Characterization of the organic contamination pattern of a hyper-saline ecosystem by rapid screening using gas chromatography coupled to high-resolution time-of-flight mass spectrometry

Roque Serrano*, Tania Portolés, Miguel A. Blanes, Félix Hernández
[a]*Research Institute for Pesticides and Water (IUPA). Avda Sos Baynat, s/n. University Jaume I, 12071 Castellón, Spain*
Juan C. Navarro, Inmaculada Varó, Francisco Amat
[b]*Institute of Aquaculture of Torre la Sal (IATS), C.S.I.C., 12595 Ribera de Cabanes, Castellón, Spain*

Abstract

In this paper, gas chromatography coupled to high-resolution time-of-flight mass spectrometry (GC-TOF MS) has been applied to evaluate organic pollution in a hyper-saline aquatic environment. Firstly, a target screening was made for a list of 150 GC-amenable organic micro-contaminants, including PAHs, octyl/nonyl phenols, PCBs, PBDEs, and a notable number of pesticides, such us insecticides (organochlorines, organophosphorus, carbamates and pyretroids), herbicides (triazines and chloroacetanilides), fungicides and several transformation products. This methodology was applied to brine samples, with a salt content from 112 g/L to saturation, and to samples from *Artemia* populations (crustacean anostraca) collected along one year from three sampling stations in saltworks bodies sited in the Ebro river delta. Around 50 target contaminants, belong to chemical families included in the list of priority substances within the framework on European Water Policy.

Additionally, a non-target analysis was performed in both types of samples with the objective of investigating the presence of other non-selected organic compounds taking advantage of the potential of GC-TOF MS (high sensitivity in full-spectrum acquisition mode, accurate mass measurements) for searching unknowns. Organophosphorus pesticides were the contaminants more frequently detected in brine samples. Other compounds usually present in urban and industrial wastewaters, like caffeine, methyl paraben, butylated-hydroxytoluene and N-butylbenzenesulfonamide were also detected in brines. The herbicide simazine and the insecticide chlorpyrifos were among the contaminants detected in *Artemia* samples.

Results of this work reveal a potential threat to vulnerable populations inhabiting the hyper-saline ecosystem. The valuable contribution of GC-TOF MS in environmental analysis, allowing the rapid screening of a large number of organic contaminants, is also proven in this paper.

Keywords: Gas Chromatography, Mass Spectrometry, Time-of-Flight, Screening, hyper-saline ecosystem, organic contaminants

* *Corresponding author. Tel. +34-964387358;Fax +34-964387368 e-mail: serrano@qfa.uji.es*

Introduction

Contamination of natural waters is a problem worldwide, which can have important consequences for the health of both man and animals. Human activities are the main factor affecting the quality of surface and groundwater through atmospheric pollution, effluent discharges, eroded soils or land use. Anthropogenic effluent discharges constitute one of the more important polluting sources, whereas surface runoff is normally a seasonal phenomenon, able to transport a variety of pollutants (Vega et al., 1996; Singh et al., 2004; Kazi et al., 2009). Particularly, the use of agricultural chemicals affects the quality of natural waters (Niemi et al., 1990; Pitarch et al., 2007). Run-off from agricultural soils may contain high concentrations of herbicides, insecticides and other types of pesticides during application periods along the year. Thus, pesticides can become a serious environmental problem, as they may have a deleterious impact on the aquatic biota (Harmon et al., 2010; Mearns et al., 2010), given that many of them are toxic to non target organisms, including fish (Varó et al., 2000; Slaninova et al, 2009) and aquatic invertebrates (Serrano et al. 1997; Varó et al. 1998; Varó et al., 2000). Given the above mentioned negative impacts, the application of reliable and rapid multi-residue methods is required for monitoring of the presence of the large variety of contaminants that can be potentially present in vulnerable aquatic ecosystems. The European framework for water policies set up in the European Directive 2000/60/EC encourages the Members States to carry out periodical trend studies on different water pollutants (Annexe V Section 2.4.4). In such context, the application of wide-scope screening procedures, able to detect the highest number of analytes as possible, is one of the most convenient approaches.

Typically, water pollution monitoring has been carried out by application of target analytical methods, focused on a limited number of analytes (Sancho et al., 2004; Pitarch

et al., 2007; Kuster et al., 2008). For determination of semi-polar and polar compounds, liquid chromatography combined with tandem mass spectrometry is the technique of choice at present (Bobeldijk et al., 2001, 2002; Kuster et al., 2008; Marin et al., 2009; Gracia-Lor et al., 2011). The other complementary approach for determination of organic pollutants in water is the application of gas chromatography combined with tandem mass spectrometry (Claver et al., 2006, Hancock et al., 2007, Pitarch et al., 2007, 2010), which allows investigating the presence of volatile and semi-volatile compounds.

The best way to have a general overview on the presence of organic pollutants in water is to apply wide-scope screening methods based on full-spectrum acquisition techniques that combine high selectivity and sensitivity. GC or LC coupled to TOF MS are among the most powerful techniques for this purpose thanks to the elevated mass resolution and accurate mass measurements of the analyte molecule and for its fragment ions. GC-TOF and LC-TOIF MS accomplish the requirements for detection and confirmation of target and also non-target compounds with the possibility of searching many other pollutants not initially selected by re-examining MS data acquired when desired, without the need of performing additional analysis (Hernández et al., 2007, 2011; Ibañez et al., 2005, 2008).

In this work, the objective was to make use of the GC-TOF MS potential for the rapid and large-scope screening of organic pollutants in a hypersaline natural protected area sited in the Ebro river delta (Spain). Samples of brines with salinities from 112 g/L to saturation were collected along one year from three saltworks in order to know the seasonal pollutants dynamics. Likewise, samples from *Artemia* populations living in these brines were collected to search for pollutants bioaccumulated. Genus *Artemia* belongs to the order Anostraca. They are passive filter feeder branchiopod crustaceans

88 that thrive in hypersaline water bodies, playing a key role in the trophic dynamics of the
89 ecosystem.

90

91 **Material and methods**

92 *Area of study and Sampling*

93 The Ebro River is used as a supply of water for agriculture, cattle breeding
94 (89.3%), domestic (7.2%) and industrial activities (3.5%) with a total supply of
95 18,217 hm^3/year in 2004. This river receives domestic and industrial wastewater from
96 numerous minor settlements along the bed. Discharges into the Ebro River vary at
97 different locations, showing an increase downstream, probably due to inputs from the
98 tributaries or natural recharge of the stream, and finally flowing into the Ebro Delta at the
99 Mediterramean sea (Bouza-Deaño et al, 2008). This estuary is a valuable protected
100 natural area with shallow waters bodies. At the south of the delta, in contact with the sea,
101 a wide extension is used for the industrial extraction of salt under the form of a traditional
102 Mediterranean saltpan. The ponds of this saltpans are the refugee of an abundant
103 waterfowl feeding on big populations of the brine shrimp *Artemia*.

