

1 Characterization of the organic contamination pattern of a hyper-saline 2 ecosystem by rapid screening using gas chromatography coupled to 3 high-resolution time-of-flight mass spectrometry

4
5 **Roque Serrano***, Tania Portolés, Miguel A. Blanes, Félix Hernández

6 ^aResearch Institute for Pesticides and Water (IUPA). Avda Sos Baynat, s/n. University Jaume I,
7 12071 Castellón, Spain

8 **Juan C. Navarro, Inmaculada Varó, Francisco Amat**

9 ^bInstitute of Aquaculture of Torre la Sal (IATS), C.S.I.C., 12595 Ribera de Cabanes, Castellón,
10 Spain

11 12 **Abstract**

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

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

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

35
36 **Keywords:** Gas Chromatography, Mass Spectrometry, Time-of-Flight, Screening, hyper-saline
37 ecosystem, organic contaminants

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

39 **Introduction**

40 Contamination of natural waters is a problem worldwide, which can have
41 important consequences for the health of both man and animals. Human activities are the
42 main factor affecting the quality of surface and groundwater through atmospheric
43 pollution, effluent discharges, eroded soils or land use. Anthropogenic effluent discharges
44 constitute one of the more important polluting sources, whereas surface runoff is
45 normally a seasonal phenomenon, able to transport a variety of pollutants (Vega et al.,
46 1996; Singh et al., 2004; Kazi et al., 2009). Particularly, the use of agricultural chemicals
47 affects the quality of natural waters (Niemi et al., 1990; Pitarch et al., 2007). Run-off
48 from agricultural soils may contain high concentrations of herbicides, insecticides and
49 other types of pesticides during application periods along the year. Thus, pesticides can
50 become a serious environmental problem, as they may have a deleterious impact on the
51 aquatic biota (Harmon et al., 2010; Mearns et al., 2010), given that many of them are
52 toxic to non target organisms, including fish (Varó et al., 2000; Slaninova et al, 2009) and
53 aquatic invertebrates (Serrano et al. 1997; Varó et al. 1998; Varó et al., 2000). Given the
54 above mentioned negative impacts, the application of reliable and rapid multi-residue
55 methods is required for monitoring of the presence of the large variety of contaminants
56 that can be potentially present in vulnerable aquatic ecosystems. The European
57 framework for water policies set up in the European Directive 2000/60/EC encourages
58 the Members States to carry out periodical trend studies on different water pollutants
59 (Annexe V Section 2.4.4). In such context, the application of wide-scope screening
60 procedures, able to detect the highest number of analytes as possible, is one of the most
61 convenient approaches.

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

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

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

81 In this work, the objective was to make use of the GC-TOF MS potential for the
82 rapid and large-scope screening of organic pollutants in a hypersaline natural protected
83 area sited in the Ebro river delta (Spain). Samples of brines with salinities from 112 g/L
84 to saturation were collected along one year from three saltworks in order to know the
85 seasonal pollutants dynamics. Likewise, samples from *Artemia* populations living in
86 these brines were collected to search for pollutants bioaccumulated. Genus *Artemia*
87 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 hm³/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 Mediterranean 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 salt pans are the refuge 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

112 high (higher than 100 g L⁻¹). It was sampled three times along the year. Station 3 (40° 34'
113 38'' N 0° 41' 43'' E) is a small pond submitted to notable environmental fluctuations,
114 separated by a sand bar located next to the open sea. The water body of Station 3 was
115 highly dependent on seasonal and antropic fluctuations and it was sampled only twice
116 along the year.

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

120 *Artemia* biomass samples were collected from Station 1 with a 160 µm mesh and
121 transported alive to the laboratory where they were further rinsed with water and stored at
122 -20°C until analysis. *Artemia* populations were present the whole year round. All
123 specimens collected were classified as *Artemia franciscana*, an alochthonous species
124 from North America introduced in saltmarsh from all around the world mainly due to its
125 widespread use as larval food in aquaculture. This species out-competes autochthonous
126 *Artemia* populations, replacing them due to its superior environmental fitness (Amat et al,
127 2005).

