the mass accuracy was not affected very much. In fact, after one way-Analysis of Variance (ANOVA) for each compound studied, we did not find significant differences in the mass error when changing lockspray parameters.

We also tested different combinations of these parameters to evaluate their joint effect on mass accuracy measurements. Thus, when an interval of 30 s or higher was used together with low scan times (lower than 1 s), higher mass errors were obtained. For example, using scan times of 0.5 s and 0.25 s, and an interval of 50 s, average mass errors were 1.38 and 1.47 mDa, respectively, i.e., two-fold higher than those given in Table 2. In contrast, when an interval of 30 s or higher was selected, the number of peaks interfered by the lock mass acquisition was minimized (less than 4% of the compounds studied were affected at the peak top). Finally, the best option selected, for both adequate peak shape and mass accuracy, was an interval of 30 seconds and a scan time of 1 s as lock mass conditions when using UHPLC.

Another parameter studied was the influence of the signal intensity of the lock mass (measured as counts per second, cps) on the mass accuracy. For this purpose, the 50 μg/L mix standard was analysed at 300, 500 and 800 cps for lock mass (these intensities were adjusted using an adequate cone voltage). It was concluded that signal intensity of the lock mass did not affect the mass accuracy of analytes very much, although a slight decrease in mass error was observed in the range 300-500 cps (data not shown). Finally, lock mass signal intensity between 400 and 500 cps was selected as the optimum value.

Effect of the ion abundance on the mass accuracy. Programmable Dynamic Range Enhancement.

Another parameter studied was the relationship between mass accuracy and analyte ion abundance. Traditional TDC detectors suffer from saturation effects, which limit some applications of the TOF mass spectrometer. The analogic-to-digital converter (ADC) detector digitises the ion current from the output of the micro-channel plate (MCP). The use of an ADC device avoids the saturation effects and allows the ion detector to render an accurate count of ions even for abundant species, although they have an inherent background noise associated with analog detection[13]. In our instrument, a TWIG is used to extend the dynamic range of the TDC detector. The entrance lens to the TWIG is used as a gate to allow only a portion of ions to go through, therefore producing an attenuated pulsed signal. The TWIG electrodes are then used to smooth out the attenuated signal and to create a homogeneous ion beam of lower intensity.

To assess the dynamic range increase when using TWIG collision cell, we compared the data obtained when acquiring with, and without, the attenuated pulsed signal (DRE) enabled. The mass accuracy, over a wide dynamic range, was also compared. Differences between the measured and theoretical accurate masses for 43 compounds at diverse ion abundances (i.e, different concentrations) were studied. In both cases (DRE-off and DRE-on), a similar mass accuracy was obtained when the ion intensity was sufficiently small to not saturate the detector (less than approximately 3500 counts). However, when ion intensity was higher than 3500 counts, pDRE-enabled acquisitions demonstrated a significant improvement in the mass accuracy compared with the acquisitions without pDRE. As an example, Figure 1 shows the effect of DRE on average mass accuracy for 8 of the compounds selected (Carbendazim, Thiabendazol, Imazalil, Simazine, Terbuthylazine, Thiobencarb, Pyridaphention and Pirimiphos-methyl) at different concentration levels. Selection of the representative compounds was performed as a function of the sensitivity in order to obtain similar saturation for the concentration levels tested. Our results demonstrated that

mass accuracy was satisfactory using the pDRE option, with mass errors lower than 1 mDa at all concentrations tested, illustrating the high degree of confidence, as regards mass accuracy, provided by the instrument.

Consequently, pDRE can be satisfactorily employed to increase the ion abundance range for which accurate mass measurements are recorded. Although the mass errors achievable were comparable when the ion intensities were lower than 3500 counts, the mass accuracy was notably improved at high concentrations (high ion abundance) when using this option. This significant improvement illustrates the its effectiveness in qualitative analysis. It is important to note that applying this approach affects the number of spectra per second recorded, decreasing the number of points per chromatographic peak. However this fact was not a problem, even using UHPLC, because of the high acquisition speed of the instrument.

Effect of the Enhanced Duty Cycle in the sensitivity and the mass accuracy

The sensitivity of TOF instruments is a key parameter in environmental and food safety analysis. The TWIG transports the ions in packets and so it is possible to synchronise the TOF pusher with individual ion packet and enhance the duty cycle over a selected *m/z* range. This mode of operation is referred as Enhanced Duty Cycle (EDC).