104 Brine samples were taken at three stations (Figure 1). Sampling was carried out
105 along one year from Station 1, following a proximate seasonal trend in March, June,
106 September and November 2007. Station 1 (40° 34’ 58’’ N 0° 40’ 49’’ E) is a reservoir
107 extended along 90 Ha used for the pre-storage of the Alfaques bay sea water, which is
108 pumped into the evaporators where brines are obtained and finally sent to the
109 crystallization salt ponds. These ponds house populations of waterfowl. Station 2 (40° 35’
110 16’’ N 0° 41’ 55’’ E) is a channel used for moving brines into the saline towards the
111 crystallisation ponds where final evaporation takes place. Salinity of this station is always

high (higher than 100 g L^{-1}). It was sampled three times along the year. Station 3 (40° 34' 38'' N 0° 41' 43'' E) is a small pond submitted to notable environmental fluctuations, separated by a sand bar located next to the open sea. The water body of Station 3 was highly dependent on seasonal and antropic fluctuations and it was sampled only twice along the year.

Brine samples were directly collected in plastic bottles from the sampling sites, roughly filtered *in situ* through a 160 µm nylon mesh, transported to the laboratory and filtered again through a 60 µm mesh and finally stored at -20°C until analysis.

Artemia biomass samples were collected from Station 1 with a 160 µm mesh and transported alive to the laboratory where they were further rinsed with water and stored at -20°C until analysis. *Artemia* populations were present the whole year round. All specimens collected were classified as *Artemia franciscana*, an alochthonous species from North America introduced in saltmarsh from all around the world mainly due to its widespread use as larval food in aquaculture. This species out-competes autochtonous *Artemia* populations, replacing them due to its superior environmental fitness (Amat et al, 2005).

Analytical procedure

Brine samples

PAHs, octyl/nonyl phenols, PCBs, PBDEs, and a notable number of pesticides, such us insecticides (organochlorines, organophosphorus, carbamates and pyretroids), herbicides (triazines and chloroacetanilides), fungicides and several metabolites are included in the GC-TOF MS screening. A qualitative validation has been carried out previously by Portoles et al. (2011). The complete list of compounds included in this research is presented in Supplementary data (Table I). Briefly, the method applied

consisted on using 100 mL of sample that were passed through a C_{18} SPE cartridge. The elution was performed by passing 5 mL of ethyl acetate:DCM (50:50). The eluate was evaporated under a gentle nitrogen stream at 40 °C and redisolved in 0.5 mL of hexane before injection into the GC-TOF MS system.

Artemia samples

Artemia samples were analyzed based on our previous works for organophosphorous and organochlorine pesticides and for PAHs and PBDEs (Hernández et al., 1998, Serrano et al., 2003, Nacher-Mestre 2009, 2010). Briefly, pools of *Artemia* specimens were thawed at room temperature. Approximately 4 g were chopped and homogenized in a mortar with the amount of anhydrous sodium sulfate necessary to remove water. Extraction was carried out by refluxing in n-hexane for 4 hours the whole sample weighted. After filtration (0.45 µm), the extract was pre-concentrated using a Kuderna-Danish until ca. 2 ml. The final residue was adjusted to 1 ml with n-hexane (4 g sample per ml hexane). Then, a clean up of the hexanic extract was carried out by normal phase HPLC using silicagel column in order to separate most of the fats from analytes. Using hexane as mobile phase (1ml/min) and changing it to ethyl acetate at minute 16, two fractions were collected: the first from 0 to 8 ml containing non polar compounds, and the second, from 8 to 20 ml, for medium polar analytes (for more details see Serrano et al., 2003). After evaporation of the eluates, the final extract were adjusted to 1-ml with hexane, and analysis were performed separately for each fraction by GC-TOF MS.

GC-TOF MS analysis

An Agilent 6890N GC system (Palo Alto, CA), equipped with an Agilent 7683 autosampler, was coupled to a GCT time-of-flight mass spectrometer (Waters Corporation, Manchester, U.K.), operating in electron impact ionization mode (EI). The

GC separation was performed using a fused-silica HP-5MS capillary column with a length of 30 m x 0.25 mm i.d. and a film thickness of 0.25 µm (J&W Scientific, Folson, CA). injection volume was 2 µl. The oven temperature was programmed as follows: 90 °C (6 min); 5 °C/ min to 300 °C (2 min). Helium was used as carrier gas at 1 mL/ min. The interface and source temperatures were set to 250 °C for all analytes studied, and a solvent delay of 3 min was selected. The time-of-flight mass spectrometer was operated at 1 spectrum/s, acquisition rate over the mass range *m*/*z* 50-650, using a multichannel plate voltage of 2500 V. TOF-MS resolution was approximately 7000 (FWHM). Heptacosa, used for the daily mass calibration and as lock mass, was injected via syringe in the reference reservoir at 30 °C for this purpose; the *m*/*z* ion monitored was 218.9856. The application manager TargetLynx, a module of MassLynx software, was used to process the qualitative and quantitative data obtained from standards and samples for target compounds. The application manager ChromaLynx, also a module of MassLynx software, was used to investigate the presence of non target compounds in samples. Library search was performed, when required, using the commercial NIST library.

Compounds considered in the screening were tested in order to prove their presence in the eluate from SPE (brines) and LC (*Artemia*). Brine and *Artemia* samples were fortified 0.05 ng/mL and 2.5 ng/g, respectively (concentration in the sample extract was 10 ng/mL), and their presence was tested in the eluate after clean up by SPE or LC, respectively, by GC TOF MS. Other unknown compounds with similar physico-chemical characteristics are expected to be included in the screening, as well.

Although the objective of this work was a qualitative screening, a semi-qualitative estimation was made for the compounds detected in samples. The estimation was made by interpolating the absolute response in calibration curves prepared with standards in solvent in the range of 1-50 ng/mL in extracts (which corresponds to 0.005-0.25 ng/mL

and 0.25-12.5 ng/g in brine and *Artemia* samples, respectively). Actual range of comparison was variable depending on sensitivity for each analyte detected.

Results and Discussion

A first characterization of the organic contamination pattern in the hypersaline ecosystem was performed by evaluating the presence of target analytes in brine and *Artemia* samples by GC-TOF MS screening.

As regards brine samples, the methodology applied for most of the compounds was quantitatively validated previously by Pitarch et al. (2007) using GC-MS/MS with triple-quadrupole and the screening methodology was qualitatively validated using GC-TOF MS by Portoles et al. (2011). After sample analysis, the presence of other selected compounds was also investigated in a post-target way (i.e. searching for target compounds after MS data acquisition), taking advantage of the full-spectrum TOF MS acquisition at accurate masses.

In the case of Artemia samples all chemical families considered were quantitatively validated previously in our laboratory (Hernández et al., 1998, Serrano et al., 2003, Nacher-Mestre 2009, 2010) for *Artemia* and other marine organisms. In both cases, brine and Artemia, a semi-quantitative estimation of the analyte concentrations in the samples has been reported by comparison of the responses with calibration curves using standards in solvent.

