Screening of Pharmaceuticals and Illicit Drugs in Wastewater and Surface Waters of Spain and Italy by High Resolution Mass Spectrometry using UHPLC-QTOF MS and LC-LTQ-Orbitrap MS

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Abstract:

The existence of pharmaceuticals and illicit drugs (PIDs) in environmental waters has led many analytical chemists to develop screening methods for monitoring purposes. Water samples can contain a huge number of possible contaminants, commonly at low concentrations, which makes their detection and identification problematic. Liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS) has proven itself effective in the screening of environmental contaminants. The present work investigates the use of the most popular HRMS instruments, quadrupole time-of-flight and linear trap quadrupole-Orbitrap, from two different laboratories. A suspect screening for PIDs was carried out on wastewater (influent and effluent) and surface water samples from Castellón, Eastern Spain, and Cremona, Northern Italy, incorporating a database of 107 PIDs (including 220 fragment ions). A comparison between the findings of both instruments and of the samples was made which highlights the advantages and drawbacks of the strategies applied in each case. In total, 28 compounds were detected and/or identified by either/both instruments with irbesartan, valsartan, benzoylecgonine and caffeine being the most commonly found compounds across all samples.

Key words: Liquid Chromatography, Screening, Pharmaceuticals, Illicit Drugs, High Resolution Mass Spectrometry

1. Introduction

The presence of pharmaceuticals and illicit drugs (PIDs) in environmental waters has become more apparent in the past decade due to the improvements in selectivity and sensitivity of modern analytical techniques. PIDs are continuously excreted or disposed into the sewer systems as the unaltered parent compound or as metabolites (Richardson, 2012). Compounds of the most concern in the environment are those found with high concentrations and those that have been proven detrimental to the aquatic life; in fact, for several antibiotics, hormones and pharmaceuticals, some effects have been shown (Brozinski et al., 2013; Kümmerer, 2009; Li, 2014). Various pharmaceuticals, antibiotics and illicit drugs and metabolites such as acetaminophen, carbamazepine, diclofenac, ciprofloxacin and benzoylecgonine, are frequently detected in waters and are considered a potential threat (Li, 2014; Luo et al., 2014; Pal et al., 2013).

Nowadays, data processing is often the most time-consuming step when screening a large number compounds. A fast screening method, referring to the easy searching of PIDs by software following full scan acquisition by high resolution mass spectrometry (HRMS), is therefore of great interest in order to gain a complete overview of possible contaminants and their fate in the aquatic environment. Another time consuming step is sample preparation. Important improvements in sensitivity and specificity of recent instrumentation have facilitated the direct injection of the samples (Anumol and Snyder, 2015; Backe and Field, 2012).

HRMS instruments, such as quadrupole time-of-flight (QTOF) MS and Orbitrap MS, provide high quality data by combining full spectrum mass data with high mass resolution and mass accuracy (Krauss et al., 2010). Powerful deconvolution software is required to facilitate sample analysis for these instruments. Each manufacturer has its own software, such as ChromaLynx (Waters) and TraceFinder (ThermoScientific). These systems allow post-target analysis, whereby the presence of an unlimited number of contaminants can be investigated through the addition of empirical or theoretical compound databases, without depending on the pre-selection of analytes or having reference standards available (Hernández et al., 2014). In the processing of numerous samples and compounds, these programs undeniably save time, and hence are a necessity for a large screening of environmental contaminants.

It is difficult to compare the individual HRMS mass analysers (i.e. TOF and Orbitrap), but each hold distinct advantages. The main advantage of Orbitrap is its high mass resolving power (>100000 full width at half maximum (FWHM)). In complex environmental matrices, co-elution of analytes with matrix interferences can result in ambiguous peaks. By utilising the ultra-high resolution capabilities, compounds with a very similar exact mass can be easily differentiated (Hernández et al., 2012). However, its main drawback is its slower scanning speed and inverse relationship to resolution. Conversely, the higher scanning speed of TOF enables it to be used with the highly efficient separation technique ultra-high performance liquid chromatography (UHPLC), resulting in shorter run times and improved sensitivity. (Hernández et al., 2014)

To achieve better performance such as higher sensitivity and mass resolving power, manufacturers have implemented hybrid mass spectrometers, two of which are used in the current work: quadrupole-TOF (QTOF) and linear trap quadrupole-Orbitrap (LTQ-Orbitrap). The UHPLC capabilities are exemplified when QTOF is employed in MS^E mode, which involves the simultaneous acquisition of exact mass data at low and high collision energy (Hernández et al., 2011). At low energy, information pertaining to the non-fragmented (de)protonated molecule is obtained, while at high energy, information regarding the fragments is attained. This means that all required information is obtained in just one injection. LTQ Orbitrap combines the tandem mass spectrometric capability of the LTQ with the high resolution and mass accuracy capability of the Fourier Transform (FT) Orbitrap (de Voogt et al., 2011). LTQ-Orbitrap is operated in data dependent acquisition (DDA) mode whereby MS and MS^n spectra are obtained simultaneously, however the number of compounds able to be acquired is limited and therefore may require subsequent injections. Some studies have been made on the identification of organic pollutants in water by QTOF MS (Gómez et al., 2010; Gonzalez-Mariño et al., 2012; Hernández et al., 2011; Ibáñez et al., 2009) and LTQ Orbitrap MS (Kosma et al., 2014; Pinhancos et al., 2011; Wille et al., 2011).