128 *Analytical procedure*

129 *Brine samples*

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

136 consisted on using 100 mL of sample that were passed through a C₁₈ SPE cartridge. The
137 elution was performed by passing 5 mL of ethyl acetate:DCM (50:50). The eluate was
138 evaporated under a gentle nitrogen stream at 40 °C and redissolved in 0.5 mL of hexane
139 before injection into the GC-TOF MS system.

140 *Artemia samples*

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

156 *GC-TOF MS analysis*

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

160 GC separation was performed using a fused-silica HP-5MS capillary column with a
161 length of 30 m x 0.25 mm i.d. and a film thickness of 0.25 μm (J&W Scientific, Folsom,
162 CA). injection volume was 2 μL . The oven temperature was programmed as follows: 90
163 $^{\circ}\text{C}$ (6 min); 5 $^{\circ}\text{C}/\text{min}$ to 300 $^{\circ}\text{C}$ (2 min). Helium was used as carrier gas at 1 mL/ min.
164 The interface and source temperatures were set to 250 $^{\circ}\text{C}$ for all analytes studied, and a
165 solvent delay of 3 min was selected. The time-of-flight mass spectrometer was operated
166 at 1 spectrum/s, acquisition rate over the mass range m/z 50-650, using a multichannel
167 plate voltage of 2500 V. TOF-MS resolution was approximately 7000 (FWHM).
168 Heptacosane, used for the daily mass calibration and as lock mass, was injected via syringe
169 in the reference reservoir at 30 $^{\circ}\text{C}$ for this purpose; the m/z ion monitored was 218.9856.
170 The application manager TargetLynx, a module of MassLynx software, was used to
171 process the qualitative and quantitative data obtained from standards and samples for
172 target compounds. The application manager ChromaLynx, also a module of MassLynx
173 software, was used to investigate the presence of non target compounds in samples.
174 Library search was performed, when required, using the commercial NIST library.

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

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

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

187

188 **Results and Discussion**

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

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

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

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

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

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

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

235 *Compounds detected in brine samples*

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

241 Regarding to the eco-toxicological implications, most of the analytes found were
242 organophosphorous pesticides (chlorpyrifos, thiabendazole, dichlorvos and diazinon).
243 Particularly, the insecticide chlorpyrifos [O,O – diethyl – O - (3,5,6-trichloro-2-pyridil)
244 phosphorothiate] is an organophosphorous pesticide widely used in the countries of the
245 European Union (Varó et al., 1998, 2000, 2006), and has been frequently detected in
246 surface waters of the Ebro river (Claver et al., 2006). This pesticide has been studied by
247 several authors as a model organophosphorus pesticide, demonstrating its high toxicity
248 and bioconcentration ability in different groups of aquatic organisms (Serrano et al.,
249 1997, Varó et al., 1998). Other organophosphorous compounds detected were the post-
250 harvest pesticides thiabendazole, diazinon (and its derivative diazoxon) and dichlorvos,
251 that show moderate acute toxicity to non-target organisms, inhibiting the acetyl-
252 cholinesterase activity (Hernández et al, 1998, Tomlin, 2006). All of them are widely
253 used in agricultural activities and are frequently detected in natural waters (Ibañez et al.,
254 2008).

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

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

280 Figure 3 shows the total concentration estimated for pollutants in each sampling
281 station along the year and the number of findings. As it can be observed, spring is the
282 season with higher load of contaminants in water coinciding, as expected, with the
283 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 compound in 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 lipophilicity. These compounds seem 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

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

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

316 In the light of the results obtained here, it seems that *Artemia* populations
317 inhabiting the area of study are exposed to a variety of contaminants, which may produce
318 an appreciable toxic stress affecting their survival. This exposure to toxicants can act as
319 one of the forces driving the success of the alochthonous *Artemia franciscana* over the
320 *Artemia parthenogenetica* and *Artemia salina* autochthonous species. Varo et al. (1997,
321 1998) reported that different *Artemia* strains show different degree of sensitivity to
322 toxicants, including organophosphorus and organochlorine compounds, and *Artemia*
323 *franciscana* is one of the most resistant forms.

