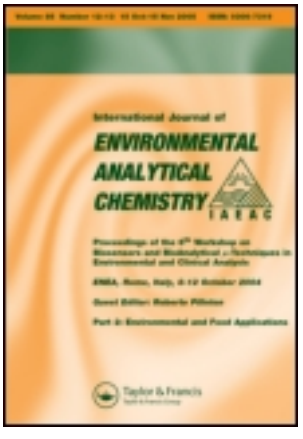

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/geac20

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Available online: 21 Oct 2011

To cite this article: Cristina Ripollés, José M. Marín, Juan V. Sancho, Francisco J. López & Félix Hernández (2011): Analytical study on ethephon residue determination in water by ion-pairing liquid chromatography/tandem mass spectrometry, International Journal of Environmental Analytical Chemistry, 91:14, 1380-1391

To link to this article: http://dx.doi.org/10.1080/03067319.2010.520131

Intern. J. Environ. Anal. Chem.
Vol. 91, No. 14, December 2011, 1380–1391

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

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(*Received 25 May 2010; final version received 27 August 2010*)

A detailed analytical study on ethephon residue determination in water, making use of ion-pairing liquid chromatography coupled to electrospray tandem mass spectrometry (LC/MS/MS), has been carried out. Ethephon is a plant growth regulator, highly polar, which is typically present in aqueous solution in anionic form due to its acid character. Both its extraction and pre-concentration from water samples and its chromatographic retention are difficult. Several approaches for sample pretreatment have been tested including direct injection into the chromatographic system, on-line solid phase extraction (SPE) and off-line SPE, with the best results being obtained after off-line SPE, using Oasis MAX cartridges (mixed-mode strong anion-exchange). After testing several ion-pairing reagents, tetrabuthylammonium acetate (TBA) was selected. This was added to the samples before LC/MS/MS analysis to facilitate ethephon chromatographic retention. The acquisition of several specific MS/MS transitions together with the evaluation of their relative intensity ratios allowed the reliable confirmation of the analyte in samples. The optimised approach was tested in low-salinity water spiked at 0.1 $\mu g\,L^{-1}$ level with satisfactory recovery, and a limit of detection of 0.02 $\mu g\,L^{-1}$. To this purpose, the water sample was partially de-ionised in an initial stage, in order to remove major ions that would have interfered in analyses. The application of this methodology to more saline/complex water samples, as surface or wastewater, was problematic and a thorough optimisation of the de-ionisation conditions would be required.

Keywords: ethephon; ion-pairing liquid chromatography; tandem mass spectrometry; 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

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ISSN 0306–7319 print/ISSN 1029–0397 online

http://dx.doi.org/10.1080/03067319.2010.520131
http://www.tandfonline.com

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 Ionisation Detector (FID) [3–5] and drinking water [6]. Despite acceptable detection limits are achieved (between 0.01 and 0.1 mg Kg^{-1}), 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 for modern analytical methodology capable of accurately determining 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 minimise interferences [7]. Another work based on GC/MS with previous extraction followed by SPE clean-up 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 analyser, based on a previous de-ionisation 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 reaching a limit of quantification of 0.1 μg L^{-1}. The need of applying a multistage procedure with lot of sample manipulation illustrates the analytical difficulties associated to this problematic analyte. The result is that the method applied turns out to be extensive, complex and involves a large amount of time to ensure a reliable quantification of the compound in water. Another method has been proposed based on ion chromatography/inductively coupled plasma mass spectrometry for the simultaneous determination of ethephon and three more polar herbicides [10]. This method proved to be simple and rapid, but its sensitivity was unsatisfactory with a limit of detection of 1.4 μg L^{-1}, as could be expected from the technique employed, not the most appropriate for pesticide residue analysis (PRA).