This work derives from a collaborative study between two laboratories within the ITN Marie Curie SEWPROF network ("SEWPROF ITN: A new paradigm in drug use and human health risk assessment: Sewage profiling at the community level," n.d.). The aim was to perform a screening of PIDs in waste- and environmental waters using two different LC-HRMS systems (UHPLC-QTOF MS and Capillary-LC-LTQ-Orbitrap MS) available in our laboratories. Normally, a given laboratory would use just one of the

instruments, but as both have a great capability for screening and identification, in this work we pursued the comparison of data from the same analyzed samples (samples collected from Spain and Italy). In addition, the complementary use allows true confirmation of the compound in the sample (Krauss et al., 2010). Although the same compounds were initially searched, the different acquisition modes may additionally detect other contaminants, and widen the scope of the screening. It is worth noting that a comparison of the instruments or methods used was not pursued, rather their use to ratify the findings of the other instrument. In this way, on the basis of the results of the present paper, the methodologies applied by each laboratory could be validated by the results obtained by the other laboratory in terms of qualitative analysis. When performing the screening, four criteria were used based on the level of confidence and on the availability of reference standards. Numerous compounds were detected and identified from samples of different origin and matrix composition (influent and effluent urban wastewater, surface water). All samples, independently of they were collected from Spain or from Italy, were analysed by both HRMS instruments (Orbitrap in Milan, and TOF in Castellon), which supports the feasibility and robustness of high-resolution screening of PIDs in complex environmental samples.

2. Experimental

2.1 Chemicals and Reagents

For information regarding the reference standards used for QTOF and LTQ Orbitrap analysis (see Electronic Supplementary Material (ESM), Section 2.1).

HPLC-grade methanol (MeOH), ammonia solution (25%), formic acid (HCOOH, 98–100%) were acquired from Scharlau (Barcelona, Spain) and acetonitrile for LC-MS, from Riedel de Haen (Seelze, Germany). HPLC-grade water was obtained by purifying demineralised water in a Milli-Q plus system from Millipore (Bedford, MA, USA). SPE cartridges used were Oasis HLB 3 mL (60 mg) from Waters (Milford, MA, USA). 0.45 μm mixed cellulose ester membrane filters and 1.6 μm GF/A glass microfiber filters were purchased from Whatman (Kent, UK).

2.2 Samples:

2.2.1 Influent and effluent wastewater

In total, 18 wastewater samples (nine influent wastewater (IWW) and effluent wastewater (EWW)) were taken from a WWTP in Castellón, Eastern Spain, and Cremona, Northern Italy. The 24h composite IWW and EWW samples were collected over seven consecutive days in March 2014 (Castellón) and two consecutive days in May (Cremona) in high density polystyrene bottles and directly transported to the lab where they were immediately filtered and stored in the dark at −20 °C until analysis.

2.2.2 Surface waters

Five grab samples were taken from the Castellon region, Eastern Spain: Almenara, Burriana Clot, Nules, and two sites in Albufera Natural Park. All samples were stored in high density polystyrene bottles at 4º C for less than 48 hours, until sample treatment.

2.3 Sample Treatment

Prior to SPE, Spanish WW samples were vacuum filtered through 0.45μm mixed cellulose ester membrane filters and the Italian samples were filtered through 1.6 μm GF/A glass microfiber filters. Surface water samples were not filtered. Subsequently, 100mL of the samples (IWW was four times diluted with MilliQ water, i.e. 25mL sample in 100mL) were extracted using SPE cartridges (Oasis HLB), which were first conditioned by washing and rinsing with 6 mL of MeOH and 6 mL of Milli-Q water. Samples were percolated through the cartridges by gravity (flow rate around 3mL min−1), and vacuum dried for approximately 15 min. After elution with methanol, extracts were evaporated to dryness at 35 °C under a gentle stream of nitrogen and reconstructed in 1 mL of 10% methanol aqueous solution (Bijlsma et al., 2014) . Two 0.5mL aliquots were taken from the extract, one each for the QTOF and Orbitrap analyses. UHPLC-MS QTOF analyses were performed by injecting 20μL of the final extract into the system. Capillary-LC-LTQ-Orbitrap analyses were performed by injecting 2μL of the final extract into the system.

2.4 Instrumentation

2.4.1 Ultra High Performance Liquid Chromatography –QTOF- Mass Spectrometry

A Waters Acquity UPLC system (Waters, Milford, MA, USA) was interfaced to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (XEVO G2 QTOF, Waters

Micromass, Manchester, UK), using a Z-Spray ESI interface operating in positive ion mode.

The chromatographic separation was performed using an Acquity UPLC BEH C_{18} 1.7 µm particle size column 100 × 2.1 mm (Waters) at a flow rate of 300µl/min. The mobile phases used were A = H_2O and B = MeOH (both with 0.01% HCOOH). The total run time was 18 minutes. The initial percentage of B was 10%, which was linearly increased to 90% in 14 min, followed by a 2 min isocratic period and, then, returned to initial conditions during 2 min. Nitrogen was used as drying gas and nebulizing gas.

MS data were acquired over an m/z range of 50–1000. A capillary voltage of 0.7 kV and cone voltage of 20 V were used. Collision gas was argon 99.995% (Praxair, Valencia, Spain). The desolvation temperature was set to 600 °C, and the source temperature to 135 °C. The column temperature was set to 40 °C.

For MS^E experiments, two acquisition functions with different collision energies were created. The low energy function (LE), selecting a collision energy of 4 eV, and the high energy (HE) function, with a collision energy ramp ranging from 15 to 40 eV in order to obtain a greater range of fragment ions. The total scan time was 0.4 s.