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

330

331 **Acknowledgments**

332 This research was supported by the Spanish Ministry for Education and Science project
333 (CGL2005-02306/BOS) “Biodiversidad de *Artemia* (*Branchiopoda*, *Anostraca*) en el
334 Mediterráneo occidental. Presencia de *A. franciscana* como especie invasora.
335 Implicaciones ecológicas y de interés en acuicultura”. The authors addressed in IUPA
336 acknowledge the financial support of Generalitat Valenciana, as research group of
337 excellence PROMETEO/2009/054. The authors also are very grateful to the Serveis
338 Centrals d’Instrumentació Científica (SCIC) of University Jaume I for use of Agilent
339 6890N GC system coupled to a GCT time-of-flight mass spectrometer.

340

341

342

343

344

345

346

347

348

349

350

351

352

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 Ibáñez 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-pollutants 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 organochlorine pesticides and PCBs in fish feed and fish tissues from aquaculture
455 activities by gas chromatography with tandem mass spectrometry. *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 Norwich;2006.

476 Varó 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 bioconcentration 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. Assessment of the efficacy
487 of *Artemia* sp (Crustacea) cysts chorion as barrier to chlorpyrifos (organophosphorus
488 pesticide) 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

507
508
509
510
511

Table 1. Compounds detected by GC-TOF MS and estimated concentrations ($\mu\text{g/L}$) 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 ($\mu\text{g/L}$)									
Sampling Compound	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Spring	Summer
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 Compound	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Spring	Summer
Caffeine	X	X	X	X	X				
BHT		X	X					X	
Methylparaben		X						X	
Diazoxon		X							
N-BBS		X	X	X		X		X	

512 *pt: post-target detection, d: detected at concentrations below the lowest point of the calibration plot, BHT: Butyl-hydroxytoluene, N-BSS:*
513 *Butylbenzenesulfonamide*
514
515
516
517
518
519
520
521

522
523
524
525
526
527

528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558

Table 2. Compounds detected by GC-TOF MS, and concentrations estimated in

Artemia samples collected in winter

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

559 **Figure captions**

560

561 **Figure 1.** Area of study

562

563

564

565 **Figure 2.** Identification of non target diazoxon in water sample by GC-TOF MS. (A)

566 extracted-ion chromatograms for two diazoxon ions used for deconvolution.

567 (B) Commercial library mass spectrum of diazoxon at nominal mass. (C)

568 Deconvoluted accurate mass spectrum of diazoxon from the water sample.

569 (D) Library forward fit and accurate mass confirmation of four fragments;

570 experimental accurate masses compared with theoretical exact masses (in

571 brackets, mass errors in mDa).

572

573

574 **Figure 3.** Sum of estimated concentrations of the organic pollutants quantified (A) and

575 number of organic contaminants detected (B) in the samplings stations along

576 the year

577

578

579

580

581 **Figure 4.** GC-TOF MS extracted ion chromatograms at different m/z (mass window

582 0.02 Da) for simazine (left) and diazinon (right) detected in artemia samples

583 and water samples, respectively. Experimental EI accurate mass spectra

584 (bottom). Q, quantitative ion; q, confirmative ion; St, reference standard; S,

585 sample;

586 ✓ Q/q ratio within tolerance limits.