A 2.5 µg/L mix standard of 43 pesticides and antibiotics (see Table 1) was selected as a compromise for detecting the majority of the analytes but avoiding saturation. The mix standard was injected with and without EDC mode at *m/z* values of 200, 300, 400, 500 and 800 Da. The objective was to evaluate the effective signal increase at mass range between 200 and 800 Da, as well as the mass interval affected. As can be seen in Figure 2, the EDC increased the analytes sensitivity between 2 and 6 times in relation to the measurements without EDC.

Regarding the mass range affected, it depended on the target mass range selected. Thus, when EDC values at *m/z* 200 or 800 were selected (i.e. located at both ends of the mass spectra), sensitivity

increased only for analytes in a range of approximately 100 Da around the *m/z* tested. However, when EDC values at *m/z* 300, 400 or 500 were selected, sensitivity in all mass range was improved. Thus to detect and identify target analytes and/or elucidate unknowns, the most useful approach to increase the sensitivity would be to use EDC on at *m/z* 400, as sensitivity for ions up to *m/z* 700 would notably increase. We also observed that the repeatability of the ion intensity measurement was not affected by the use of the EDC.

The possible influence of the EDC on the mass accuracy was also investigated. Although a direct relationship was not expected between these two parameters, data obtained indicated some kind of correlation because a slight loss of mass accuracy was observed when the EDC function was selected. The mass error was around two-fold higher when the EDC function was switch-on, but it was lower for those analytes with *m/z* close to the selected EDC value, as shown in Table 3.

Finally, the EDC was not selected for screening of compounds that might be present in samples in a wide range of concentrations. As the EDC cannot be applied simultaneously with the pDRE and because of the possibility of saturation occurring for the most abundant compounds, the latter option (pDRE) was preferred. If EDC had to be used, two separate injections would be required.

Effect of calibration on the mass accuracy

When data from section *"Effect of the lockspray parameters on the mass accuracy"* were studied in detail, a remarkable increase in the mass errors was observed for those compounds ranging from *m/z* 270 to 310. After considering possible reasons, we noticed that the highest mass error in the calibration function (see Figure 3a) was also produced in this interval. For mass axis calibration of the time-of-flight mass spectrometer, adducts of sodium formiate (obtained from NaOH 0.05M:HCOOH 5% diluted 1:25 in water:ACN (20:80)) have been traditionally used in our laboratory. From the calibration report using this solution, we observed that the ion at *m/z* 294 presented notable lower intensity. This led to higher mass deviations in the area around 300 Da that

could not be corrected by the calibration function of fifth polynomial order, therefore affecting the accurate mass measurements.

To solve this problem, different compounds with *m/z* around 300 Da were studied. The fungicide imazalil (*m/z* $[M+H]^+$ 297.0561) was tested at different concentration levels (5, 50 and 500 µg/L) in the calibration solution, and 500 µg/L was finally selected as the best option. In this way, the peaks envelope was smoothed and mass errors were lower (Figure 3b). As illustrative examples, pesticides metolachlor (*m/z* 284.1417) and buprofezin (*m/z* 306.1640) had mass errors of 0.7 and 1.5 mDa respectively, when only sodium formiate adducts were used. However, after adding imazalil to the calibration solution, the mass errors decreased to 0.2 and 0.7 mDa, respectively.

Effect of the V and W ion optic modes on the mass accuracy. Matrix effect

Two mass spectrometry acquisition methods were compared selecting V and W ion optics modes. A separate calibration file was recorded for each mode. The effect of using V mode (resolution 10000 FWHM) or W mode (resolution 17500 FWHM) on mass accuracy was evaluated for five different matrices: two food matrices (cucumber, pepper) and three waters (surface water, influent and effluent urban wastewater). Samples were spiked with 10 organic contaminants (see Table 1, compounds marked as "b") with $[M+H]^+$ ranging from *m/z* 192 to 350, at 50 µg/L level each and analysed by triplicate. As shown in Table 4, slightly lower mass errors were typically obtained when the W-mode was used. As an example, average errors for influent wastewater (the most complex water matrix) decreased from 1.30 mDa (V-mode) to 0.81 mDa (W-mode).