QTOF MS data was processed using ChromaLynx XS application manager (within MassLynx v 4.1; Waters Corporation). The following parameters were used: Target RT Tolerance (1.50min) from empirical retention time (obtained from injecting standards), Mass tolerance 0.020Da (for positive ID 0.010 Da), Peak width at 5% height: 6 seconds, Peak-to-peak baseline noise: 1000, Threshold absolute area: 250 for SW, 500 for IWW and EWW.

2.4.2 Liquid Chromatography- LTQ Obritrap Mass Spectrometry

Mass spectrometry analysis was performed on a high resolution LTQ-Orbitrap XL instrument, interfaced to the HPLC system through a Desorption Electrospray Ionisation (DESI) source, used in nano-spray mode (Prosolia, Indianapolis, IN, USA). The instrument was operated at 60,000 resolution for full MS scans and at 15,000 resolution for MS^2 scans, after CID fragmentation in the linear ion trap cell. The scan time was 0.85 seconds and the cycle time was 4.8 seconds. Every sample was analysed in a data-dependent acquisition mode (DDA), in which the first data event was a full scan MS, while the following 4 data events were MS^2 scans of the 4 most intense ions recorded in

the first event. A subsequent injection in MS/MS mode was made to find further fragments, based on target lists (see Section 3.1).

The chromatographic separation was performed using a Zorbax SB-C_{18} 5µm particle size column 150 × 0.5mm (Agilent Technologies) at a flow rate of 10µL/min. The mobile phases used were A: H_2O (0.1% HCOOH) and B: Acetonitrile. The total run time was 46 minutes. The initial percentage of B was 1% and was linearly increased to 99% in 24 minutes, followed by a 10 minute isocratic period and then reduced back to initial conditions in 2 minutes which were kept for 10 minutes (post-run) to equilibrate the column.

Processing of the Orbitrap data was made using TraceFinder EFS and QualBrowser software (Thermo Scientific). The following parameters were used: $[M+H]^+$ intensity (5,000), signal-to-noise ratio threshold (=5), retention time window override for confirmation (90 seconds), fragment intensity (1,000 with 10ppm mass tolerance); isotopic pattern (60% fit threshold, 5ppm mass deviation, 10% intensity deviation); library search (20% score threshold) using the Toxicology HCD 30-70-110eV Library of Thermo Scientific. This library contained the information of 1700 compounds, but not all PIDs compounds selected in our list were included in the Library.

2.5 Criteria for detection/identification

Up to four levels were considered for detection and identification of the compounds based on the availability of reference standard and on the reliability of the data obtained. These levels were derived from a recent article exploring the means of identification of small molecules (Schymanski et al., 2014) and the European Commission Decision 2002/657/EC for the confirmation of contaminants in samples of animal origin, commonly employed in environmental studies. The criteria used included the presence of the protonated molecule at accurate mass, fragment ion(s) at accurate mass, retention time and isotopic pattern.

2.5.1 Selected criteria for QTOF MS

The standards injected on each instrument were those available at the respective laboratories (See SI Section 2.1). They may be different; however, as explained above, a comparison between the labs is not proposed.

Standard available (11 illicit drug and 61 pharmaceutical standards, see SI section 2.1)

1. Detection: protonated molecule $[M+H]^+$ at accurate mass (<5ppm), and retention time in agreement with reference standard (±2.5%).

2. Identification: protonated molecule $[M+H]^+$ at accurate mass (<5ppm), at least one fragment ion, commonly in HE (<5ppm), retention time agreement with reference standard (±2.5%).

Standard not available:

3. Tentative detection (potential positive): presence in the extracted ion chromatogram of the protonated molecule [M+H]+ at accurate mass (<10 ppm), typically in the LE function

4. Tentative identification: protonated molecule $[M+H]^+$ at accurate mass (<10ppm), and at least one fragment ion, commonly in HE function (<10 ppm), in agreement with data reported from literature or online databases.

2.5.2 Selected criteria for LTQ-Orbitrap MS

Standard available (25 illicit drug and 27 pharmaceutical standards, see SI section 2.1)

1. Detection: protonated molecule $[M+H]^+$ at accurate mass (<5 ppm), retention time agreement with standard (±2.5%), isotopic pattern (60% fit threshold, 5ppm mass deviation, 10% intensity deviation)

2. Identification: protonated molecule $[M+H]^+$ at accurate mass (<5 ppm, intensity >5000), at least one fragment ion in MS/MS (intensity > 1000, <10 ppm), retention time agreement with standard ((±2.5%), isotopic pattern (60% fit threshold, 5 ppm mass deviation, 10% intensity deviation); library search (20% score threshold) using the Toxicology HCD 30-70-110eV Library.

Standard not available

3. Tentative detection (potential positive): protonated molecule $[M+H]^+$ at accurate mass (<10ppm, intensity >5000)

4. Tentative identification: protonated molecule $[M+H]^+$ at accurate mass (<10ppm intensity >5000), at least one fragment ion (intensity > 1,000, <10ppm mass tolerance),

comparable with literature or online databases, isotopic pattern (60% fit threshold, 5ppm mass deviation, 10% intensity deviation).

3. Results and Discussion

The methodologies followed for these analyses are based on the generic configuration employed by each laboratory. We acknowledge that the Orbitrap configuration is not optimal for screening of PIDs in water, as its use is primarily in the field of proteomics, which justifies the use of a capillary column and DESI source. Therefore, we do not intend to show a comprehensive comparison of the instruments, but to determine whether both laboratories and instruments can detect and identify the same compounds using their individual workflows.

