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1 **Analytical study on ethephon residue determination in water by ion-pairing liquid**
2 **chromatography/tandem mass spectrometry**

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35 **ABSTRACT**
36 A detailed analytical study on ethephon residue determination in water, making use of ion-pairing
37 liquid chromatography coupled to electrospray tandem mass spectrometry (LC/MS/MS), has been
38 carried out. Ethephon is a plant growth regulator, highly polar, which is typically present in aqueous
39 solution in anionic form due to its acid character. Both, its extraction and preconcentration from
40 water samples and its chromatographic retention are difficult. Several approaches for sample
41 pretreatment have been tested including direct injection into the chromatographic system, on-line
42 solid phase extraction (SPE) and off-line SPE, with the best results being obtained after off-line
43 SPE, using Oasis MAX cartridges (mixed-mode strong anion-exchange). After testing several ion-
44 pairing reagents, tetrabuthylammonium acetate (TBA) was selected. This was added to the samples
45 before LC/MS/MS analysis to facilitate ethephon chromatographic retention. The acquisition of
46 several specific MS/MS transitions together with the evaluation of their relative intensity ratios
47 allowed the reliable confirmation of the analyte in samples. The optimized approach was tested in
48 low-salinity water spiked at 0.1 µg/L level with satisfactory recovery, and a limit of detection of
49 0.02 µg/L. To this aim, the water sample was partially de-ionized in an initial stage, in order to
50 remove major ions that would have interfered in analyses. The application of this methodology to
51 more saline/complex water samples, as surface or wastewater, was problematic and a thorough
52 optimization of the de-ionization conditions would be required.
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66 **Keywords:** Ethephon, ion-pairing liquid chromatography, tandem mass spectrometry,
67 tetrabuthylammonium, water analysis.

## 1. Introduction

Ethephon (2-chloroethylphosphonic acid) is the common name of a plant growth and maturity regulator with systemic properties, which it is also used as a ripening accelerator in the post-harvest of fruit and vegetables. Its mode of action is via liberation of ethylene (its active metabolite) which is absorbed by the plant and interferes in the growth process, including seed germination, fruit maturation, flower wilt, etc. This compound is stable in aqueous solutions below pH 4-4.5, and its rate of degradation to ethylene, phosphate and chlorine ion increases with pH and temperature [1]. Ethephon can easily reach ground and surface waters as a result of its highly polar and hydrophilic nature. Therefore, it is crucial to develop reliable and sensitive analytical methodology capable of determining ethephon at sub-ppb levels in water to be in compliance of European regulations on water quality [2].

Most of reported methods for ethephon residues are based on their indirect determination by the analysis of liberated ethylene under basic conditions and/or high temperature. These methods are usually based on headspace/gas chromatography both for vegetable samples, using Flame Ionization Detector (FID) [3-5] and drinking water [6]. Despite acceptable detection limits are achieved (between 0.01 and 0.1 mg/Kg), indirect methods are poorly reproducible, time-consuming and unspecific. Besides, for monitoring purposes the relevant residues of ethephon consist of the sole parent compound. Ethephon residues can not be determined by commonly used multiresidue methods, mainly due to its high polarity and acidic character, which lead this compound to be present in aqueous samples as its anionic form. Thus, there is a need of modern analytical methodology able to accurately determine ethephon in water at sub-ppb levels.

Only a few studies have been reported on direct determination of ethephon residues in fruit and vegetables. A methodology based on the use of microcolumn liquid chromatography and capillary electrophoresis (CE) coupled to flame photometric detector (μLC/FPD and CE/FPD) has been reported, making use of large volume injection (LVI) in order to enhance limit of detection and minimize interferences [7]. Another work based on GC/MS with previous extraction followed by SPE cleanup was described by Takenaka [8]. Both methods resulted in very laborious multi-stage procedures.

More recently, Royer *et al* [9] have developed a procedure for the determination of ethephon in drinking and surface water by GC/MS[3] with ion-trap analyzer, based on a previous de-ionization with an anion/cation-exchange resin followed by SPE using anion-exchange extraction disks and redisolution of the eluate into acetonitrile after evaporation and silylation with MTBSTFA. The method allows to reach a limit of quantification of 0.1 μg/L. The need of applying a multistage

105 procedure with lot of sample manipulation illustrates the analytical difficulties associated to this
106 problematic analyte. The result is that the method applied turns out extensive, complex and involves
107 much time to ensure a reliable quantification of the compound in water. Another method has been
108 proposed based on ion chromatography/inductively coupled plasma mass spectrometry for the
109 simultaneous determination of ethephon and three more polar herbicides [10]. This method proved
110 to be simple and rapid, but their sensitivity was unsatisfactory with a limit of detection of 1.4 μg/L,
111 as could be expected from the technique employed, not the most appropriate for pesticide residue
112 analysis (PRA).

113

114 In recent years, LC in combination with tandem mass spectrometry (LC/MS/MS) has
115 become a powerful tool in PRA. The excellent selectivity and sensitivity reached in selected
116 reaction monitoring (SRM) mode makes it an ideal technique for determining most of the polar
117 and/or ionic contaminants in environmental waters at low detection levels [11]. LC/MS/MS has
118 played an important role in analyzing modern pesticides, which are less persistent, low volatile as
119 well as more polar than old ones [12,13] together with their transformation products (TPs) [13-15].
120 Despite the high sensitivity of this technique a preconcentration step in normally required, e.g.
121 using SPE [12-16] or LLE [15-17], in order to meet water regulation requirements.