However, a significant decrease in sensitivity was also observed (up to 3-fold in some cases) when using the W-mode. In food matrices with important matrix suppression effect, like pepper, the mass errors were even higher due to the sensitivity decrease. Thus, the W-mode was considered as a good approach for the analysis of complex matrices in those cases when sufficient sensitivity was maintained.

Optimization of the chromatographic conditions

Another alternative to reduce the mass errors in complex matrices analysis would be the use of longer chromatographic columns. All experiments described until now were carried out with a 50 mm UHPLC column. At this point, we tested the effect of using a three-fold longer column, i.e. a 150 mm. In this way, a better separation between analytes and interferents, and consequently better mass accuracy, was expected. Using the 150 mm column and working in V-mode, mass errors were comparable to those of the W-mode for all the matrices tested, but without sensitivity loss. Using a longer column, it was also possible to increase the injection volume. We tested a 50 µL loop, instead of the 20 µL initially used, obtaining excellent peak shape while injecting 2.5 times more sample. As a consequence, the sensitivity could be increased by a factor of around 2.5 times although with the disadvantage of longer chromatographic runs. Finally, a 150 mm UHPLC column (Acquity UPLC BEH C18) was selected for the empirical spectra library creation and for further screening experiments. Regarding organic mobile phase, we tested the use of MeOH and ACN, as weel as, the addition of HCOOH. For most of compounds studied, sensitivity was better using MeOH with 0.01% HCOOH (see 2.2 Instrumentation).

Study of the fragmentation conditions

To improve the confidence in the identification of positive findings, analyte fragmentation was promoted, in this way rendering richer mass spectra, which is more useful for identification/ elucidation purposes. The objective was to find a compromise between producing enough fragment ions and still observing the $[M+H]^+$ ion. Different approaches were considered using both in-source and in-collision cell fragmentation.

Different cone voltages were tested to evaluate sensitivity (10, 20 and 30 V) as well as in-

source fragmentation (40, 50 and 60 V). Regarding sensitivity, this was maximized at cone voltages ranging from 20 to 30 V. However, it was unfeasible to find a common voltage for the pesticides and antibiotics studied at which fragmentation was acceptable for all compounds. Thus, two different cone voltage ramps, 10-70 V and 20-60 V, were tested in order to find a fragmentation compromise. The results revealed a considerable loss of mass accuracy, with mass errors mostly exceeding the value of 5 ppm normally required for a correct identification, even with increasing the scan time (Figure 4). In addition, the cone voltage ramps did not produce fragmentation as efficiently as a single high voltage, probably because the algorithm of the ramp is not linear.

To improve the identification potential of (Q)TOF MS, in-collision cell fragmentation was evaluated. As stated before, our instrument is equipped with a TWIG collision cell, designed to always work with an Argon flow through. This permits increasing the entrance energy in the TWIG promoting collision induced dissociation (CID), although working in TOF MS mode. After testing the possibility of using single collision energy (CE) to fragment the analytes (no satisfactory compromise CE was found), four CE ramps were tested: 10-30 eV, 10-50 eV, 10-70 eV, and 15-40 eV. A CE ramp of 15-40 eV was finally chosen as it involved a smaller voltage change, with improved fragmentation.

Two simultaneous acquisition TOF MS functions were monitored: the first one with 4 eV collision energy (low energy function, LE, poor or none fragmentation) and the second one with a collision energy ramp ranging from 15 to 40 eV (high energy function, HE, promoting fragmentation). The LE and HE functions scan time were set to 0.2 s and 0.15 s, respectively, with an interscan delay of 0.05s. This type of acquisition is known as MS^E. Furthermore, when HE and MS/MS acquisitions were compared, the spectra were quite similar in most cases (see Figure 5). Moreover, additional information such as isotopic pattern and adduct formation is retained (not available with MS/MS experiments). For example, chlorine pattern for fungicide imazalil ([M+H]

$^{+}$, *m/z* 297) is also shown for fragments at *m/z* 158, 176 and 255, demonstrating that this approach is excellent for identification purposes[23].

Finally, for the creation of the in-house empirical spectra library, different standard pollutant solutions (see Table 1) at approximately 100 µg/L were injected into the UHPLC-(Q)TOF MS system under the conditions previously established, i.e. pDRE-on (EDC-off), V ion optics mode using lock mass intensity around 500 cps with 1 s scan time and 30 s acquisition interval, and 150 mm UPLC column. In total, 115 compounds were included in the empirical library to facilitate screening in water and food samples. Different families of pesticides, antibiotics, pharmaceuticals, drugs of abuse and mycotoxins commonly detected were included in this list, as well as some transformation products. All compounds were acquired in positive ion electrospray mode.