Furthermore, in this work, we do not set out to perform a wide-scope screening of a huge number of compounds, but rather to focus on a limited number of well-known, widely detected PIDs in wastewater to test the usefulness of the different approaches and instruments used and also to compare the countries.

3.1 Selection of PIDs

The compounds to be investigated were initially based on a database similar to that made by Diaz *et al.* (Díaz et al., 2011), but focussing solely on pharmaceuticals and illicit drugs. The database contained the compound name, associated fragments (if known), retention time (if standard was available) and molecular formula. A qualitative validation was previously performed using a similar analytical procedure based on LC-QTOF MS measurements, and the empirical screening detection limits (SDL) and limits of identification (LOI) in different type of water samples were established for some of the compounds included in the present work. For most compounds evaluated SDLs were 0.02 or 0.1 µg/L (Diaz et al., 2013; Hernández et al., 2015). For compounds where no standards were available, the database included data regarding fragment ions and molecular formula from literature or online databases such as MassBank (Horai et al., 2010).

To ensure national significance, compounds found from previous studies of these regions were investigated and added to the database (Spain (Gracia-Lor et al., 2011, 2010) ; Italy (Al Aukidy et al., 2012; Zuccato et al., 2010, 2006)). In total, 107 PIDs and 220 fragment ions were incorporated in the database (See **ESM Table S1**).

As stated in Section 2.4.2, each sample was injected twice in the Orbitrap. The first injection enabled full-scan acquisition. In this data, the original database of 107 PIDs was searched using TraceFinder and potential positive compounds were then selected, based

on retention time and exact mass, as unfortunately the expected fragment ion information of the targeted compounds was not among the four most intense ions detected within the data events. These "positives" were then grouped into target lists, according to whether they were found in the Spanish (40 compounds) or Italian (52 compounds) samples (See **ESM Tables S2 and S3**). The second injection incorporated these target lists, thereby enabling their product ions to be seen.

Within the software of the QTOF and Orbitrap, a threshold was given for intensity and mass error, outside which peaks were disregarded as noise. The majority of compounds were able to be automatically identified or detected thanks to the ChromaLynx and TraceFinder software. However, in some cases, some compounds were only able to be detected (i.e. no fragment ions found). This demonstrated the complementariness of the instruments: when one instrument was solely able to detect a compound (only the precursor ion found), the other was able to at least tentatively identify the compound with fragments. The raw data of both instruments was manually inspected in these situations to ratify the fragments (see **Table 1**). This secondary examination also reduced the possibility that one of the software had erroneously eliminated some compounds. The raw data investigations were only required for a small number of compounds, which is testament to the utility of the software. However, by decreasing the identification thresholds used, the need for manual inspection could be avoided, which is especially recommended for wide-scope screening methods incorporating thousands of compounds.

3.2 Comparison of results from Spain and Italy

Table 1 shows the number of compounds identified (according to identification criteria 2 and 4, from Section 2.5) and detected (according to detection criteria 1 and 3, from Section 2.5**)** in each sample. In general, more compounds were able to be identified (up to 18) or detected (up to 25) in the Spanish samples than the Italian. However, some compounds were only able to be detected and not identified in some samples, due to low analyte concentration. For example, ofloxacin in Castellón IWW was identified in only three samples by QTOF, but detected in the all, while it was identified in all samples by Orbitrap. In addition, trimethoprim was only detected in all Spanish and Italian EWW samples by both instruments. These results show that these compounds were likely present in all the detected samples, but unable to be identified due to a combination of

their low concentrations and the difference in MS/MS acquisition between the instruments.

There is little national difference, with just cotinine, gabapentin, oxazepam, temazepam and a metabolite of metamizole (4-aminoantipyrine), not identified in the Italian samples. This is not to say that these compounds are not in the samples, rather that their concentration is lower than that necessary for detection or identification. In fact, 4-aminoantipyrine was the only compound in the aforementioned list not detected in at least one of the Italian samples. Metamizole (also known as dipyrone) is a non-steroidal anti-inflammatory drug. In some countries such as Sweden, UK, Canada or United States metamizole has been banned or restricted for human use due to its association with diseases like agranulocytosis (Gómez et al., 2008). However in many other countries including Spain and Italy it can be purchased without a medical prescription ("Metamizolo: una lunga storia tra luci ed ombre," n.d.). Metamizole is a widely consumed pharmaceutical in Spain and some years ago it was among the ten most consumed pharmaceuticals (considering the amount of prescriptions), according to the information published by the Spanish Ministry of Health (Prescription data: IT del Sistema Nacional de Salud Volumen 30, 2006). In Italy, metamizole is also highly consumed but is not included in the list of the most consumed pharmaceuticals (Farmaco, 2013). This apparent difference in consumption could reflect the difference in its detection in the samples of Spain and Italy.

The most commonly detected compounds mirrored those found in similar studies (Gracia-Lor et al., 2012; Hernández et al., 2011; Zuccato et al., 2010, 2006). Irbesartan, ofloxacin, sulfamethoxazole and valsartan were identified in IWW and EWW of both Spain and Italy. Caffeine, irbesartan, valsartan and venlafaxine were also found in a high enough concentration in surface water to be identified by at least one of the two instruments. Several previous studies have also shown that these compounds are not fully removed in WWTPs and can be present in surface waters (Ferrer and Thurman, 2012; Kasprzyk-Hordern et al., 2007; Li, 2014; Luo et al., 2014), which indicates that further investigation into their potential fate and harm to the environment is necessary.