122

123 Regarding the acidic character of ethephon, its deprotonated anionic form in found to be
124 difficult to retain in the most commonly applied reversed-phase LC columns. Thus, ion-pairing
125 chromatography is an appropriate approach for increasing the retention of ionic compounds like
126 ethephon [18-21]. Ion-pairing reagents used for anionic analytes generally have a positively charged
127 quaternary nitrogen with a bulky hydrophobic part that contains alkyls with 4-18 carbon atoms (e.g.
128 tetrabuthylammonium or hexadecyltrimethylammonium) in order to favor the retention of the
129 negatively charged analyte when applying reversed-phase LC approach [21,22]. In our research
130 group, we have developed a rapid, sensitive and selective method for the determination of ethephon
131 residues in vegetables (apple, cherry, tomato) based on ion-pairing LC/MS/MS using
132 tetrabuthylammonium as ion-pairing reagent [22]. The aim of the present work is to investigate the
133 potential of this approach, which gave excellent results in fruits and vegetables, for the direct
134 determination of ethephon residues in water, with special attention to the unequivocal confirmation
135 of positive samples.

136

137 **2. Experimental**

138 ***2.1. Reagents and Chemicals***

139

140 The ethephon reference standard (98.5%) was purchased from Dr. Ehrenstorfer (Augsburg,
141 Germany). Tetrabuthylammonium acetate (TBA, 97%), tetradecyltrimethylammonium bromide
142 (TDTA, ≥99%) and tetraoctylammonium bromide (TOA, ≥99%) were obtained from Sigma-
143 Aldrich (St. Louis, MO, USA). The AG 501-X8 anion/cation-exchange mixed bed resin was
144 purchased from Bio-Rad Laboratories (Hercules, CA, USA). Reagent-grade formic acid (>98%),
145 acetic acid (>99%), ammonium acetate (98%), sodium chloride (99.8%), hydrochloric acid (35%),
146 acetone for residue analysis, HPLC-grade acetonitrile and HPLC-grade methanol were supplied by
147 Scharlab (Barcelona, Spain). HPLC-grade water was obtained by purifying demineralized water in a
148 Milli-Q Gradient A10 system (Millipore, Bedford, MA, USA).

149 The stock standard solution of ethephon was prepared by dissolving around 50 mg powder,
150 accurately weighed, in 100 mL of acetone obtaining a final concentration of 500 mg/L, and stored
151 in a freezer at < -18 ºC. Working solutions were prepared from stock solution by dilution in
152 acetonitrile for concentrations higher than 5 mg/L, and using aqueous formic acid (pH 3) for lower
153 concentrations. The working standards were stored at 4 ºC.

154 TBA was prepared by dissolving 7.77 g of reagent in 50 mL of HPLC-grade water obtaining
155 a final concentration of 500 mM. Aqueous formic acid (pH 3) was prepared by dilution of 5 mL of
156 10% formic acid in 500 mL of HPLC-grade water.

157 TOA and TDTA individual solutions were prepared by diluting 1.36 g and 0.84 g
158 respectively, in 2.5 mL of MeOH resulting in a final concentration of 1 M.

159

160 ***2.2. Instrumentation***

161

162 A Quattro Micro triple quadrupole mass spectrometer (Waters, Milford, MA, USA) was
163 interfaced using an orthogonal Z-spray-electrospray ion source to an HPLC system based on a
164 Waters Alliance 2695 (Waters) quaternary pump used for the chromatographic separation. Nitrogen
165 generated from pressurized air in a high-purity nitrogen generator (NM30LA 230Vac Gas Station
166 from Peak Scientific, Inchinnan, UK) was employed as drying and nebulising gas. The cone gas and
167 the desolvation gas flows were set to approximately 60 L/h and 600 L/h, respectively. For operation
168 in MS/MS mode, collision gas was Argon 99.995% (Praxair, Valencia, Spain) with a pressure of
169 approximately $1\times10^{-4}$ mbar in the collision cell. Electrospray needle capillary voltage of 3.2 kV
170 was selected in negative ionization mode. The desolvation temperature was set to 350 ºC and the
171 source temperature to 120 ºC. Infusion experiments were performed using the built-in syringe pump
172 directly connected to the ion source at a flow rate of 10 µL/min. Dwell time of 300 ms was chosen.

173 A solvent delay of 7.5 min was selected to give an additional clean-up using the built-in divert valve
174 controlled by the Masslynx NT v 4.0 software (Waters).

175 Cartridges used for off-line SPE experiments were Oasis HLB (60 mg) and Oasis MAX (60
176 and 150 mg), from Waters. For on-line experiments, $C_{18}$ and polymeric phase Hamilton (PRP) (both
177 10 × 2 mm, 10 µm; Teknokroma, Barcelona, Spain) and Oasis HLB (20 × 2.1 mm, 25 µm; Waters)
178 cartridges were checked.