For each compound two library entries were created, one for HE and the other for LE acquisition. Entries included compound name, nominal mass, exact mass and molecular formula. Any information field could be used to filter the "hit list" and facilitate the assignment of the right candidate.

3.2 Evaluation of the empirical library for screening purposes

Once the library was created, it was tested for the analysis of real-world samples. For this test an effluent wastewater sample was 50-fold pre-concentrated by passing 50 mL of sample through an SPE Oasis HLB 60 mg cartridge. The 5mL-eluted MeOH extract was evaporated to dryness and reconstituted with 1mL H_2O:MeOH (90:10). Then, 50 µL of the final extract were injected in the UPLC-(Q)TOF MS system under the chromatographic conditions explained in the paper. As shown in Figure 6, the TIC was too complex to detect the trace level compounds; so data processing was required. We used ChromaLynx software in a non-target mode for TIC deconvolution in the LE

function, thus facilitating the detection of trace level components present in the sample. To illustrate the strategy applied, Figure 6 shows the detection and identification of the fungicide carbendazim in effluent wastewater. The LE spectrum of the compound given by the software was searched against the in-house mass spectra library and carbendazim was proposed as the first candidate. In addition, the higher fragmentation observed in the HE spectrum allowed us to reliably improve the identification process.

At present, this non-target approach using an empirical spectral library, is being compared with the target-approach. For the target approach, a large list of compounds are specifically searched for in the full-scan TOF spectra after MS data acquisition (i.e. post-target way) in different types of water and food matrices.

4. CONCLUSIONS

An extensive investigation of the different factors that can affect mass accuracy, sensitivity and dynamic range in a UHPLC-QTOF MS instrument has been carried out. Additionally, conditions for building mass spectra library have been studied with the aim of improving the sensitivity and the analyte fragmentation information.

The results reported in this article have allowed us to improve knowledge about the capabilities, limitations and robustness of the instrument. This is necessary to perform an efficient wide-scope screening analysis in a later stage. Establishing the optimum experimental conditions to build an empirical home-made spectral library is essential to optimize accuracy and future results of screening. Sensitivity and dynamic range of the instrument are now well-known, which is also crucial when dealing with the elucidation of unknown compounds. Under optimum conditions, mass errors below 1 mDa have been obtained for the standard solutions of the compounds investigated, and below 2 mDa for several food and water spiked samples. Robustness in terms of mass accuracy and sensitivity has been proven for an adequate concentration range (at least three orders of magnitude) thanks to the DRE option.

A home-made compound spectra library has been created for 115 compounds, including pesticides, antibiotics, other pharmaceuticals, drugs of abuse and toxins, as well as several transformation products. Using this empirical library allows the rapid screening of samples making use of the MS^E approach (simultaneous acquisition at low and high collision energy), as both the detection and the confirmation of the identity of the analyte can be achieved simultaneously, without the need of sample re-analysis.

5. ACKNOWLEDMENTS

This work has been developed with financial support from the Ministry of Education and Science, Spain (CTQ 2009-12347). R. Diaz is very grateful to Conselleria d'Educació (Generalitat Valenciana) for his pre-doctoral grant. The authors are grateful to Serveis Centrals d'Instrumentació Científica (SCIC) of University Jaume I for the use of UPLC-QTOF-MS (QTOF Premier) and to Generalitat Valenciana for the financial support (Research Group of Excellence, Prometeo/2009/054)