3.3 Use of QTOF and LTQ-Orbitrap

There was little difference between the number of compounds able to be identified by QTOF and Orbitrap. However, there are some discrepancies between the numbers of compounds in each sample that are identified by each instrument. For example, naproxen is identified using the software in all seven Spanish IWW samples by QTOF, while in only four by the Orbitrap. Alternatively, with Orbitrap, bezafibrate and morphine are identified in seven and four Spanish EWW samples respectively, but in none by QTOF. In both cases, the compounds were detected and compared with standards. **Figures 1 and 2** show the examples of naproxen and bezafibrate respectively.

Figure 1 provides an example of raw data investigation, touched upon in Section 3.1. In an IWW sample, the QTOF data was searched with ChromaLynx and able to identify naproxen, without having to refer to the raw data **(Figure 1 (a))**. The protonated molecule (231.1017) and primary fragment ion (185.0971) were seen in the LE and HE spectra within the thresholds of mass error for identification. To gain further confidence in this finding, the Orbitrap data was searched with TraceFinder. However, the software could not identify naproxen in this IWW sample. A subsequent search of the raw data by QualBrowser revealed its presence **(Figure 1 (b))**.

Figure 2 exemplifies the compatibility of the two HRMS instruments. ChromaLynx was unable to identify bezafibrate through the use of fragments, so the raw data was checked (**Figure 2 (a**), right). Again, only the precursor was able to be found. A comparison with the standard is also made, showing the three fragments of 316.1105, 276.0788 and 138.9947 (**Figure 2 (a**), left). These fragments were searched in the HE spectrum of the sample, but no suitable peaks were found (**Figure 2 (a**), right). It was thought that bezafibrate was below the detection threshold for the QTOF, which has a known relationship between intensity and mass error (i.e. at low concentration, the mass error would be high) and therefore at a low concentration, the fragment ions would fall outside the given mass error threshold. Conversely, Orbitrap is able to maintain its mass accuracy, even at low concentrations. Hence, for the same sample, using Orbitrap, bezafibrate was able to be identified with all three fragments **(Figure 2 (b),** right**)**.

A further example of the use of these instruments was in the identification of benzoylecgonine in an IWW sample. The protonated ion was seen in the QTOF data with a mass error of 0.07 ppm, a retention time of Δ 0.01 min. from that of the standard, and only one fragment with a mass error of 6.31ppm – just outside the criteria used for identification (see Section 2.5). Although we were confident this was in fact benzoylecgonine, and did tentatively assign it as "identified", it did not quite fulfil our set requirements. To appease our doubts, we also checked the Orbitrap data. Here, the protonated molecule and fragment ion were found within the threshold of 5ppm and the retention time differed by 0.1 minutes from the standard. The isotopic pattern fit was above the 60% threshold and the library search score of 50%, above the threshold set. Therefore, full confidence in identification was gained for this compound. Although identification could also have been possible by manually scouring the raw data, as the fragment ion was only just outside the given threshold it was thought better to compare with the Orbitrap data.

The mere fact that further information can be gleaned from these two instruments shows their complementariness. The SANCO guidelines on analytical quality control and validation procedures for pesticide residues analysis in food and feed ("Guidance document on analytical quality control and validation procedures for pesticides residues in food and feed," 2013), which are also applied for environmental samples in the absence of specific guidelines for the environmental field, state that "confirmation is the combination of two or more analyses that are in agreement with each other." The use of two HRMS instruments thus follows these SANCO guidelines, and as shown in Section 3.3 and **Table 1**, confirmation (i.e. identification) of numerous compounds is made. The examples given thus demonstrate the complementary use of QTOF and Orbitrap in the suspect screening of PIDs in environmental and wastewaters.

4. Conclusions:

In this paper, two LC-HRMS instruments (QTOF and LTQ-Orbitrap) used in different laboratories were used to screen for PIDs in samples of wastewater and surface water from Spain and Italy. A database of compounds frequently detected in water was used by both instruments, incorporating compounds of national significance. The samples were first investigated through the use of the manufacturer's software (ChromaLynx or TraceFinder), which allowed the detection of various compounds and in some cases the positive identification with fragment ions. Following this strategy, up to 18 compounds were identified with fragment ions across both instruments, while 28 compounds were at least detected (only the most abundant ion present) across all samples. In particular cases where identification was not automatic using software, the raw data was manually investigated. This was only necessary for a few compounds and occurred when the compound was detected but could not be identified. There was no significant difference between the countries, the metamizole metabolite, 4-aminoantipyrine, being the only compound found solely in Spanish waters.

The screening results obtained by QTOF MS and LTQ-Orbitrap MS were very similar, with the number of compounds detected/identified only differing by up to four across all samples. Although the objective of this work was not to compare the methods, rather the results in terms of detection and reliable identification, it is worth noting that the LTQ-Orbitrap was able to identify a few more compounds without having to manually search for fragment ions. On the whole, this paper shows that these powerful instruments, used in these configurations, are suitable for the detection and identification of numerous environmental contaminants in waste and environmental waters. The general agreement in data obtained will serve to validate the methodologies commonly used by our laboratories for qualitative screening of emerging contaminants in waters.

Acknowledgements

R. Bade, N. I. Rousis and E. Gracia-Lor acknowledge the European Union's International Training Network SEWPROF [Marie Curie-FP7-PEOPLE Grant #317205] for their Early Stage Researcher (R. Bade and N. I. Rousis) and Experienced Researcher (E. Gracia-Lor) contracts. The financial support from the Spanish Ministry of Economy and Competitiveness (Ref CTQ2012-36189), and Generalitat Valenciana, Spain (research group of excellence PROMETEO II/2014/023; ISIC 2012/016) is also acknowledged.