The detection and identification of target analytes in the samples was carried out by evaluating the presence of at least two (maximum five) *m*/*z* ions at accurate mass for every compound. In addition to the accurate masses for analyte identification, experimental ion intensity ratios in samples were evaluated by comparing with those of standards in solvent (Hernández et al., 2007, Portoles et al., 2007). The use of narrow

mass windows (0.02 Da) allowed to notable increase sensitivity and selectivity in analysis.

In addition, the presence of unknowns (non target analysis) was investigated making use of the full-acquisition accurate-mass data acquired without the need of re-analyzing the sample. In this approach unknown compounds, different to those included in the target list, eluting from the analytical column and ionized in the EI interface are detected and identified applying a component detection algorithm and deconvolution software . Obviously, only those compounds fulfilling the sample treatment (extraction and clean up and GC-MS analysis requirements) could be detected, being excluded contaminants that are not GC-amenable. Combination of target and post target screening is one of the most appropriate approaches for environmental samples that may content many different contaminants, where high cost and time consuming analysis would be necessary if they had to be targeted individually.

Investigation of non target compounds in complex-matrix samples is a laborious challenging and time-consuming task. In this work, the deconvolution package ChromaLynx Application Manager was used to automatically process data in non target analysis. Four abundant ions were selected for a reliable identification of the analytes (Hernandez et al., 2007). When a peak was found to satisfy user defined parameters (such as scan width, spectra rejection factor, peak width at 5% height) the software displayed its deconvoluted mass spectrum, which was submitted to an automatic library search routine (NISTH 02 mass spectral library). Components were reduced to a list of possible candidates by using the fit factor from the mass library search. A library match >70% was required for non target compounds identification. After that, Accurate mass measurements of up to five of the most abundant ions are evaluated for confirmation (or rejection) of the finding.

Compounds detected in brine samples

Table 1 shows the contaminants identified using target and non-target approaches in the brine samples. Chlorpyrifos was the only analyte detected from the target list considered in the present work (Table I, Suplementary data). However, although not included in the target list, Thiabendazole, dichlorvos and diazinon could be detected by means of post-target investigation, when searching specifically for them.

Regarding to the eco-toxicological implications, most of the analytes found were organophosphorous pesticides (chlorpirifos, thiabendazole, dichlorvos and diazinon). Particularly, the insecticide chlorpyrifos [O,O – diethyl – O - (3,5,6-trichloror-2-pyridil) phosphorothiate] is an organophosphorous pesticide widely used in the countries of the European Union (Varó et al., 1998, 2000, 2006), and has been frequently detected in surface waters of the Ebro river (Claver et al., 2006). This pesticide has been studied by several authors as a model organophosphorus pesticide, demonstrating its high toxicity and bioconcentration ability in different groups of aquatic organisms (Serrano et al., 1997, Varó et al., 1998). Other organophosphorous compounds detected were the post-harvest pesticides thiabendazole, diazinon (and its derivative diazoxon) and dichlorvos, that show moderate acute toxicity to non-target organisms, inhibiting the acetyl-cholinesterase activity (Hernández et al, 1998, Tomlin, 2006). All of them are widely used in agricultural activities and are frequently detected in natural waters (Ibañez et al., 2008).

Figure 2 shows a positive finding of the diazinon metabolite diazoxon, detected in a brine sample when using the deconvolution process. The accurate mass measurement of four ions in the EI spectrum, together with library fit, allowed a confidence confirmation of the identity of the compound detected.

Priority pollutants constitute only part of the large chemical pollution puzzle; there is a diverse group of unregulated pollutants, including industrial sub-products, drugs, pharmaceuticals and personal care products, raising an increasing concern on the risks they pose on humans and the environment (Daughton and Ternes, 1999). For these compounds, urban and industrial wastewaters are the main route of emission to the environment (Daughton and Ternes, 1999, Ternes et al., 2004, Muñoz et al., 2008). We have detected some of them in this work using the non-target approach, like caffeine, methyl paraben, butylated-hydroxytoluene and N-butylbenzenesulfonamide. Caffeine is nowadays usually detected in urban waste waters. Its presence in the saline waters is an indicator of its arrival through the mainstream and further pumping into the area studied (Muñoz et al., 2008; Swati et al., 2008). This fact can also explain the presence of other contaminants in the brine samples. Methyl-paraben belongs to the chemical group of parabens, used in cosmetics and pharmaceutical industry as preservative due to its bacteriocidal and fungicidal properties. Parabens are considered to be safe because of their low toxicity profile and their long history of safe use (Soni et al., 2002, 2005). Butylated-hydroxytoluene is an antioxidant widely used as food aditive not included in offical lists of dangerous contaminants. It is noteworthy the frequent presence of N-butylbenzenesulfonamide (N-BBS) in the brine samples. This plasticizer presents neurotoxic effects and has been detected in different environmental compartments. Its production and application was stopped in Germany, but occurrence in the environment will still continue due to its persistence (Huppert et al., 1998).

Figure 3 shows the total concentration estimated for pollutants in each sampling station along the year and the number of findings. As it can be observed, spring is the season with higher load of contaminants in water coinciding, as expected, with the application of herbicides and insecticides in agricultural crops (mainly rice fields) along

284 the river, some of them near the protected area of the Ebro delta. Station 1, a reservoir
285 used for the pre-storage of water before evaporation and crystallization processes,
286 presented the highest number of organic contaminants findings, probably as consequence
287 of the concentration by evaporation during storage.

288 *Compounds detected in Artemia populations*

289 The organophosphorus pesticide chlorpyrifos was the only contaminant detected
290 in water that was also present in *Artemia* samples (Table 2). This insecticide was found in
291 all brine samples, collected in winter, spring and summer. This fact reveals an intensive
292 use of this compoundin the area under study, and a potential threat to vulnerable
293 populations inhabiting the waters of the saline ecosystem.

294 Non-polar organochlorine contaminants, pp'DDE and PCBs, were detected in
295 *Artemia* specimens but not in water, due to their lipophility. These compounds seems to
296 be ubiquitous in the biotic compartment of aquatic environments and have been detected
297 in *Artemia* specimens in previous works (Wang and Simpson, 1998).

298 Besides, the non-target derivative of naphthalene, the 2,6-diisopropylnaphthalene,
299 was also been detected in *Artemia*. This compound is used as post-harvest pesticide for
300 potatoes storage and it is also a sub-product of industrial processes. Several authors have
301 reported that it might affect vertebrate physiology and reproduction (Terasaki et al.,
302 2008).

303 The herbicide simazine, widely used in the area of study and frequently detected
304 in surface water of the Ebro river (Claver et al., 2006), was also detected in the
305 organisms. Unexpectedly, this herbicide was not detected in the water samples collected.
306 A more detailed study on the presence of simazine in the waters of this area would be

necessary to determine the dynamics of this compound in the different compartments of the ecosystem studied.

As an illustrative example, Figure 4 shows positive findings of the herbicide simazine and the insecticide diazinon in *Artemia* samples. In both cases, we observed the 5 characteristic ions at the expected retention time in the 0.02 Da nw-XICs. The attainment of all 4 Q/qi ratios within accepted tolerances led to the unequivocal confirmation of both compounds. The accurate mass spectra of both sample peaks are shown together with mass errors for the five ions. Mass errors were calculated as the difference between experimental accurate masses and theoretical exact mass of the fragment ions.