179 LC columns tested for chromatographic separation were: Discovery $C_{18}$ (50 × 2.1 mm, 5
180 µm; Sigma); Sunfire $C_{18}$ (50 × 2.1 mm, 5 µm; Waters), Mediterranea $SEA_{18}$ (50 × 2.1 mm, 5 µm;
181 Teknokroma) as well as Acquity UPLC HSS T3 (50 mm × 2.1mm, 1.8 µm; Waters) for UHPLC
182 analysis.

183 Masslynx NT v 4.0 (Waters) software was used to process the quantitative data obtained
184 from calibration standards and from water samples.

185

186 ***2.3. Procedure***

187 Water samples (100 mL) were de-ionized by adding 0.1 g AG 501-X8 resin, stirring
188 strongly for 10 min by using a magnetic bar. Then, samples were loaded onto an Oasis MAX
189 cartridge (150 mg, 6 mL), previously conditioned by passing 6 mL 2% HCl in methanol, 6 mL
190 methanol and 6 mL HPLC water. After loading the sample, the cartridge was dried by passing air
191 using vacuum for at least 20 min. The elution was performed with 1 mL 2% HCl in methanol and
192 the extract was diluted with HPLC water up to a final volume of 5 mL. An aliquot of 880 µL of the
193 final extract was transferred to a 2 mL-vial, which contained 120 µL 500 mM TBA solution (giving
194 a final concentration of 60 mM in TBA). Finally, 100 µL were directly injected into the
195 LC(ESI)MS/MS system, employing a Mediterranea $SEA_{18}$ column (50 × 2.1 mm i.d., 5 µm) for
196 chromatographic separation. A binary water/methanol gradient elution was applied changing
197 linearly the percentage of methanol as follows: 0 min, 10%; 1 min 10%; 6 min, 50%; 7 min, 50%; 8
198 min, 10%; 10 min, 10%. The flow rate was kept at 0.2 mL/min and the chromatographic run time
199 was 15 min. The selection of the mobile phase was based on our previous work [22], where water
200 and methanol without any additive gave the best results in terms of peak shape and sensitivity.

201 Calibration was carried out in the range 0.5–50.0 µg/L, from standards prepared in water
202 acidified at pH 3 (formic acid) by adding 880 µL of each standard solution into a vial containing
203 120 µL 500 mM TBA solution.

204 LC/MS/MS analysis was performed acquiring five MS/MS transitions; *m/z* 107>79 for
205 quantification (Q) and *m/z* 143>107 ($q_1$), 143>79 ($q_2$), 145>107 ($q_3$) and 145>79 ($q_4$) for
206 confirmation. Confirmation of the identity of ethephon was carried out by comparison of Q/q ratios
207 between standards and samples.

208

209 **3. Results and discussion**

210 ***3.1. MS optimization***

211

212 The negative electrospray full-scan spectra of ethephon was obtained by infusion of 2.5
213 µg/mL standard solution in acetonitrile:water (50:50 v/v), at a flow rate of 10 µL/min (Figure 1).
214 Two ions at *m/z* 143 and *m/z* 145 corresponding to deprotonated ethephon with $^{35}Cl$ and $^{37}Cl$
215 isotopes respectively were observed and optimized at a cone voltage of 15 V (Figure 1(a)). When
216 *m/z* 143 was used as precursor, two product ions were observed in the MS/MS spectrum. The most
217 abundant (*m/z* 107) was optimized at 5 eV collision energy (Figure 1(c), bottom), and it could be
218 explained by the loss of HCl. The other product ion (*m/z* 79) was optimized at 15 eV (Figure 1(c),
219 top) and corresponded to the loss of $C_2H_4$ (ethylene) from the *m/z* 107 fragment. The proposed
220 fragmentation pathway [22] is in agreement with the ions observed in the MS/MS spectra. Taking
221 advantage of the chlorine presence in the ethephon molecule, *m/z* 145 could also be used as
222 precursor leading to the same product ions (*m/z* 107 and 79). Notice that none of the product ions
223 contain chlorine in their chemical structure, explaining that both precursor ions gave the same
224 products after the loss of HCl.

225

226 In order to improve sensitivity, in-source fragmentation was promoted by increasing the
227 cone voltage to 25 V (Figure 1(b)). Under these conditions, *m/z* 107 was by far the most abundant
228 ion. The MS/MS fragmentation of this in-source ion generated the *m/z* 79 product ion, which was
229 optimized at 10 eV collision energy (Figure 1(d)). This transition (*m/z* 107>79) was the most
230 sensitive, and consequently it was selected for quantification purposes.

231

232 The optimized MS conditions are summarized in Table 1. According to the abundance of the
233 different transitions obtained in the SRM mode, the transition *m/z* 107>79 was chosen for
234 quantification, and the transitions *m/z* 143>107, *m/z* 143>79, *m/z* 145>107 and *m/z* 145>79 were all
235 selected for confirmation purposes. Q/q ratios were obtained from injection in sextuplicate of an
236 aqueous standard at a concentration of 0.5 µg/L. As expected from relative abundances of $^{35}Cl$ and
237 $^{37}Cl$, the $q_1$ and $q_2$ transitions from *m/z* 143 precursor ion were more sensitive than from *m/z* 145 ($q_3$
238 and $q_4$), with the result that lower values of Q/q ratios were obtained (Q/q ratio 1 means that Q and
239 q intensities are similar).