We would like to thank Thermo Scientific who provided the software Trace Finder and particularly Claudia Martins for technical support.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Table 1: All compounds detected/identified in wastewater and surface water samples of Spain and Italy

Compound	Spain IWW (n=7)		Spain EWW (n=7)		Spain SW (n=5)		Italy IWW (n=2)		Italy EWW (n=2)	
	QTOF	Orbitrap	QTOF	Orbitrap	QTOF	Orbitrap	QTOF	Orbitrap	QTOF	Orbitrap
4-aminoantipyrine[a]	7/1[b]	6/5	7/7	7/3						
Acetaminophen	7/7	7/7 (7)[c]					2/2	2/2 (2)		
Atorvastatin			2/0	5/0						
Benzoylecgonine	7/7	7/7	7/7 (7)	7/7	1/0		2/2 (2)	2/2		
Bezafibrate		3/3	7/0	7/7			2/1 (1)	2/2		1/1
Caffeine[a,d]	7/7	6/6	7/1	7/7	4/3	5/5	2/2	2/2		
Carbamazepine	3/0	7/7	7/7	7/7			2/2	2/1	2/2	2/2
Ciprofloxacin	7/6 (6)	6/6	6/5 (5)	6/5			2/2 (2)	2/2		
Clarithromycin		1/0	5/5 (5)	6/6			2/1 (1)	2/1	2/1(1)	2/1
Cocaine	7/3 (3)	3/3	2/0	1/0			2/2 (2)	1/1		
Codeine	7/7 (7)	7/7	7/4 (4)	6/6			2/1 (1)	2/1	2/1(1)	1/1
Cotinine[a,d]	7/6		7/7		2/0		2/0			
Diclofenac		3/3	7/7	7/7			2/2	2/2	2/2	2/2
Enalapril	1/0									
Gabapentin[a]	5/0		6/6						2/0	
Gemfibrozil								2/0		
Irbesartan[a]	7/7	7/7	7/7	7/7	2/1	2/2	2/2	2/2	2/2	2/2
Ketoprofen	7/6 (6)	6/5	6/2	2/2			2/2			
Morphine	7/0	4/4	2/0	4/4			2/0	2/2	2/0	2/0
Moxifloxacin			2/0							
Naproxen	7/7	7/7 (3)	4/0				2/1	1/1 (1)		
Ofloxacin	7/3	7/7	7/7	7/7	1/0		2/2	2/2	2/2	2/2
Oxazepam[a,d]		3/3	7/7	5/5				1/1		1/0
Sulfamethoxazole	3/2 (2)	6/6	7/5	5/4			2/2 (2)	2/2	2/1(1)	2/2
Temazepam[a,d]	3/3 (3)	6/5	7/5	7/7				1/0		1/1
Trimethoprim	1/0		7/0	7/0			2/0		2/0	2/0
Valsartan[a]	7/7	7/7	7/7	7/7	2/0	3/3	2/2	2/2	1/1	2/2
Venlafaxine[a]	7/7 (6)	7/7	7/7	7/7		1/0	2/0	2/2	2/2	2/2
Total	21/16	23/18	25/18	21/18	6/2	4/4	20/16	20/16	12/9	12/10

[a]: no reference standard for Orbitrap analysis

[b]: number of compounds detected/identified

[c]: number in brackets refers to the number of samples manually checked in raw data for missing fragment ions