In the light of the results obtained here, it seems that *Artemia* populations inhabiting the area of study are exposed to a variety of contaminants, which may produce an appreciable toxic stress affecting their survival. This exposure to toxicants can act as one of the forces driving the success of the alochthonous *Artemia franciscana* over the *Artemia parthenogenetica* and *Artemia salina* authocthonous species. Varo et al. (1997, 1998) reported that different *Artemia* strains show different degree of sensitivity to toxicants, including organophosphorus and organochlorine compounds, and *Artemia franciscana* is one of the most resistant forms.

The detection of several contaminants and derivatives from anthropogenic activities in brine and organisms, some of them not specifically searched, demonstrates the valuable contribution of GC-TOF MS for rapid wide scope screening of organic contaminants in the environment. The results obtained reveal a potential threat to vulnerable populations inhabiting the ecosystem under study, as a consequence of the presence of several toxic compounds.

Acknowledgments

This research was supported by the Spanish Ministry for Education and Science project (CGL2005-02306/BOS) “Biodiversidad de *Artemia (Branchiopoda, Anostraca)* en el Mediterráneo occidental. Presencia de *A. franciscana* como especie invasora. Implicaciones ecológicas y de interés en acuicultura”. The authors addressed in IUPA acknowledge the financial support of Generalitat Valenciana, as research group of excellence PROMETEO/2009/054. The authors also are very grateful to the Serveis Centrals d’Instrumentació Científica (SCIC) of University Jaume I for use of Agilent 6890N GC system coupled to a GCT time-of-flight mass spectrometer.

353 **References**

354 Amat F, Hontoria F, Ruiz O, Green A, Sánchez M, Figuerola J, Hortas F. The American
355 brine shrimp as an exotic invasive species in the Western Mediterranean. Biological
356 Invasions 2005;7:37-47.

357 Bobeldijk I, Stocks PGM, Vissers JPC, Enke E, van Leerdam JA. Surface and wastewater
358 quality monitoring: combination of liquid chromatography with (geno)toxicity detection,
359 diode array detection and tandem mass spectrometry for identification of pollutants. J
360 Chromatogr A 2002;970:167-181.

361 Bobeldijk I, Vissers JPC, Kearney G, Major H, van Leerdam JA. Screening and
362 identification of unknown contaminants in water with liquid chromatography and
363 quadrupole-orthogonal acceleration-time-of-flight tandem mass spectrometry.
364 J Chromatogr A 2001;929:63-74.

365 Bouza-Deaño R, Ternero-Rodríguez, M, Fernández-Espinosa AJ. Trend study and
366 assessment of surface water quality in the Ebro River (Spain). J Hydrol, 2008;361:227-
367 239.

368 Claver A, Peña Ormad, LR, Ovelleiro, JL Study of the presence of pesticides in surface
369 waters in the Ebro river basin (Spain). Chemosphere 2006;64:1437-1443.

370 Daughton CG, Ternes TA. Pharmaceuticals and personal care products in the
371 environment: agents of subtle change?, Environ. Health Perspect. 1999;107:907–938.

372 European Union. Directiva del Parlamento Europeo y del Consejo 2000/60/CE de 23
373 Octubre de 2000 por la que se establece el marco comunitario de acción en política de
374 aguas. Diario oficial L017. 19/01/2001:p0039-0039 IT.

375 Gracia-Lor E, Sancho JV, Hernández F. Multi-class determination of around 50
376 pharmaceuticals, including 26 antibiotics, in environmental and wastewater samples by

377 ultra-high performance liquid chromatography-tandem mass spectrometry. J Chromatogr
378 A 2011;1218:2264-2275.

379 Hancock P, Leandro CC, Keely BJ, Fussell RJ. Waters Application Note 720002027EN;
380 2007. (http://www.waters.com/waters/library.htm?cid=511436&lid=1512761).

381 Harmon, SM, Wiley, FE. Effects of pollution on fresh water organisms. Water Environ
382 Res 2010;82:1945-2000.

383 Hernández F, Serrano R, Pitarch E, López FJ. Automated sample clean up procedures for
384 organophosphorus pesticides in several aquatic organisms using normal phase liquid
385 chromatography. Anal Chim Acta 1998;374:215-229.

386 Hernández F, Portolés T, Pitarch E, Lopez FJ. Target and non target screening of organic
387 micropollutants in water by solid-phase microextraction combined with gas
388 chromatography/high-resolution time-of-flight mass spectrometry. Anal Chem
389 2007;79:9494-9504.

390 Hernández F, Bijlsma L, Sancho JV, Díaz R, Ibáñez M. Rapid wide-scope screening of
391 drugs of abuse, prescription drugs with potential for abuse and their metabolites in
392 influent and effluent wastewater by ultrahigh pressure liquid-chromatography-
393 quadrupole-time-of-flight-mass spectrometry. Anal Chim Acta 2011;684:96-106.

394 Huppert N, Wurtele M, Hahn HH. Determination of the plasticizer N-
395 butylbenzenesulfonamide and the farmaceutical ibuprofen in waste waters using solid
396 phase microextraction (SPME). Fresenius J Anal Chem 1998;362:529-536.

397 Ibañez M, Sancho JV, Pozo OJ, Hernández F, Niessen WMA. Use of quadrupole time-of-
398 flight mass spectrometry in the elucidation of unknown compounds present in
399 environmental waters. Rapid Comm Mass Spectr 2005;19:169-178.

400 Ibañez M, Sancho JV, McMillan D, Rao R, Hernández F. Rapid non-target screening of
401 organic pollutants in water by ultraperformance liquid chromatography coupled to time-
402 of-flight mass spectrometry. Trends Anal Chem 2008;27:481-489.

403 Kazi TG, Arain MB, Jamali MK, Jalbani N, Afridi HI, Sarfraz RA, Baig JA, Shah AQ.
404 Assessment of water quality of polluted lake using multivariate statistical techniques: A
405 case study. Ecotoxicol Environ Safety 2009;72:301-309.

406 Kuster M, López de Alba M J, Barata C, Raldúa D, Barceló D. Analysis of 17 polar to
407 semi-polar pesticides in Ebro river delta during the main growing season of rice by
408 automated on-line solid-phase extraction-liquid chromatography-tandem mass
409 spectrometry. Talanta 2008,75:390-401.

410 Marín JM, Gracia-Lor E, Sancho JV, López F J, Hernández F. Application of ultra-high-
411 pressure liquid chromatography - tandem mass spectrometry to the determination of
412 multi-class pesticides in environmental and wastewater samples: Study of matrix effects.
413 J. Chromatogry A 2009;1216:1410-1420.

414 Mearns AJ, Reish DJ, Oshida PS, Ginn T. Effects of pollution on marine organisms.
415 Water Environ Res 2010;82:2001-2046.