587

588

589

590

591

592

593

594

595

596

597

598

599

600

601

602

603

604

605

606

607

608

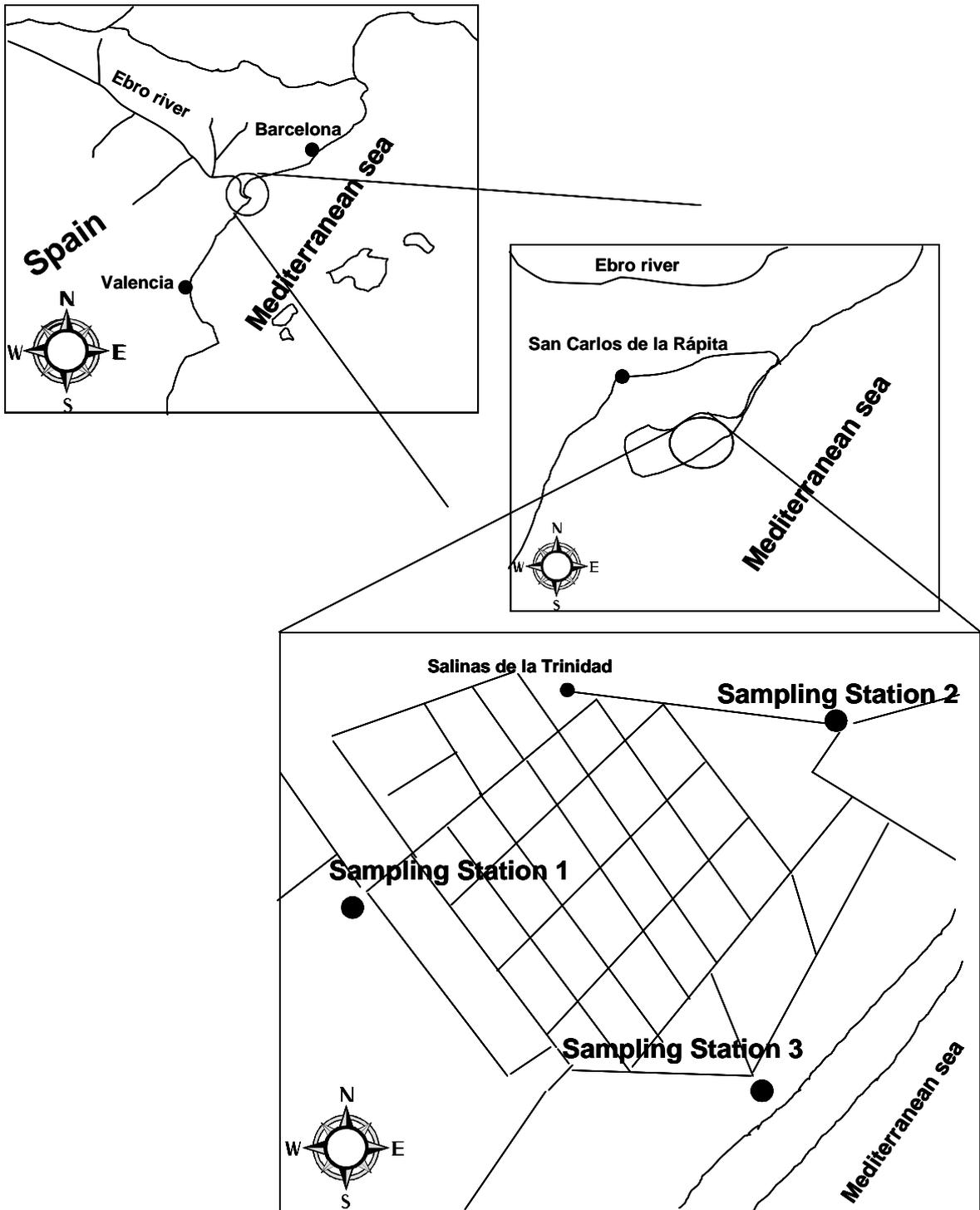
609

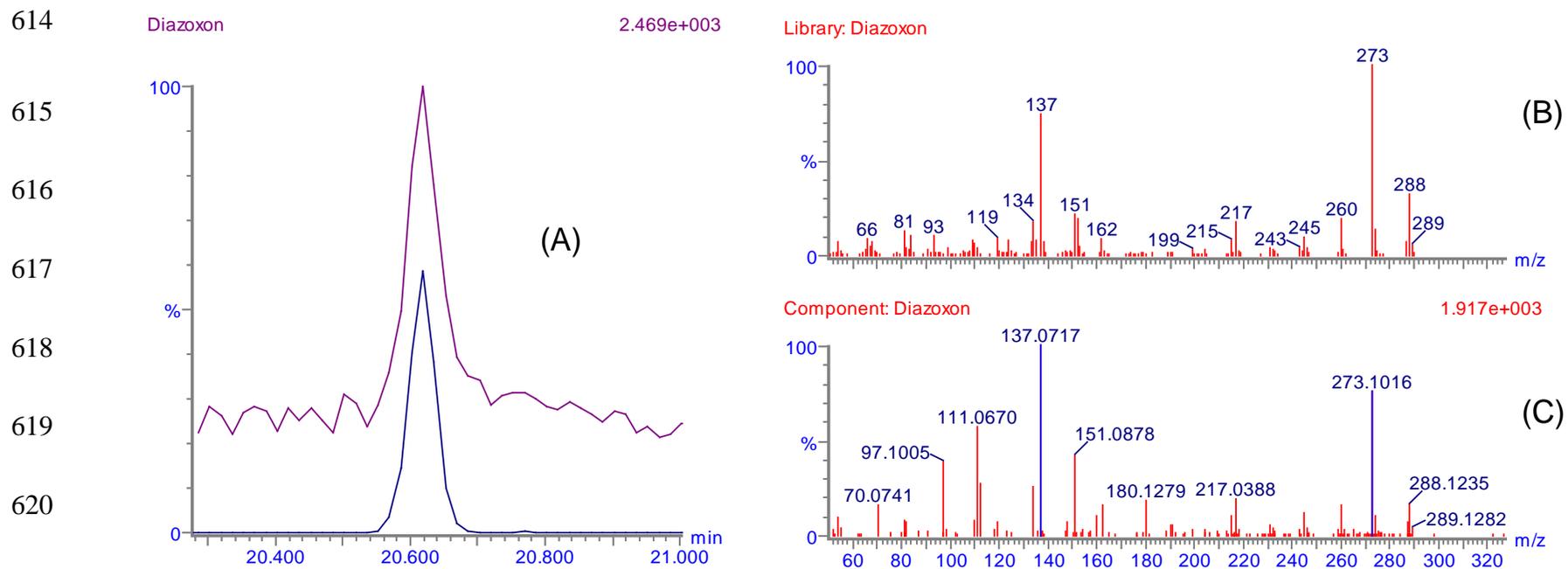
610

611