[d]: no reference standard for QTOF analysis

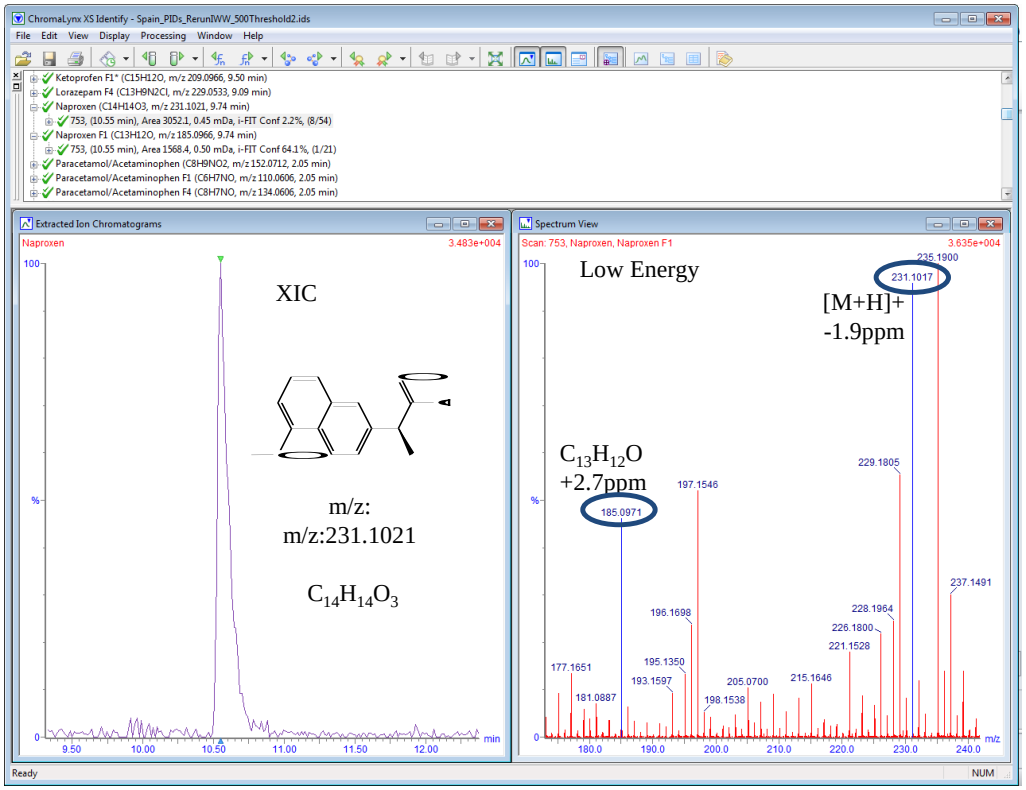
Captions for Figures

Figure 1: (a): Identification of naproxen in an IWW sample by QTOF (ChromaLynx); LE spectrum shows the parent and fragment of naproxen (blue at 231.1017 and 185.0971). (b): Identification of naproxen in the same IWW sample by Orbitrap (raw data, using QualBrowser) based on the fragment of 185.0961, compared with the standard (left).
The QTOF data is from the single MS^E injection, while the Orbitrap data is from the second MS/MS injection.

Figure 2: (a) Detection of bezafibrate in an EWW sample by QTOF (raw data). The XICs (left) and related mass spectrum (bottom) of the standard show the fragments (316.1104, 276.0791 and 138.9951), and mass errors. The sample (right) shows none of the related fragments at a similar peak shape or retention time to the precursor (362.1159).
(b) Identification of bezafibrate in the same EWW sample (right) by Orbitrap (QualBrowser), compared with the standard (left). The fragment ions of 316.1102, 276.0787 and 138.9946 are clear in both, as is the chromatographic peak at ~20.85 minutes.
The QTOF data is from the single MS^E injection, while the Orbitrap data is from the second MS/MS injection.

(a)

(b)


ChromaLynx XS Identify - Spain_PIDs_RerunIWW_500Threshold2.ids
Extracted Ion Chromatograms
Naproxen
XIC
m/z:
m/z:231.1021
C14H14O3
Spectrum View
Low Energy
[M+H]+
-1.9ppm
C13H12O
+2.7ppm