416 Muñoz I, Gómez MJ, Molina-Díaz A, Huijbregts MAJ, Fernández-Alba AR, García-
417 Calvo E. Ranking potential impacts of priority and emerging pollutants in urban
418 wastewater through life cycle impact assessment.
419 Chemosphere 2008;74:37-44.

420 Nacher-Mestre J, Serrano R, Portoles T, Hernández F, Benedito-Palos L, Pérez-Sánchez
421 J. A reliable analytical approach based on gas chromatography coupled to triple
422 quadrupole and time of flight analyzers for the determination and confirmation of

423 polycyclic aromatic hydrocarbons in complex matrices from aquaculture activities. Rapid
424 Comm Mass Spectr 2009;23:2075-2086.

425 Nacher-Mestre J, Serrano R, Portoles T, Hernández F, Benedito-Palos L, Pérez-Sánchez
426 J. Determination of PBDEs in complex matrices from aquaculture activities by GC-QqQ-
427 NCI. Comparison between NPLC and SPE clean up procedures. Anal Chim Acta
428 2010;664:190-198.

429 Niemi GJ, Devore P, Detenbeck N, Taylor D, Lima A. Overview of case studies on
430 recovery of aquatic systems from disturbance. Environ Management 1990;14:571-587.

431 Pitarch E, Medina C, Portolés T, López FJ, Hernández F. Determination of priority
432 micro-polutants in water by gas chromatography coupled to triple quadrupole mass
433 spectrometry. Anal Chim Acta 2007;583:246-258.

434 Pitarch E, Portolés T, Marín JM, Ibáñez M, Albarrán F, Hernández F. Analytical strategy
435 based on the use of liquid chromatography and gas chromatography with triple
436 quadrupole and time-of-flight MS analyzers for investigating organic contaminants in
437 wastewater. Anal Bioanal Chem 2010;39:2763-2776.

438 Portolés T, Pitarch E, López FJ, Sancho JV, Hernández F. Methodological approach for
439 the use of GC-TOF MS for screening and confirmation of organic pollutants in
440 environmental water. J Mass Spectr 2007;42:1175-1185.

441 Portolés T, Pitarch E, López FJ, Hernández F. Development and validation of a rapid and
442 wide-scope qualitative screening method for detection and identification of organic
443 pollutants in natural water and waste water by gas chromatography time-of-flight mass
444 spectrometry. J Chromatogr A 2011;1218:303-315.

445 Sancho JV, Pozo O, Hernández F. Liquid Chromatography and Tandem Mass
446 Spectrometry: a Powerful Approach for the Sensitive and Rapid Multiclass

447 Determination of Pesticides and Transformation Products in Water. The Analyst
448 2004;129:38-44.
449 Serrano R, Hernández F, López FJ, Peña JB. Study on bioconcentration of chlorpyrifos,
450 chlorfenvinfos and methidathion in Mytilus galloprovincialis. Relationships with
451 physicochemical properties and biotransformation. Bull Environ Contam Toxicol
452 1997;59:968-975.
453 Serrano R, Barreda M, Pitarch E, Hernández F. Determination of low concentrations of
454 oganochlorine pesticides and PCBs in fish feed and fish tissues from aquaculture
455 activities by gas chromatography with tandem mass espectrometry. J Separation Sci
456 2003;26:75-86.
457 Singh KP, Malik A, Mohan D, Sinha S. Multivariate statistical techniques for the
458 evaluation of spatial and temporal variations in water quality of Gomti river (India): a
459 case study. Water Res 2004;38:3980-3992.
460 Slaninova A, Smutna M, Modra H, Svoboda Z. A review: Oxidative stress in fish induced
461 by pesticides. Neuroendocrinol Letters 2009;30:2-12.
462 Soni M.G, Taylor SL, Greenberg NA, Burdock GA. Evaluation of the health aspects of
463 methyl paraben: a review of the published literature. Food Chem Toxicol 2002;40:1335-
464 1373.
465 Soni MG, Carabin IG, Burdock GA. Safety assessment of esters of p-hydroxybenzoic
466 acid (parabens). Food Chem Toxicol 2005;43,985-1015.
467 Swati M, Rema T, Kurian J. Hazardous organic compounds in urban municipal solid
468 waste from a developing country. J Hazard Mat 2008;160:213-219.

469 Terasaki M, Fukazawa H, Tani Y, Makino M. Organic pollutants in paper-recycling
470 process water discharge areas: First detection and emission in aquatic environment.
471 Environ Pollut 2008;151:53-59.

472 Ternes TA, Joss A, Siegrist H. Scrutinizing pharmaceuticals and personal care products
473 in wastewater treatment. Environ Sci Technol 2004;38**:** 392–399.

474 Tomlin CDS. The Pesticide Manual. British Crop Production Council. Fourteenth edition.
475 Norwic;2006.

476 Varo I, Taylor AC, Ferrando MD, Amat F. Effect of endosulfan pesticide on the oxygen
477 consumption rates of nauplii of different Spanish strains of *Artemia*. J Environ Sci Health
478 B 1997;32**:**363-375.

479 Varó I, Serrano R, Navarro JC, López FJ, Amat F. Acute lethal toxicity of the
480 organophosphorus pesticide chlorpyrifos to different species and strains of *Artemia*. Bull
481 Environ Contam Toxicol 1998;61:778-785.

482 Varó I, Serrano R, Pitarch E, Amat F, López FJ, Navarro JC. Toxicity and
483 biocncentration of chlorpyrifos in aquatic organisms: *Artemia parthenogenetica*
484 (Crustacea), *Gambusia affinis* and *Aphanius iberus* (Pisces). Bull Environ Contam
485 Toxicol 2000;65:623-630.

486 Varó I, Amat F, Navarro JC, Barreda M, Pitarch E, Serrano R. Assesment of the efficacy
487 of Artemia sp (Crustacea) cysts chorion as barrier to chlorpyrifos (organosphosphorus
488 pesticida) exposure. Effect on hatching and survival. Sci Total Environ 2006;366:148-
489 153.

490 Vega M, Pardo R, Barrado E, Deban L. Assessment of seasonal and polluting effects on
491 the quality of river water by exploratory data analysis. Water Res, 1996;32:3581-3592.

492 Wang JS, Simpson KL. Accumulation and depuration of DDTs in the food chain from
493 Artemia to brook trout (Salvelinus fontinalis). Bull Environ Contam Toxicol
494 1998;56**:**888-895.
495
496
497
498
499
500
501
502
503
504
505
506

Table 1. Compounds detected by GC-TOF MS and estimated concentrations (µg/L) by applying the target and non-target methodology in hypersaline water samples.