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245 ***3.2. Direct injection***

246 The first approach considered for determination of ethephon residues was the direct
247 injection of water samples in the chromatographic system. Taking into account the ionic character
248 of ethephon, ion-pairing chromatography was considered the best option for ethephon separation on
249 a reversed phase LC column. A Discovery column (50 × 2.1 mm, 5 µm) and an injection volume of
250 100 µL were employed to carry out these experiments.

251

252 In our own experience, TBA can be satisfactory used as an ion-pairing reagent for anionic
253 analytes in LC/MS/MS based procedures [22-25]. However, the presence of TBA in the mobile
254 phase causes a noticeably decrease of sensitivity due to the continuous entrance of TBA salts into
255 the MS source. Therefore, the ion-pairing reagent was only added into the sample vial, just before
256 injection into the chromatographic system in order to form the ion pair but avoiding the use of TBA
257 in the mobile phase. The optimal concentration of this reagent was found to be 60 mM, as a
258 compromise between chromatographic behavior and sensitivity. Despite obtaining reproducible
259 results and adequate peak shape, the sensitivity achieved under these conditions was insufficient to
260 determine ethephon at sub-pbb levels.

261

262 In order to enhance ion-pair retention and to increase sensitivity, two more ion-pairing
263 reagents were tested: TDTA, chosen due to its longer alkyl chain ($C_{14}$), and TOA, which has four
264 intermediate-length alkyl chains ($C_8$). Optimum concentration for both reagents was found to be 50
265 mM, reaching similar sensitivity than TBA. Taking into account the problems derived from their
266 low solubility in water and low volatility, together with the poor reproducibility observed with both
267 TDTA and TOA, TBA was finally selected as ion-pairing reagent for further experiments.

268

269 Trying to reach the sensitivity required for water analysis, we also tested the direct injection
270 of the TBA ion-pair in ultra high pressure liquid chromatography (UHPLC) coupled to tandem mass
271 spectrometry using an Acquity UPLC HSS T3 column (50 mm × 2.1 mm, 1.8 µm) but using an
272 injection loop of 20 µL. Results obtained in terms of sensitivity were not satisfactory and this option
273 was discarded.

274

275 Another option considered to improve the sensitivity was performing a derivatization step. A
276 possible esterification of the phosphonic acid group was kept in mind, but it was finally discarded
277 due to the lack of confidence to carry out this reaction, in a simple and rapid way, in aqueous media.

278

279 In consequence, to obtain the sensitivity needed for the determination of ethephon residues
280 in water, a pre-concentration step seemed necessary.

281

282 ***3.3. Preconcentration step***

283 *3.3.1 On-line SPE/LC*

284

285 Firstly, we applied an on-line SPE pre-concentration step in an attempt to reach the
286 appropriate sensitivity. Three different stationary phases were tested for the SPE cartridges, $C_{18}$,
287 PRP and Oasis HLB, using in all cases 50 × 2.1 mm, 5 μm Discovery $C_{18}$ as analytical column.
288 Different sample loops were used (500, 750 and 2500 μL) for sample loading. The transfer of the
289 ethephon from the SPE cartridge to the LC column was carried out in backflush mode to avoid peak
290 broadening, and several water/methanol percentages were used for this purpose.

291

292 Experiments were carried out using the three ion-pairing reagents indicated above and
293 performing their addition both to the sample vial and/or to the SPE mobile phase. We did not
294 observe a significant sensitivity improvement at any of the concentrations employed for the ion-
295 pairing reagents. Oasis HLB cartridges gave better results with the three ion-pair reagents, but the
296 insufficient focusing of the ion-pair in all cases led to excessive band broadening resulting on
297 unsatisfactory behavior as regards peak shape and sensitivity.

298

299 Additionally, large volume injection in combination with coupled-column liquid
300 chromatography (LVI/LC/LC) using two analytical columns was also tested, searching for a better
301 ion-pair focusing on the first analytical column. However, this option was finally discarded due to
302 the difficult retention of ethephon ion-pair when using this approach injecting 2500 μL of sample.

303

304 *3.3.2 Off-line SPE*

305

306 Regarding to the off-line SPE process, two stationary phases were tested in the SPE
307 cartridges: Oasis HLB (Hydrophilic-Lipophilic Balanced) and Oasis MAX (Mixed-mode strong
308 Anion-eXchange), both containing a poly (divinylbenzene-co-N-vinylpyrrolidone) copolymer and
309 the last one also containing strong anion-exchange quaternary amine groups on the surface. TBA
310 was selected as ion-pairing reagent and added to the vials before injection into LC/MS/MS. Both,
311 the Discovery $C_{18}$ and the Mediterranea $SEA_{18}$ analytical columns were also tested along the
312 experiments. As can be seen in Figure 2, the Mediterranea $SEA_{18}$ (50 × 2.1 mm, 5 μm) led to better
313 peak shape, higher retention and sensitivity. Therefore, this column was selected for the LC
314 separation in further experiments.