612 Figure 1

613



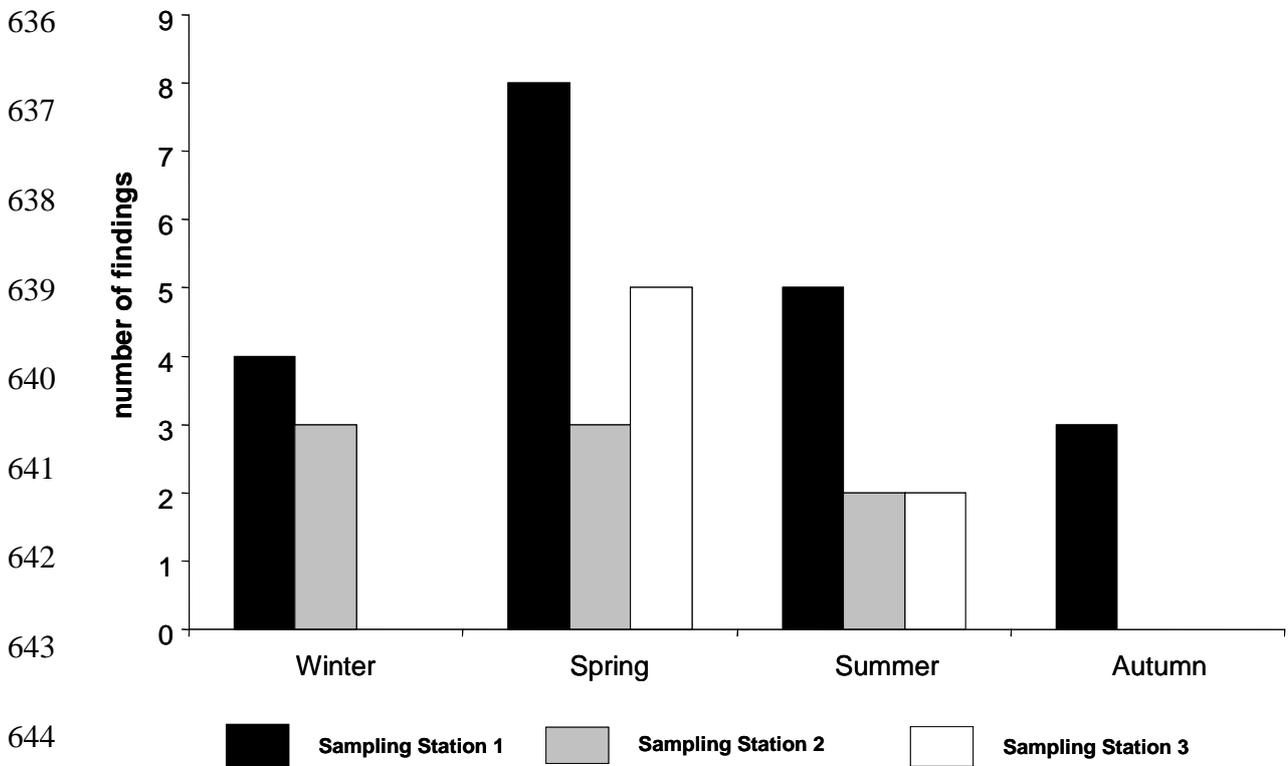
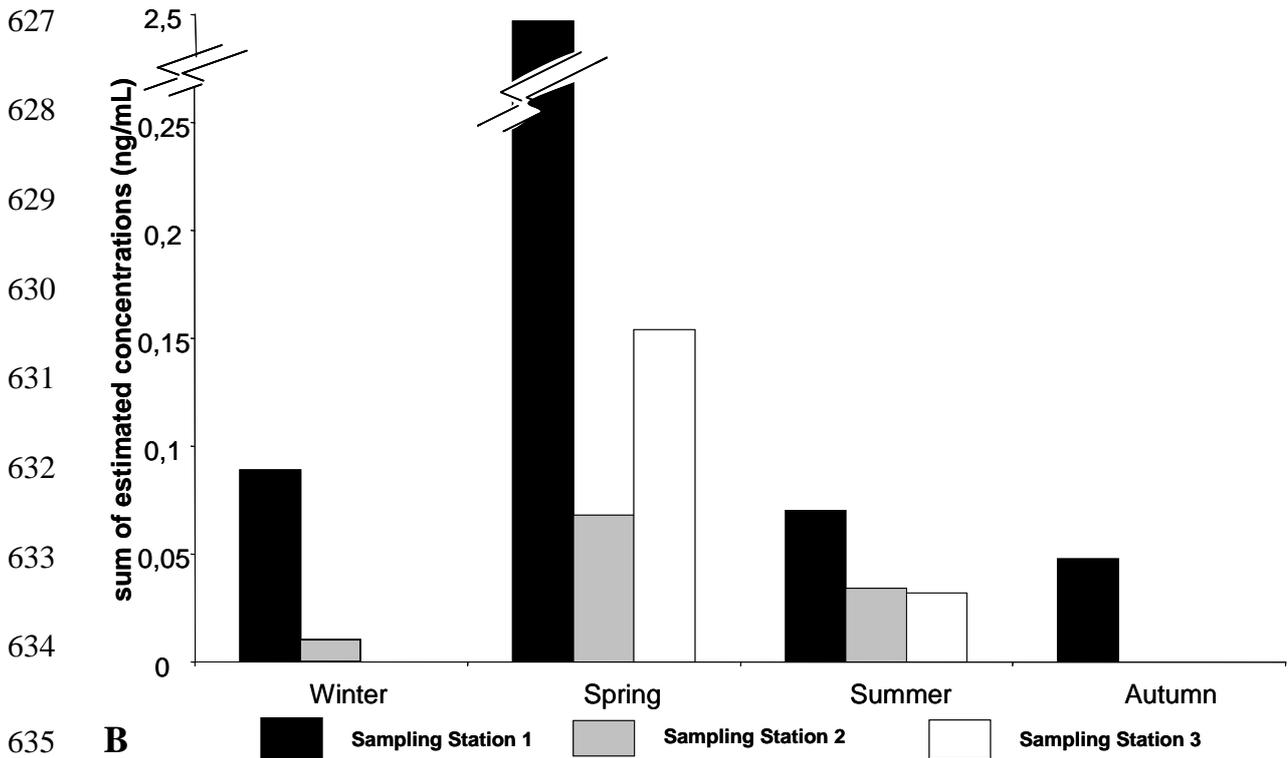