	Sampling Station 1				Sampling Station 2			Sampling Station 3	
Target-Post target analysis (µg/L)									
Sampling	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Spring**	**Summer**
Compound									
Chorpyriphos	0.011	0.026	d	-	0.010	d	d	d	d
Thiabendazole[pt]	0.078	-	-	-	-	-	-	-	-
Dichlorvos[pt]	-	d	-	-	-	-	-	-	-
Diazinon[pt]	-	2.414	0.070	0.048	-	0.068	0.034	0.154	0.032
Non target analysis									
Sampling	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Spring**	**Summer**
Compound									
Caffeine	**X**	**X**	**X**	**X**	**X**				
BHT		**X**	**X**					**X**	
Methylparaben		**X**						**X**	
Diazoxon		**X**							
N-BBS		**X**	**X**	**X**		**X**		**X**	

pt: post-target detection, d: detected at concentrations below the lowest point of the calibration plot, BHT: Butyl-hydroxytoluene, N-BSS: Butylbenzenesulfonamide

Table 2. Compounds detected by GC-TOF MS, and concentrations estimated in *Artemia* samples collected in winter

Target analysis (ng/g fresh weight)	
Compound	
pp'DDE	0.8
PCB 28	d
Simazine	81
Chlorpyrifos	6.0
Non target analysis	
Compound	**Confirmation**
BHT	X
2,6-diisopropylnaphtalene	X

Figure captions

Figure 1. Area of study

Figure 2. Identification of non target diazoxon in water sample by GC-TOF MS. (A) extracted-ion chromatograms for two diazoson ions used for deconvolution. (B) Commercial library mass spectrum of diazoxon at nominal mass. (C) Deconvoluted accurate mass spectrum of diazoxon from the water sample. (D) Library forward fit and accurate mass confirmation of four fragments; experimental accurate masses compared with theoretical exact masses (in brackets, mass errors in mDa).

Figure 3. Sum of estimated concentrations of the organic pollutants quantified (A) and number of organic contaminants detected (B) in the samplings stations along the year

Figure 4. GC-TOF MS extracted ion chromatograms at different m/z (mass window 0.02 Da) for simazine (left) and diazinon (right) detected in artemia samples and water samples, respectively. Experimental EI accurate mass spectra (bottom). Q, quantitative ion; q, confirmative ion; St, reference standard; S, sample;

✓ Q/q ratio within tolerance limits.

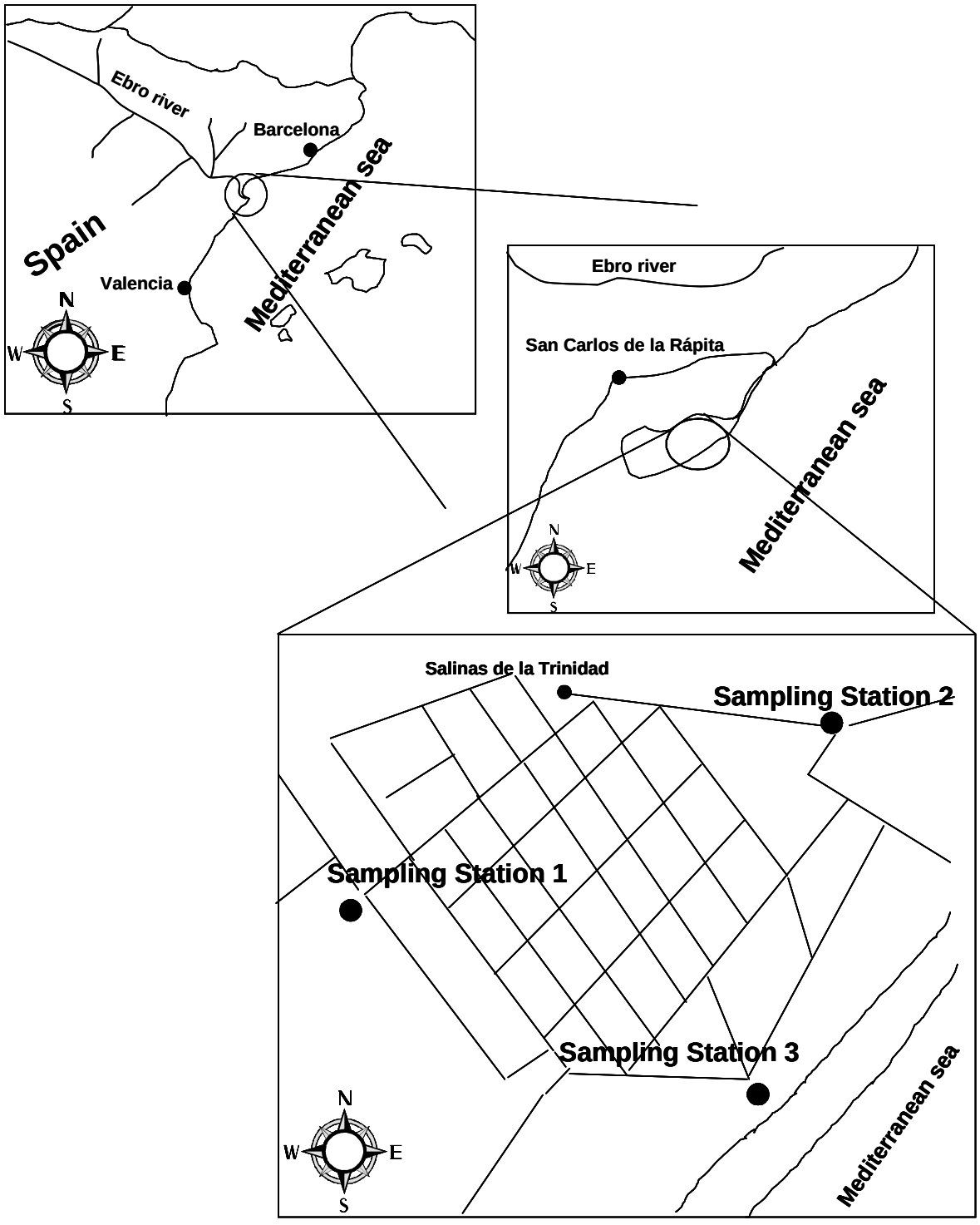
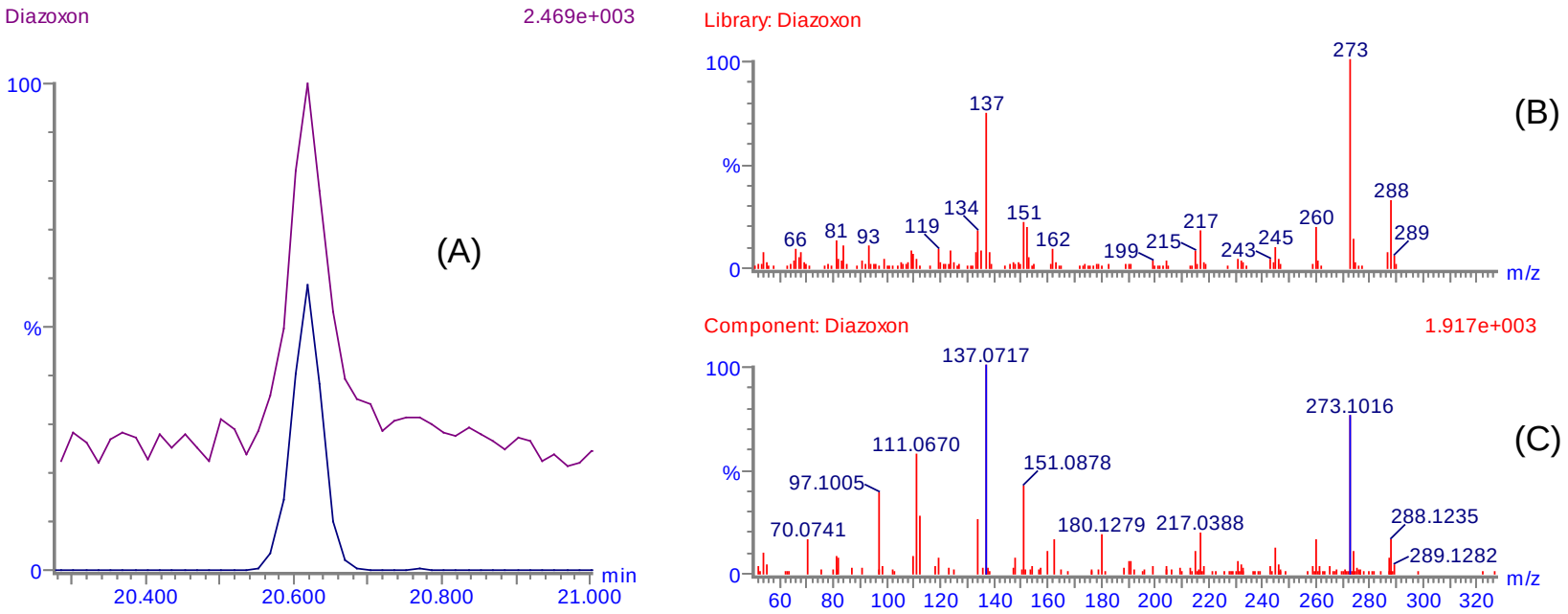