315

316 When using Oasis HLB cartridges, pre-formation of the ion-pair previously to SPE was
317 required to favor the ethephon retention onto the cartridge. The general procedure applied was as
318 follow: pre-conditioning of the cartridge by passing methanol, acetone, methanol and TBA 50 mM

319 in HPLC water (3 mL of each one); loading 10 mL of water sample containing TBA (50 mM); air-
320 drying under vacuum, and elution with 2 mL acetone. Several experiments, under different
321 conditions, were carried out in order to evaporate the eluate and to change the solvent before
322 injection into the LC/MS/MS system. Results were not satisfactory, proving in this way that losses
323 of ethephon took place along the evaporation process. The best results were obtained when the SPE
324 eluate was 5-fold diluted with HPLC water and injected (after addition of TBA into the vial), but
325 recoveries were always lower than 50% and poorly reproducible.

326

327 Other approach considered was the use of Oasis MAX cartridges, where the anionic
328 molecule of ethephon could be retained without the need of ion-pairing formation. The elution of
329 analytes in these cartridges is performed with acidic solvents. Conditioning of cartridges was made
330 by passing 6 mL 2% HCl in methanol, 6 mL methanol and 6 mL HPLC water, being crucial to use
331 acidified methanol when pre-conditioning for obtaining satisfactory recoveries and suitable peak
332 shapes. In order to optimize the SPE process, we studied the effect of sample volume and the
333 elution solvent. The effect of sample volume was studied in the range 10-200 mL, the optimum
334 being found 100 mL without observing losses by breaktrough. Methanol and acetone with different
335 HCl contents were tested as elution solvents. Results with acidified acetone were worse than those
336 with methanol in terms of sensitivity. Finally, the best recovery was obtained using 1 mL 2% HCl
337 in methanol. Then, the SPE eluate was diluted with HPLC water up to 5 mL and analyzed by
338 LC/MS/MS. Therefore, a 20-fold preconcentration took place in the SPE process. Elution with
339 mixtures water:acidified methanol and their direct injection in the LC/MS/MS system was also
340 assayed, but sensitivity obtained was insufficient.

341

342 This optimized procedure led to satisfactory results when it was applied to HPLC water
343 spiked with ethephon at 0.1 μg/L level, obtaining satisfactory recovery (average value for five
344 replicates was 93%), with a relative standard deviation (RSD) of 12%. Linearity was studied by
345 injecting aqueous standards at seven concentrations in the range 0.5-50 μg/L, obtaining correlation
346 coefficients higher than 0.999. It corresponded to a linear range of 0.025-2.5 μg/L in water samples.
347 The instrumental limit of detection (LOD), calculated for a signal-to-noise ratio of three from the
348 chromatograms corresponding to the lowest standard analyzed, was found to be 0.4 μg/L, which
349 corresponds to a LOD of 0.02 μg/L in the water sample.

350

351 When the method was applied to the analysis of groundwater, mineral and surface water
352 samples, fortified at 0.1 μg/L level, recoveries obtained were not satisfactory, varying between 30
353 and 40%. The reason might be that the amount of major anions present in the samples prevented
354 ethephon to be retained into the MAX cartridges. At this point, we considered to include a de-

355 ionization step prior to SPE, as reported Royer *et al.* [9], in order to remove major anions. De-
356 ionization was carried out by stirring the sample with an anion/cation-exchange mixed bed resin
357 (AG 501-X8), which must be added in an amount that ensure partial de-ionization only. At the
358 typical pH values of natural waters, ethephon is mainly found as its deprotonated anionic forms
359 $ClCH_2$-$CH_2$-$PO_2(OH)^-$ and $ClCH_2$-$CH_2$-$PO_3^{2-}$, which should not be removed from the samples when
360 mixing with the resin. An optimization of the amount of resin used was required for each type of
361 water sample in order to remove anions with highest affinity for the anion-exchange sites, while
362 anions with lower affinity, as ethephon, remain in the sample. We found this step critical and one of
363 the main key aspects to be solved in ethephon residues determination.
364
365 The optimization of this de-ionization step was carried out for low conductivity mineral
366 water samples (< 500 μS/cm). The optimal amount of resin for 100 mL of sample was found to be
367 0.1 g, with a stirring time of 10 minutes. LC/MS/MS chromatograms corresponding to a mineral
368 water sample spiked with ethephon at 0.1 μg/L after applying the de-ionization step is depicted in
369 Figure 3(b). Average recovery (n=5) in mineral water was 77% with 18% RSD.
370
371 The high amount of TBA injected in comparison to other previous ion-pair LC/MS/MS
372 based methods [20,22-24] (injection volume 100 μL of 60 mM TBA in the present work compared
373 to 10-20 μL of 20-40 mM in previous works) led to a deterioration in the LC/MS/MS
374 chromatograms when increasing the number of injections. This fact might affect the limit of
375 detection of the procedure. However, despite this deterioration, both the quantitative (Q) and
376 confirmative ($q_1$) transitions could be observed and Q/q ratios were accomplished after 30 injections
377 in the same LC-column, allowing the confirmation of ethephon in the sample at 0.1 μg/L level
378 (Figure 3 (c,d)). Present research is focused on the analysis of more saline water samples, in order
379 to optimize the previous de-ionization step and to establish the adequate amount of resin to remove
380 most anions but remaining ethephon in the sample. Sample treatment for this kind of matrices, e.g.
381 surface water, saline groundwater, or wastewater, seems to be the most problematic step, once the
382 LC/MS/MS analysis has been optimized.
383