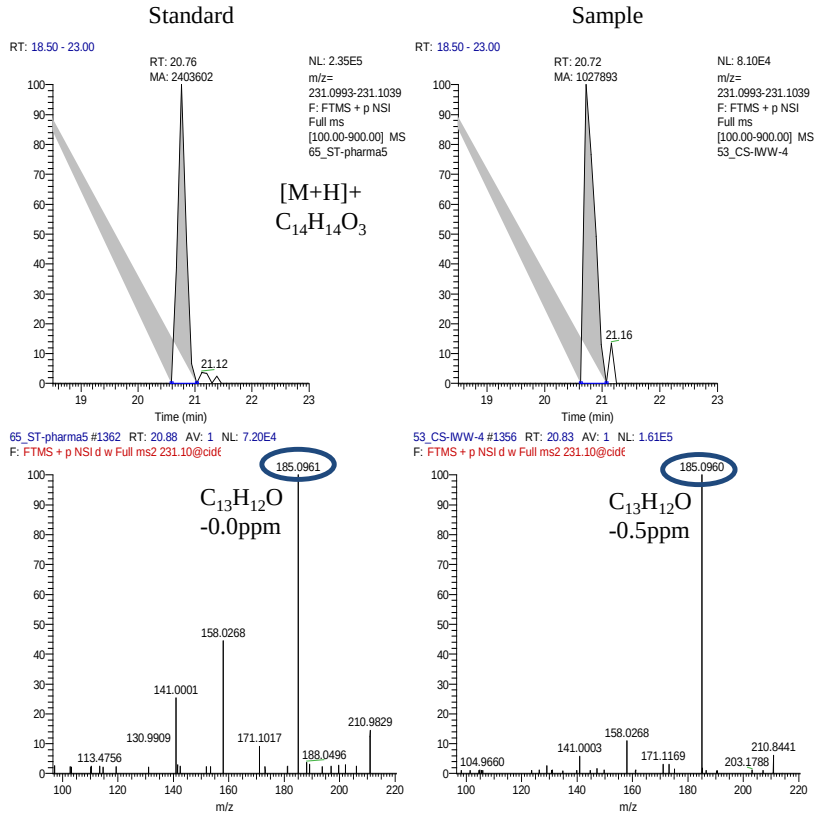

Standard
Sample
[M+H]+
C14H14O3
C13H12O
-0.0ppm
C13H12O
-0.5ppm
Time (min)
m/z

Figure 2

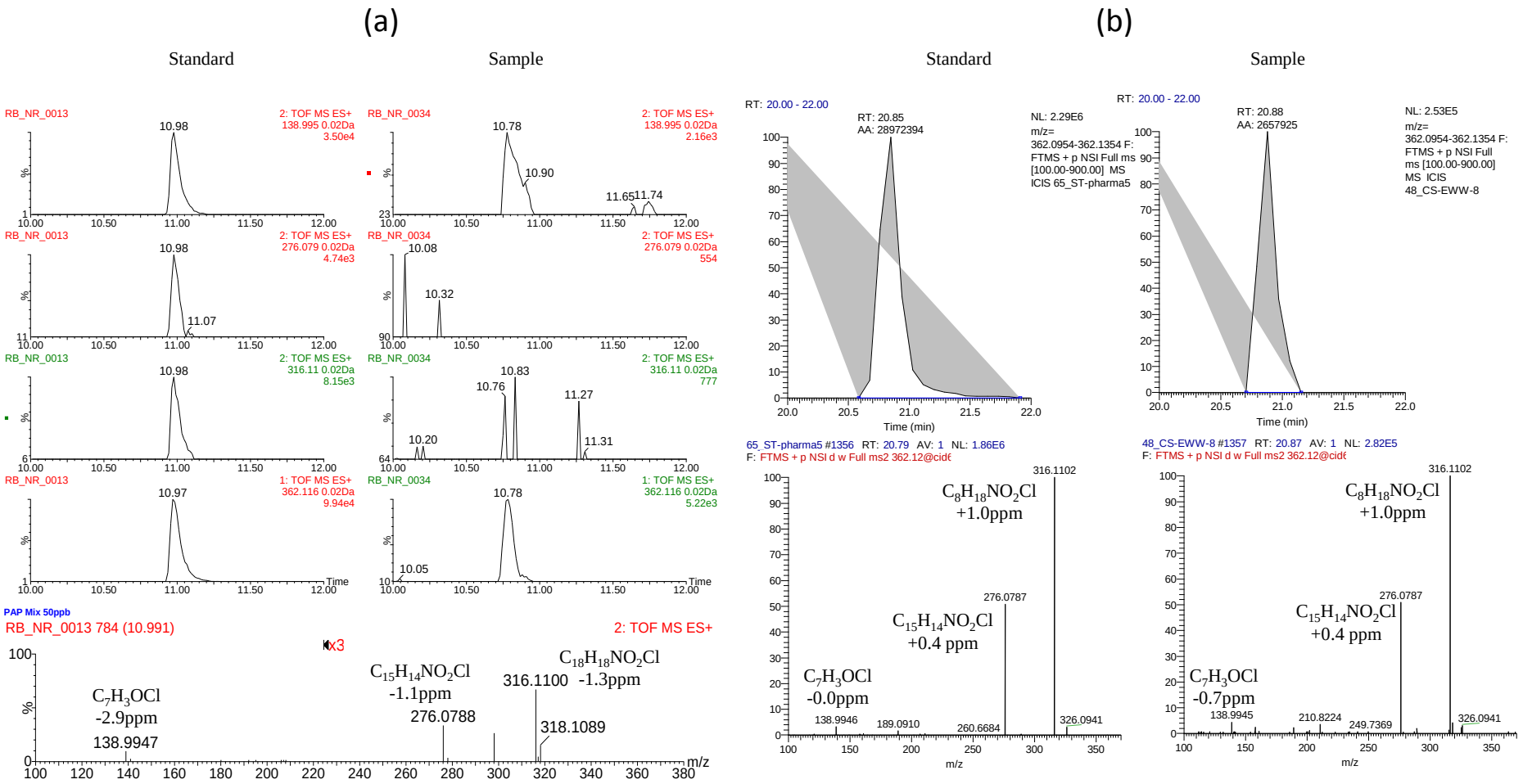

(a)
Standard
Sample
(b)
Standard
Sample
RB_NR_0013
2: TOF MS ES+
138.995 0.02Da
3.50e4
10.98
RB_NR_0034
2: TOF MS ES+
138.995 0.02Da
2.16e3
10.78
10.90
11.65
11.74
RB_NR_0013
2: TOF MS ES+
276.079 0.02Da
4.74e3
10.98
11.07
RB_NR_0034
2: TOF MS ES+
276.079 0.02Da
554
10.08
10.32
RB_NR_0013
2: TOF MS ES+
316.11 0.02Da
8.15e3
10.98
RB_NR_0034
2: TOF MS ES+
316.11 0.02Da
777
10.83
10.76
11.27
10.20
11.31
RB_NR_0013
1: TOF MS ES+
362.116 0.02Da
9.94e4
10.97
RB_NR_0034
1: TOF MS ES+
362.116 0.02Da
5.22e3
10.78
10.05
Time
PAP Mix 50ppb
RB_NR_0013 784 (10.991)
x3
2: TOF MS ES+
C7H3OCl
-2.9ppm
138.9947
C15H14NO2Cl
-1.1ppm
276.0788
C18H18NO2Cl
316.1100 -1.3ppm
318.1089
m/z
RT: 20.00 - 22.00
RT: 20.85
AA: 28972394
NL: 2.29E6
m/z=
362.0954-362.1354 F:
FTMS + p NSI Full ms
[100.00-900.00] MS
ICIS 65_ST-pharma5
Time (min)
RT: 20.00 - 22.00
RT: 20.88
AA: 2657925
NL: 2.53E5
m/z=
362.0954-362.1354 F:
FTMS + p NSI Full
ms [100.00-900.00]
MS ICIS
48_CS-EWW-8
Time (min)
65_ST-pharma5 #1356 RT: 20.79 AV: 1 NL: 1.86E6
F: FTMS + p NSI d w Full ms2 362.12@cid
316.1102
C8H18NO2Cl
+1.0ppm
276.0787
C15H14NO2Cl
+0.4 ppm
C7H3OCl
-0.0ppm
138.9946
189.0910
260.6684
326.0941
m/z
48_CS-EWW-8 #1357 RT: 20.87 AV: 1 NL: 2.82E5
F: FTMS + p NSI d w Full ms2 362.12@cid
316.1102
C8H18NO2Cl
+1.0ppm
276.0787
C15H14NO2Cl
+0.4 ppm
C7H3OCl
-0.7ppm
138.9945
210.8224
249.7369
326.0941
m/z

Figure 2