Ebro river
Barcelona
Spain
Valencia
Mediterranean sea
N
W
E
S
Ebro river
San Carlos de la Rápita
Mediterranean sea
N
W
E
S
Salinas de la Trinidad
Sampling Station 2
Sampling Station 1
Sampling Station 3
Mediterranean sea
N
W
E
S

Figure 1

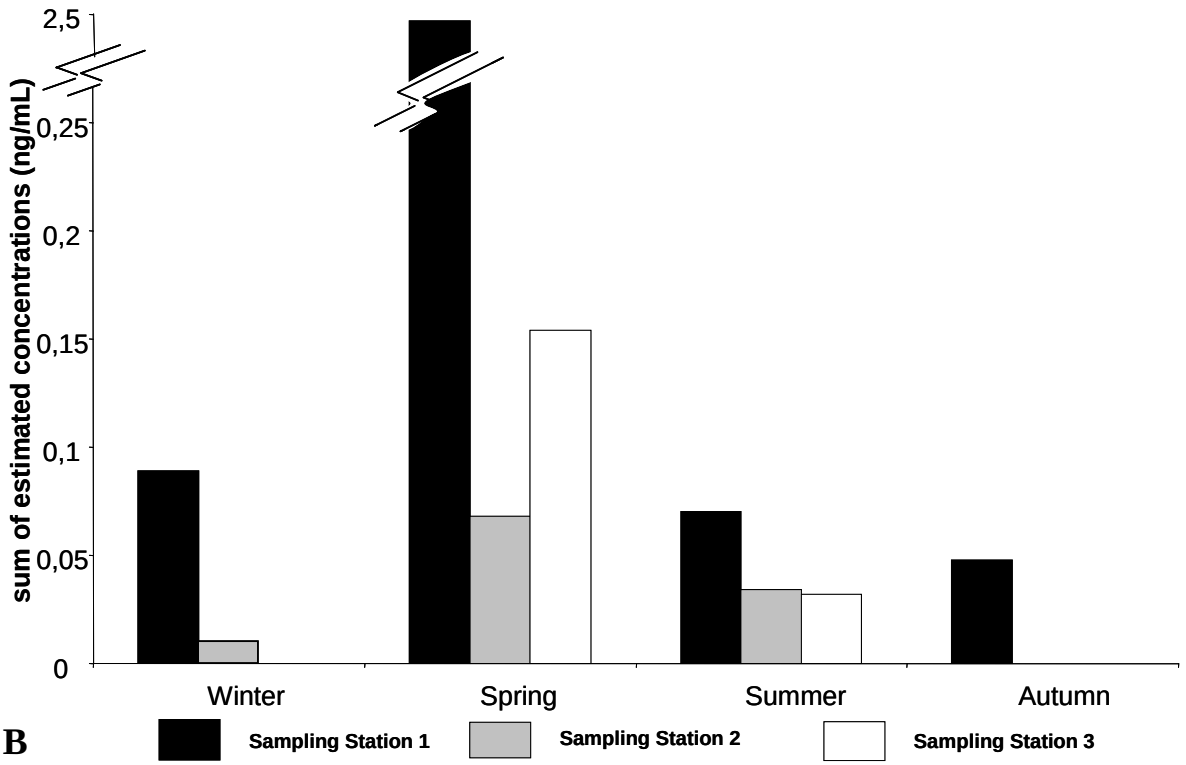


(D)

Compound	Molecular Formula	Molecular Mass	Forward Fit	m/z_1	m/z_2	m/z_3	m/z_4
Diazoxon	$C_{12}H_{21}N_2O_4S$	288.1239	718	$C_7H_9N_2O$ 137.0717 (0.2)	$C_{11}H_{18}N_2O_4P$ 273.1016 (1.2)	$C_8H_{11}N_2O$ 151.0878 (1.2)	$C_{12}H_{21}N_2O_4S$ 288.1235 (-0.4)

Figure 2

A


sum of estimated concentrations (ng/mL)
2,5
0,25
0,2
0,15
0,1
0,05
0
Winter
Spring
Summer
Autumn
Sampling Station 1
Sampling Station 2
Sampling Station 3