384 **4. Conclusions**

385 Determination of ethephon in water at sub-ppb levels is a difficult task due to its highly acid
386 and polar character together with small molecular size. As a result, very few analytical methods
387 have been reported for this pesticide in water samples. Despite the efforts made, the result is that the
388 analytical methodology developed until now is mostly low specific, not much sensitive and notably
389 time-consuming, with laborious sample treatments. In this work, we have performed a detailed
390 study on the potential of ion-pairing liquid chromatography coupled to tandem MS for determining
391 residue levels of ethephon in water. In addition, several approaches have been tested for the
392 extraction/pre-concentration step, selecting finally off-line SPE with Oasis MAX cartridges as the
393 most efficient system. A partial de-ionization of the sample using an anion/cation-exchange mixed
394 bed resin was required in order to remove major anions in water [9] that would negatively affect the
395 LC/MS/MS ethephon determination as an ion-pair.
396
397 Ion-pairing LC/MS/MS has been proven a useful approach for the sensitive determination of
398 ethephon in water, allowing the determination of this compound in low-conductivity water at 0.1
399 μg/L level. Sample treatment for high-salinity complex water matrices was found the most critical
400 step, in order to get the partial de-ionization of the sample, once the LC-MS/MS analysis has been
401 optimized in the present work.
402

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408

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443 **Table 1.** MS optimized conditions for the LC/MS/MS determination of ethephon

444

| Precursor ion (*m/z*) | Cone voltage (V) | Product ion (*m/z*) | Collision energy (eV) | Q/q ratio |
|---|---|---|---|---|
| 107 | 25 | 79 (*Q*) | 10 | - |
| 143 | 15 | 107 ($q_1$) | 5 | 4.4 |
| | | 79 ($q_2$) | 15 | 7.8 |
| 145 | 15 | 107 ($q_3$) | 5 | 14.2 |
| | | 79 ($q_4$) | 15 | 27.0 |

445

446 (Q) - Quantification transition, (q) – confirmation transition

447 **Figure captions**

448

449 **Figure 1.** Negative ESI full-scan mass spectra of ethephon at cone voltages of (a) 15 V and (b) 25
450 V. Product ion spectra for (c) precursor ion *m/z* 143 at a collision energy of 5 eV (bottom) and 15
451 eV (top). Product ion spectrum for (d) precursor ion *m/z* 107 at 10 eV.

452

453 **Figure 2.** LC/MS/MS chromatograms of 10.0 µg/L ethephon standard using two different analytical
454 columns: (a) Discovery $C_{18}$ and (b) Mediterranea $SEA_{18.}$

455

456 **Figure 3.** LC/MS/MS chromatograms of (a) ethephon standard of 2.5 µg/L (b) mineral water spiked
457 with ethephon at 0.1 µg/L (corresponding to 2 µg/L in the final extract) (c) and (d) correspond to (a)
458 and (b) after 30 injections in the LC system.

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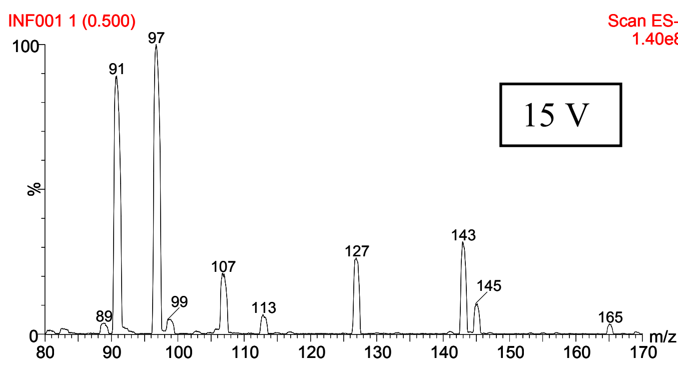
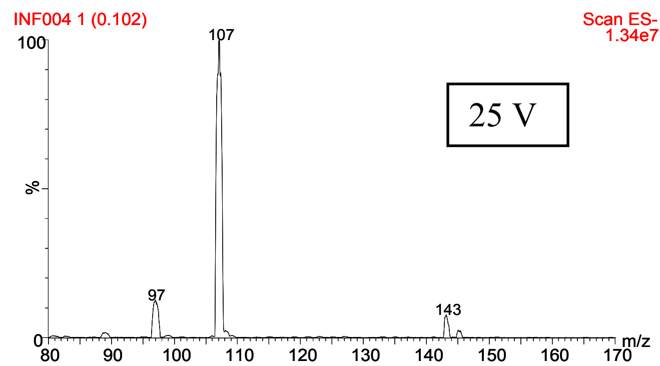
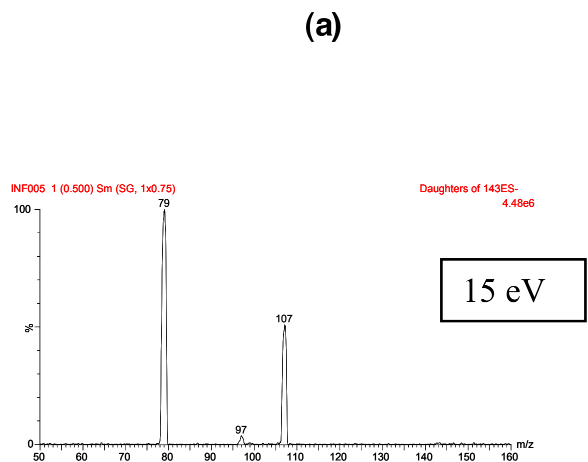
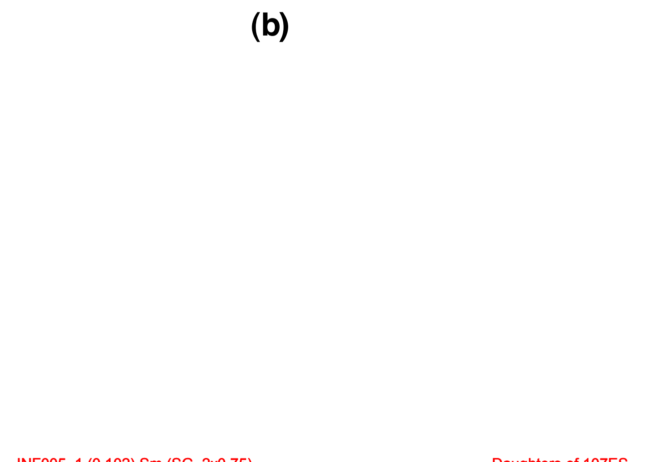