6. REFERENCES

1. Kenseth JR, Coldiron SJ. *Curr Opin Chem Biol* 2004; **8**: 418.
2. Dettmer K, Aronov PA, Hammock BD. *Mass. Spectrom. Rev.* 2007; **26**: 51.
3. Want EJ, Cravatt BF, Siuzdak G. *ChemBioChem.* 2005; **6**: 1941.
4. Grimalt S, Pozo OJ, Sancho JV, Hernández F. *Anal. Chem.* 2007; **79**: 2833.
5. Hernández F, Sancho JV, Ibáñez M, Grimalt S. *TrAC Trends Anal.Chem.* 2008; **27**: 862.
6. Hernández F, Grimalt S, Pozo OJ, Sancho JV. *J. Sep. Sci.* 2009; **32**: 2245.
7. Thurman ME, Ferrer I, Malato O, Fernández-Alba AR. *Food Addit. Contam.* 2006; **23**: 1169.
8. Sancho JV, Pozo ÓJ, Ibáñez M, Hernández F. *Anal. Bioanal. Chem.* 2006; **386**: 987.
9. Ibáñez M, Sancho JV, Pozo OJ, Niessen W, Hernández F. *Rapid Commun. in Mass Spectrom.* 2005; **19**: 169.
10. Ibáñez M, Sancho JV, Pozo ÓJ, Hernández F. *Anal. Chem.* 2004; **76**: 1328.
11. Krauss M, Singer H, Hollender J. *Ana.l Bioanal. Chem.* 2010; DOI 10.1007/s00216-010-3608-9
12. Bristow T, Constantine J, Harrison M, Cavoit F. *Rapid Commun. Mass Spectrom.* 2008; **22**: 1213.
13. Chernushevich IV, Loboda AV, Thomson BA. *J Mass Spectrom* 2001; **36**: 849.
14. Ferrer I, Thurman EM. *Liquid Chromatography Time-of-Flight Mass spectrometry. Principles, Tools and Applications for Accurate Mass Analysis.* John Wiley and Sons, Inc., 2009; 261.
15. Weaver PJ, Laures AM-, Wolff J-C. *Rapid Commun. Mass Spectrom.* 2007; **21**: 2415.
16. Farré M, Gros M, Hernández B, Petrovic M, Hancock P, Barceló D. *Rapid Commun. Mass Spectrom.* 2008; **22**: 41.
17. Wolff J-C, Eckers C, Sage AB, Giles K, Bateman R. *Anal. Chem.* 2001; **73**: 2605.
18. Calbiani F, Careri M, Elviri L, Mangia A, Zagnoni I. *J Mass Spectrom* 2006; **41**: 289.
19. Wolff J-C, Fuentes TR, Taylor J. *Rapid Commun. Mass Spectrom.* 2003; **17**: 1216.
20. Laures AM-, Wolff J-C, Eckers C, Borman PJ, Chatfield MJ. *Rapid Commun. Mass Spectrom.* 2007; **21**: 529.
21. Hernández F, Pozo OJ, Sancho JV, López FJ, Marín JM, Ibáñez M. *TrAC Trends Anal. Chem.* 2005; **24**: 596.
22. Ibáñez M, Sancho JV, Hernández F, McMillan D, Rao R. *TrAC Trends Anal. Chem.* 2008; **27**: 481.
23. Hernández F, Bijlsma L, Sancho JV, Díaz R, Ibáñez M. *Anal.Chim. Acta* 2010; submitted.

Table 1. List of compounds included in the empirical spectra library.