B

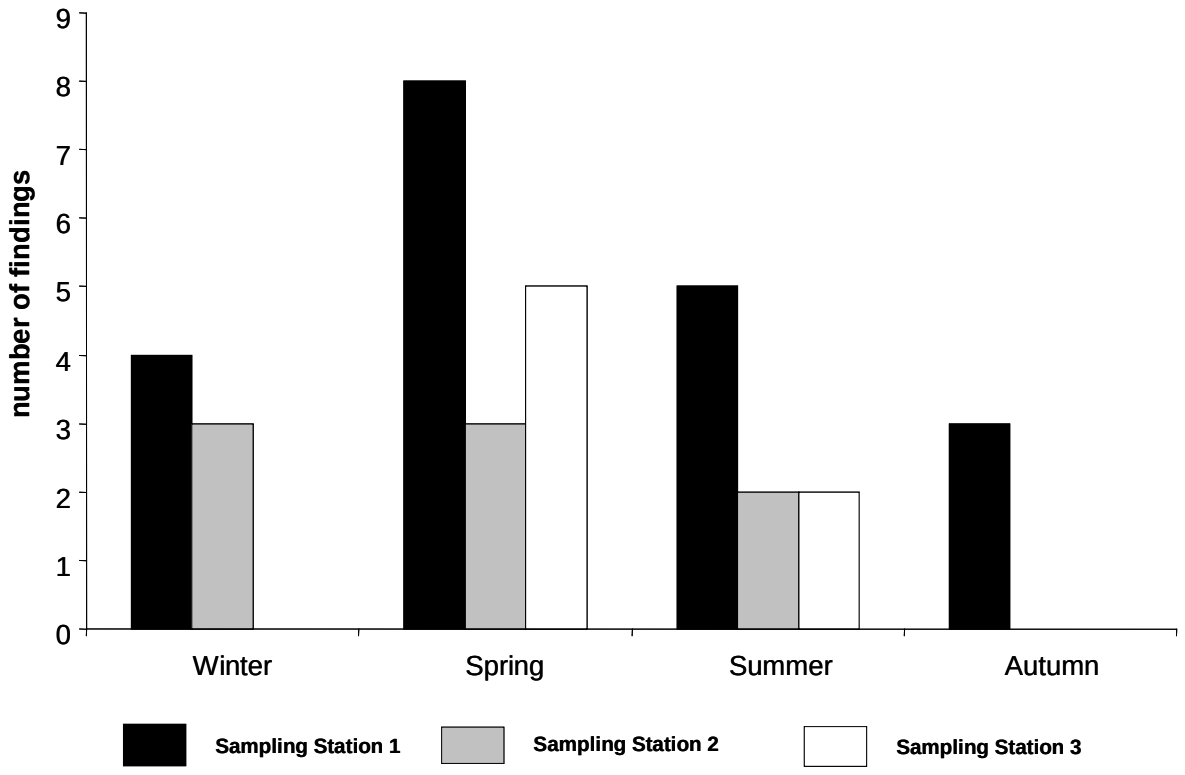

number of findings
9
8
7
6
5
4
3
2
1
0
Winter
Spring
Summer
Autumn
Sampling Station 1
Sampling Station 2
Sampling Station 3

Figure 3

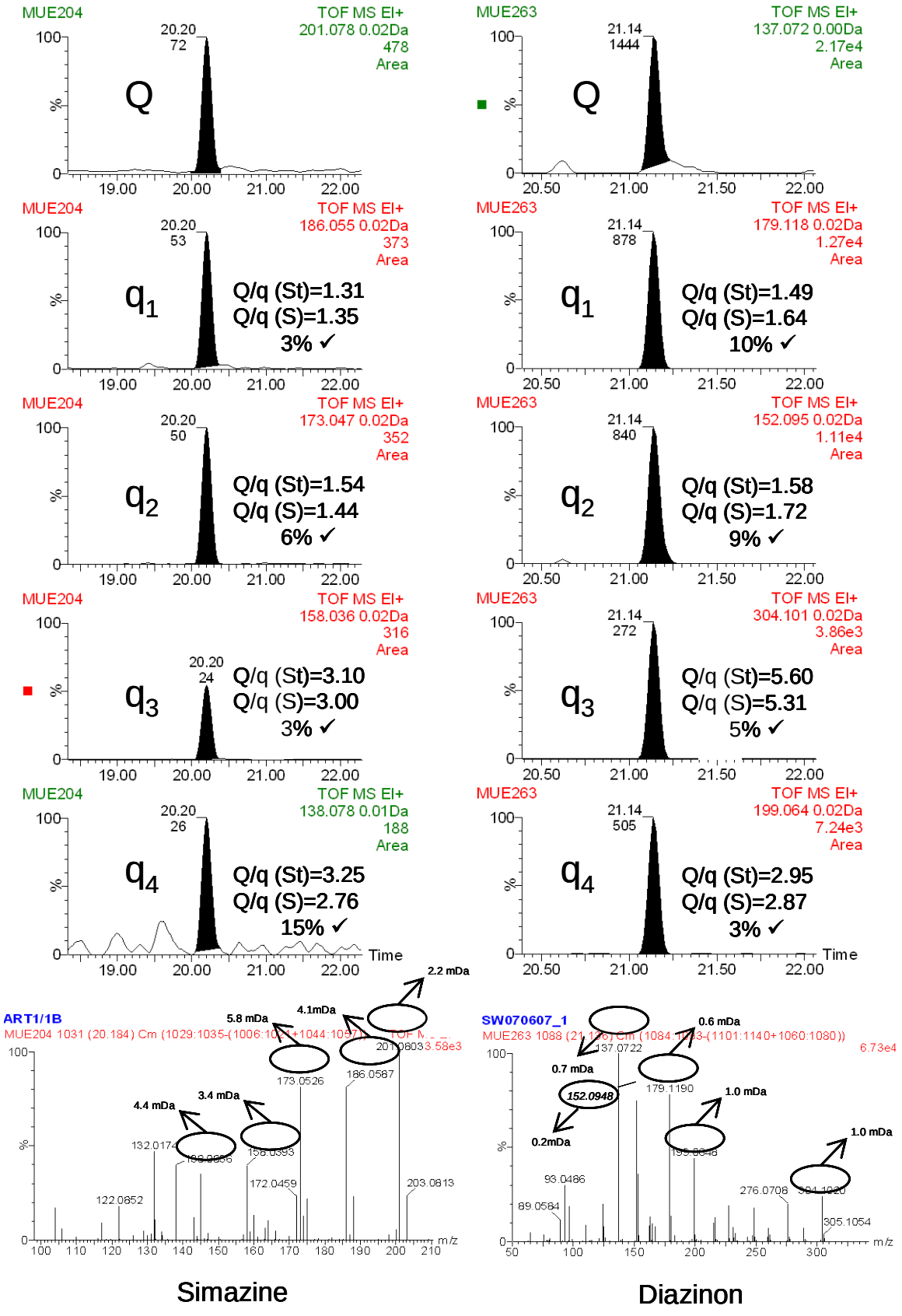

MUE204
TOF MS EI+
201.078 0.02Da
478
Area
Q
MUE263
137.072 0.00Da
2.17e4
186.055 0.02Da
373
q1
Q/q (St)=1.31
Q/q (S)=1.35
3% ✓
179.118 0.02Da
1.27e4
Q/q (St)=1.49
Q/q (S)=1.64
10% ✓
173.047 0.02Da
352
q2
Q/q (St)=1.54
Q/q (S)=1.44
6% ✓
152.095 0.02Da
1.11e4
Q/q (St)=1.58
Q/q (S)=1.72
9% ✓
158.036 0.02Da
316
q3
Q/q (St)=3.10
Q/q (S)=3.00
3% ✓
304.101 0.02Da
3.86e3
Q/q (St)=5.60
Q/q (S)=5.31
5% ✓
138.078 0.01Da
188
q4
Q/q (St)=3.25
Q/q (S)=2.76
15% ✓
199.064 0.02Da
7.24e3
Q/q (St)=2.95
Q/q (S)=2.87
3% ✓
Time
ART1/1B
SW070607_1
Simazine
Diazinon

Figure 4