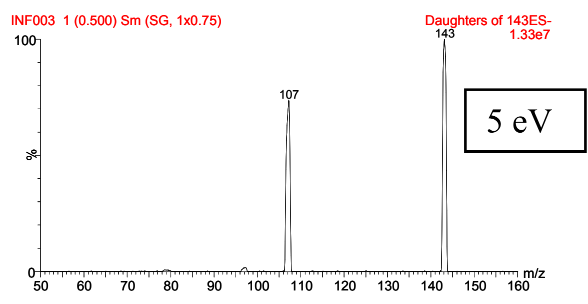

(a)




(b)






(c)

(d)

**Figure 1**

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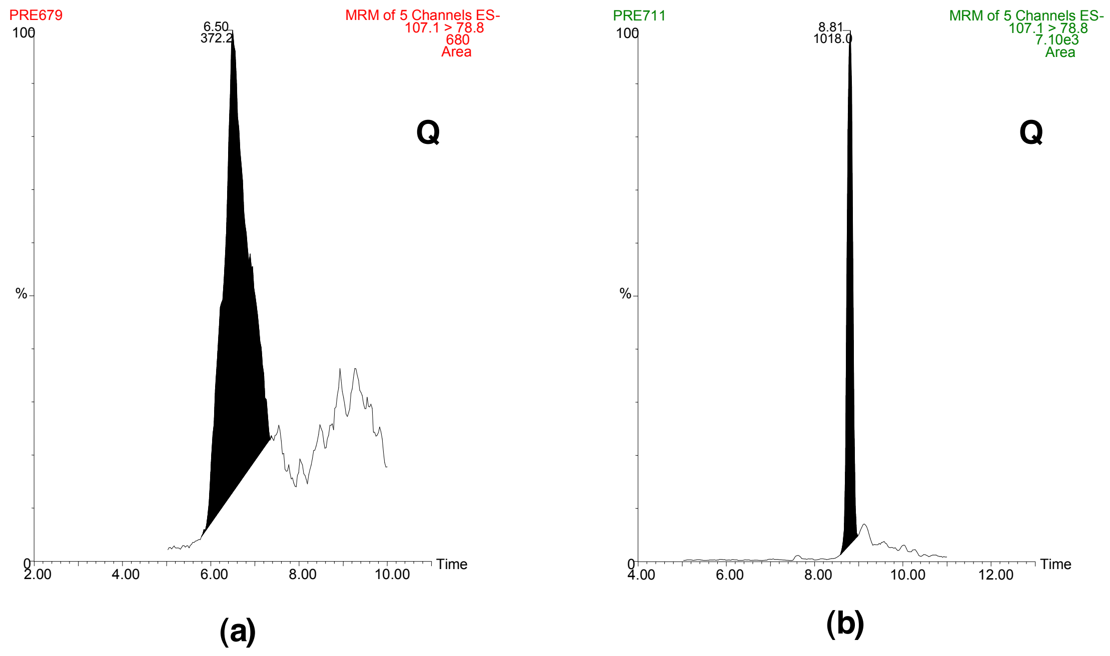