Compounds		t_R (min)	Molecular Formula	Compounds		t_R (min)	Molecular Formula
2-hydroxy-Simazine		3.75	C7H13N5O	**Lorazepam**		10.22	C15H10Cl2N2O2
2-hydroxy-terbuthylazine		6.37	C9H17N5O	**Malathion**	*a*	11.42	C10H19O6PS2
3,4-dichloraniline		9.31	C6H5Cl2N	**Marbofloxacin**		3.43	C17H19N4O4F
4-aminoantipyrin		5.34	C11H13N3O	**MDA**		3.08	C10H13NO2
Acetaminophen		2.7	C8H9NO2	**MDMA**		3.14	C11H15NO2
Aflatoxin B1		8.19	C17H12O6	**Methamphetamine**		3.15	C10H15N
Aflatoxin B2		7.79	C17H14O6	**Methiocarb**		11.09	C11H15NO2S
Aflatoxin G1		7.36	C17H12O7	**Metolachlor**	*a*	12.34	C15H22ClNO2
Aflatoxin G2		6.93	C17H14O7	**Molinate**		11.64	C9H17NOS
Aflatoxin M1		7.02	C17H12O7	**Moxifloxacin**	*a*	5.93	C21H24N3O4F
Alachlor	*a*	12.22	C14H20ClNO2	**Naproxen**		10.84	C14H14O3
Alprazolam		10.32	C17H13ClN4	**Nitrofurantoin**		3.67	C8H6N4O5
Amphetamine		3.06	C9H13N	**Nitrofurazone**		3.9	C6H6N4O4
Ampicillin	*a*	4.52	C16H19N3O4S	**Nor-benzoylecgonine**		5.28	C15H17NO4
Atorvastatin		12.4	C33H35FN2O5	**Nor-cocaine**		5.06	C16H19NO4
Atrazine	*a*	9.86	C8H14N5Cl	**Norfloxacin**	*a*	3.93	C16H18N3O3F
Azoxystrobin		10.97	C22H17N3O5	**Ochratoxin**		11.65	C20H18ClNO6
Benzoylecgonine		5.06	C16H19NO4	**Ofloxacin**	*a*	3.78	C18H20N3O4F
Bezafibrate		11.06	C19H20ClNO4	**Olanzapine**		4.84	C17H20N4S
Bromacil		8.35	C9H13BrN2O2	**Omeprazol**		9.08	C17H19N3O3S
Buprofezin	*a,b*	14.25	C16H23N3OS	**Omethoate**		2.92	C5H12NO4PS
Carbendazim	*a,b*	5.24	C9H9N3O2	**Oxacillin**		9.51	C19H18N3NaO5S
Carbofuran	*a,b*	8.61	C12H15NO3	**Oxonilic acid**		6.74	C13H11NO5
Cephalexin	*a*	4.29	C16H17N3O4S	**Oxytetracyclin**	*a*	4.83	C22H24N2O9
Cephataxim	*a*	4.52	C16H17N5O7S2	**Pantoprazol**		8.91	C16H15F2N3O4S
Chloramphenicol		6.46	C11H12Cl2N2O5	**Paroxetin**		8.57	C19H20FNO3
Chlorpyriphos		14.6	C9H11Cl3NO3PS	**Pefloxacin**	*a*	3.92	C17H20N3O3F
Chlorpyriphos-methyl		13.44	C7H7Cl3NO3PS	**Penicillin G**		8.5	C16H18N2O4S
Chlortetracyclin	*a*	5.07	C22H23ClN2O8	**Pipedimic acid**	*a*	3.3	C14H17N5O3
Ciprofloxacin	*a*	4.23	C17H18N3O3F	**Piperalacin**	*a*	8.3	C23H27N5O7S
Clarythromycin	*a*	10.34	C38H69NO13	**Pirimicarb**	*a, b*	9.15	C11H18N4O2
Clindamycin	*a*	7.82	C18H33N2O5SCl	**Pravastatin**		10.25	C23H36O7
Cloxacyllin	*a*	9.93	C19H18ClN3O5S	**Propanil**		10.96	C9H9Cl2NO
Cocaethylene		6.14	C18H23NO4	**Pirimiphos-methyl**	*a, b*	13.21	C11H20N3O3PS
Cocaine		4.97	C17H21NO4	**Pyriproxifen**	*a, b*	14.46	C20H19NO3
Deethylterbuthylazine		8.93	C7H12N5Cl	**Risperidone**		6.45	C23H27FN4O2

Deoxynivalenol		3.72	C15H20O6	**Roxythromycin**	*a*	10.57	C41H76N2O15
DIA		4.87	C5H8N5Cl	**Simazine**	*a*	8.44	C7H12ClN5
Diazinon		13.05	C12H21N2O3PS	**Simvastatin**		14.63	C25H38O5
Diclofenac		12.59	C14H11Cl2NO2	**Spinosyn A**		12.44	C41H65NO10
Dicloxacillin	*a*	10.54	C19H17Cl2N3O5S	**Sulfadiazine**	*a*	2.87	C10H10N4O2S
Diflubenzuron		12.32	C14H9ClF2N2O2	**Sulfamethazine**	*a*	4.51	C12H14N4O2S
Dimethoate	*a*	5.76	C5H12NO3PS2	**Sulfamethiazol**		4.14	C9H10N4O2S2
Diuron	*a*	9.96	C9H10N2OCl2	**Sulfamethoxazol**		4.88	C10H11N3O3S
Enalapril		8.77	C20H28N2O5	**T-2 toxin**		11.08	C22H32O8
Erythromycin	*a*	9.41	C37H67NO13	**Tebufenozide**		12.54	C22H28N2O2
Flumequine		8.6	C14H12NO3F	**Terbacil**		8.72	C9H13ClN2O2
Fumonisin B1		10.13	C34H59NO15	**Terbumetone**	*a, b*	10.34	C10H19N5O
Fumonisin B2		11.66	C34H59NO14	**Terbuthylazine**	*a*	11.28	C9H16ClN5
Furazolidone	*a*	3.84	C8H7N3O5	**Terbutryn**	*a*	11.83	C10H19N5S
Hexythiazox		14.64	C17H21ClN2O2S	**Thiabendazol**	*a, b*	5.1	C10H7N3S
HT-2 toxin		10.16	C22H32O8	**Thiobencarb**		13.39	C12H16ClNOS
Imazalil	*a,b*	9.12	C14H14Cl2N2O	**Trichlorfon**		5.83	C4H8Cl3O4P
Imidachlorprid	*a,b*	5.17	C9H10ClN5O2	**Trimethoprim**	*a*	3.78	C14H18N4O3
Isoproturon	*a*	9.99	C12H18N2O	**Tylosin A**		9.32	C46H77NO17
Ketoprofen		10.57	C16H14O3	**Venlafaxin**		6.71	C17H27NO2
Lincomycin	*a*	3.79	C18H34N2O6S	**Zearalenone**		11.48	C18H22O5
11-nor-9-carboxy-Δ9-THC		14.46	C21H28O4				