(D)

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 ₂ O ₄ S	288.1239	718	C ₇ H ₉ N ₂ O 137.0717 (0.2)	C ₁₁ H ₁₈ N ₂ O ₄ P 273.1016 (1.2)	C ₈ H ₁₁ N ₂ O 151.0878 (1.2)	C ₁₂ H ₂₁ N ₂ O ₄ S 288.1235 (-0.4)

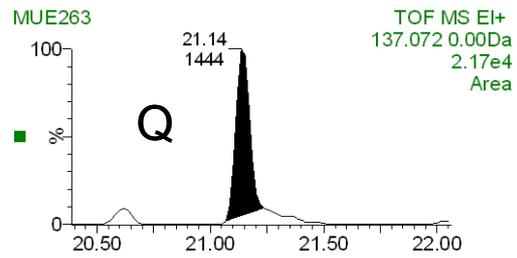
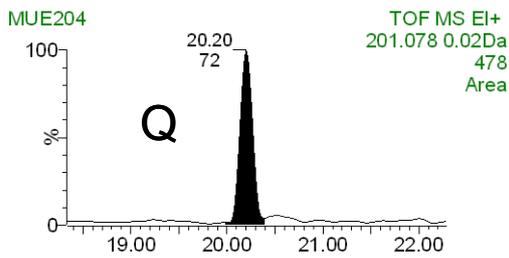
625 Figure 2

626 **A**



646 Figure 3

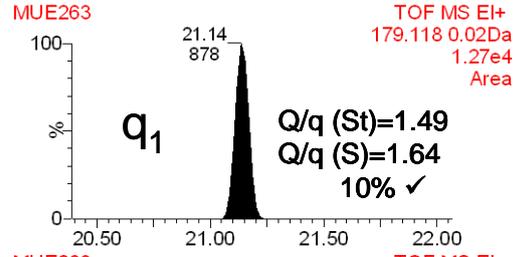
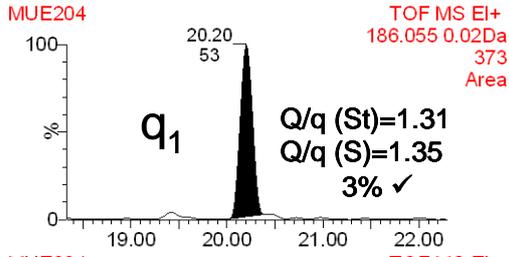
647



648

649

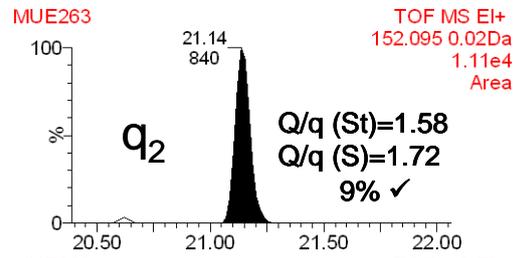
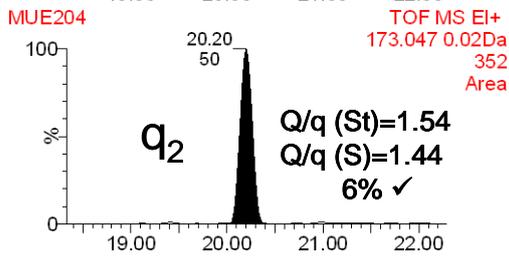
650