**Figure 2**

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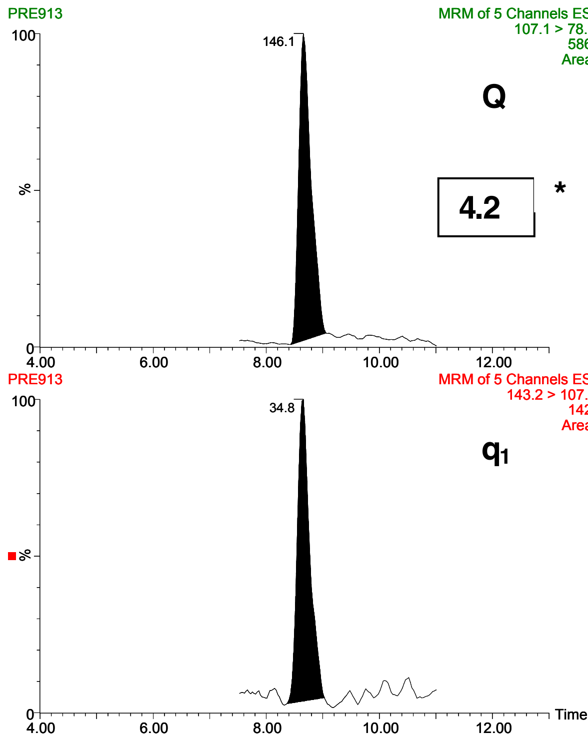


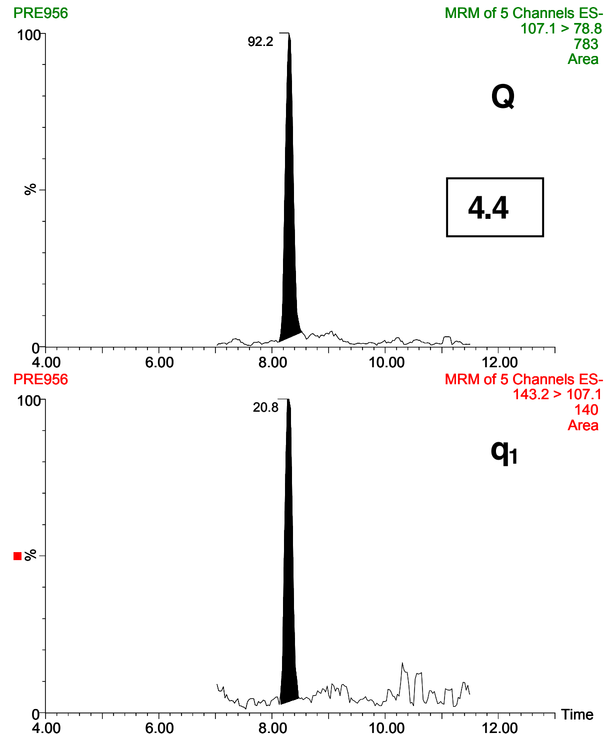


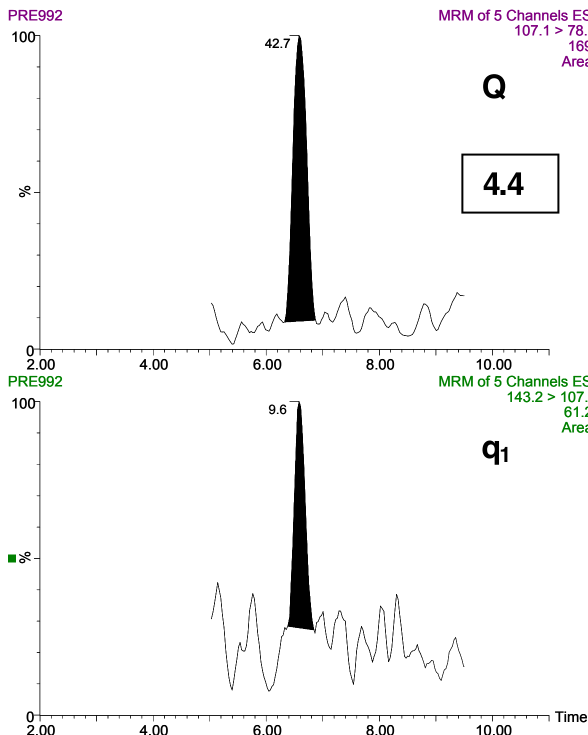


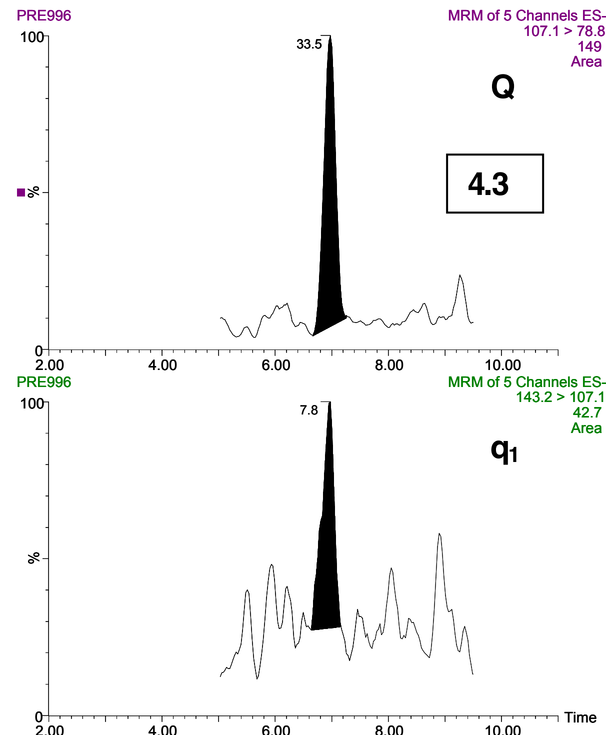


* Q/q ratios

**Figure 3**