a. Compounds studied in section "Effect of lockspray parameters on mass accuracy", section "Effect of Enhanced Duty Cycle in sensitivity and mass accuracy", section "Optimization of chromatographic conditions" and section "Study of fragmentation conditions"

b. Compounds studied in the section "Effect of V and W ion optic modes on mass accuracy. Matrix effect"

Table 2. Effect of the frequency (s) and scan time (s) of lock mass acquisition in mass accuracy.

Interval (s) at 1 s scan time	Mean error ± SD (mDa)	Scan time (s) at 10 s interval	Mean error ± SD (mDa)
10	0.63 ± 0.45	1.0	0.63 ± 0.45
20	0.67 ± 0.44	0.5	0.65 ± 0.57
30	0.64 ± 0.52	0.25	0.54 ± 0.50
50	0.49 ± 0.41	0.1	0.60 ± 0.41

Table 3. Average mass error for 30 selected compounds as a function of the mass range and EDC applied

Mass range (Da)	No EDC	EDC 200 Da	EDC 300 Da	EDC 400 Da	EDC 500 Da	EDC 800 Da
< 250 Da	0.7	0.8	2.1	2.5	2.3	2.4
250-350 Da	0.5	2.8	1.0	1.3	1.6	1.4
350-450 Da	0.6	-*	3.3	1.0	1.1	1.3

(*) For those analytes with m/z higher than 350 Da, no peaks were detected or sensitivity was too low for correctly calculating the mass error

Table 4. Average mass errors for 10 selected compounds (n=3) analysed in the five food and environmental matrices, in both V and W ion optics acquisition modes (EWW = Effluent Wastewater, IWW = Influent Wastewater, SW = Surface Water).

	Mean mass errors (mDa) (n=3)					
	Solvent	**Cucumber**	**Pepper**	**EWW**	**IWW**	**SW**
V-mode	1.09	1.15	1.25	1.33	1.30	1.41
W-mode	0.78	1.03	1.30	0.99	0.81	0.93

FIGURE CAPTION

Figure 1: Effect of DRE on mass accuracy for 8 of the pesticides studied (Carbendazim, Thiabendazol, Imazalil, Simazine, Terbuthilazine, Thiobencarb, Pyridaphention and Pirimiphos-methyl) at different concentrations (n=2)

Figure 2: Effect of EDC at different *m/z* values on the sensitivity of the TOF MS signal for several organic pollutants with *m/z* ranging from 192 to 838 Da.

Figure 3. Calibration report obtained using (a) sodium formiate and (b) sodium formiate + Imazalil. RMS Residuals for *m/z* range 226 to 362, which affected to mass measurements deviations, are highlighted.

Fig.4. Evaluation of the mass error (mDa) as a function of the cone voltage (ramp 10-70 V and 30 V) and scan time.

Figure 5: Comparison of fragmented spectra obtained for (A) fungicide imazalil and (B) antibiotic trimethoprim reference standards by MS^E (top) and MS/MS (bottom).

Figure 6: Effluent Wastewater sample positive to Carbendazim. **Left**: Total ion and Extracted ion chromatograms for the compound detected. **Right:** Comparison of Carbendazim library spectra and suspected compound spectra in LE (bottom) and HE (top) functions.