651

652

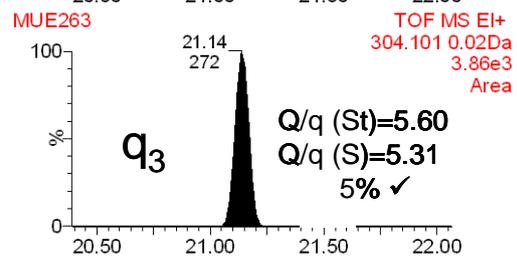
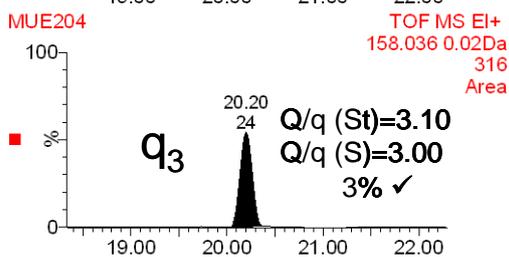
653



654

655

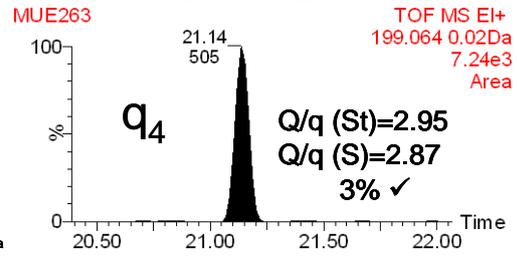
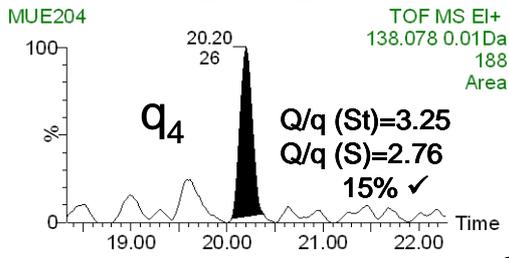
656



657

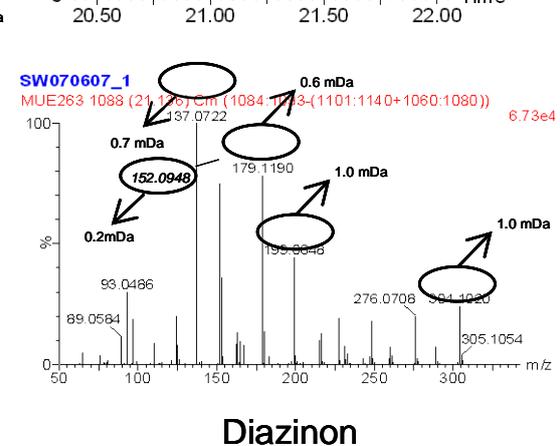
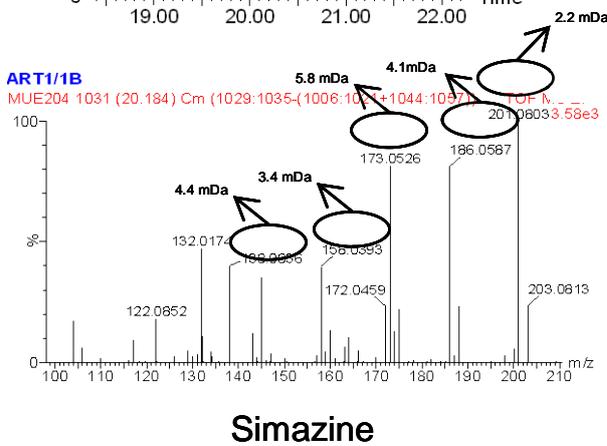
658

659



660

661



662

663

664

665

666

667 Figure 4