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

Regarding the acidic character of ethephon, its deprotonated anionic form in found to be difficult to retain in the most commonly applied reversed-phase LC columns. Thus, ion-pairing chromatography is an appropriate approach for increasing the retention of ionic compounds like ethephon [18–21]. Ion-pairing reagents used for anionic analytes generally

have a positively charged quaternary nitrogen with a bulky hydrophobic part that contains alkyls with 4–18 carbon atoms (e.g. tetrabuthylammonium or hexadecyltrimethylammonium) in order to favour the retention of the negatively charged analyte when applying reversed-phase LC approach [21,22]. In our research group, we have developed a rapid, sensitive and selective method for the determination of ethephon residues in vegetables (apple, cherry, tomato) based on ion-pairing LC/MS/MS using tetrabuthylammonium as ion-pairing reagent [22]. The aim of the present work is to investigate the potential of this approach, which gave excellent results in fruits and vegetables, for the direct determination of ethephon residues in water, with special attention to the unequivocal confirmation of positive samples.

2. Experimental

2.1 *Reagents and chemicals*

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

The stock standard solution of ethephon was prepared by dissolving around 50 mg powder, accurately weighed, in 100 mL of acetone obtaining a final concentration of 500 mg L^{-1}, and stored in a freezer at <−18°C. Working solutions were prepared from stock solution by dilution in acetonitrile for concentrations higher than 5 mg L^{-1}, and using aqueous formic acid (pH 3) for lower concentrations. The working standards were stored at 4°C.

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

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

2.2 *Instrumentation*

A Quattro Micro triple quadrupole mass spectrometer (Waters, Milford, MA, USA) was interfaced using an orthogonal Z-spray-electrospray ion source to an HPLC system based on a Waters Alliance 2695 (Waters) quaternary pump used for the chromatographic separation. Nitrogen generated from pressurised air in a high-purity nitrogen generator (NM30LA 230Vac Gas Station from Peak Scientific, Inchinnan, UK) was employed as drying and nebulising gas. The cone gas and the desolvation gas flows were set to approximately 60 L h^{-1} and 600 L h^{-1}, respectively. For operation in MS/MS mode, collision gas was Argon 99.995% (Praxair, Valencia, Spain) with a pressure of approximately 1×10^{-4} mbar in the collision cell. Electrospray needle capillary voltage

of 3.2 kV was selected in negative ionisation mode. The desolvation temperature was set to 350°C and the source temperature to 120°C. Infusion experiments were performed using the built-in syringe pump directly connected to the ion source at a flow rate of 10 μL min^{-1}. Dwell time of 300 ms was chosen. A solvent delay of 7.5 min was selected to give an additional clean-up using the built-in divert valve controlled by the Masslynx NT v 4.0 software (Waters).

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

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

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

2.3 *Procedure*

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

Calibration was carried out in the range 0.5–50.0 μg L^{-1}, from standards prepared in water acidified at pH 3 (formic acid) by adding 880 μL of each standard solution into a vial containing 120 μL 500 mM TBA solution.

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

3. Results and discussion

3.1 *MS optimisation*

The negative electrospray full-scan spectra of ethephon was obtained by infusion of 2.5 μg mL^{-1} standard solution in acetonitrile : water (50 : 50 v/v), at a flow rate of

$10\,\mu L\,min^{-1}$ (Figure 1). Two ions at m/z 143 and m/z 145 corresponding to deprotonated ethephon with ^{35}Cl and ^{37}Cl isotopes respectively were observed and optimised at a cone voltage of 15 V (Figure 1(a)). When m/z 143 was used as precursor, two product ions were observed in the MS/MS spectrum. The most abundant (m/z 107) was optimised at 5 eV collision energy (Figure 1(c), bottom), and it could be explained by the loss of HCl. The other product ion (m/z 79) was optimised at 15 eV (Figure 1(c), top) and corresponded to the loss of C_2H_4 (ethylene) from the m/z 107 fragment. The proposed fragmentation pathway [22] is in agreement with the ions observed in the MS/MS spectra. Taking advantage of the chlorine presence in the ethephon molecule, m/z 145 could also be used as precursor leading to the same product ions (m/z 107 and 79). Notice that none of the product ions contain chlorine in their chemical structure, explaining that both precursor ions gave the same products after the loss of HCl.

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

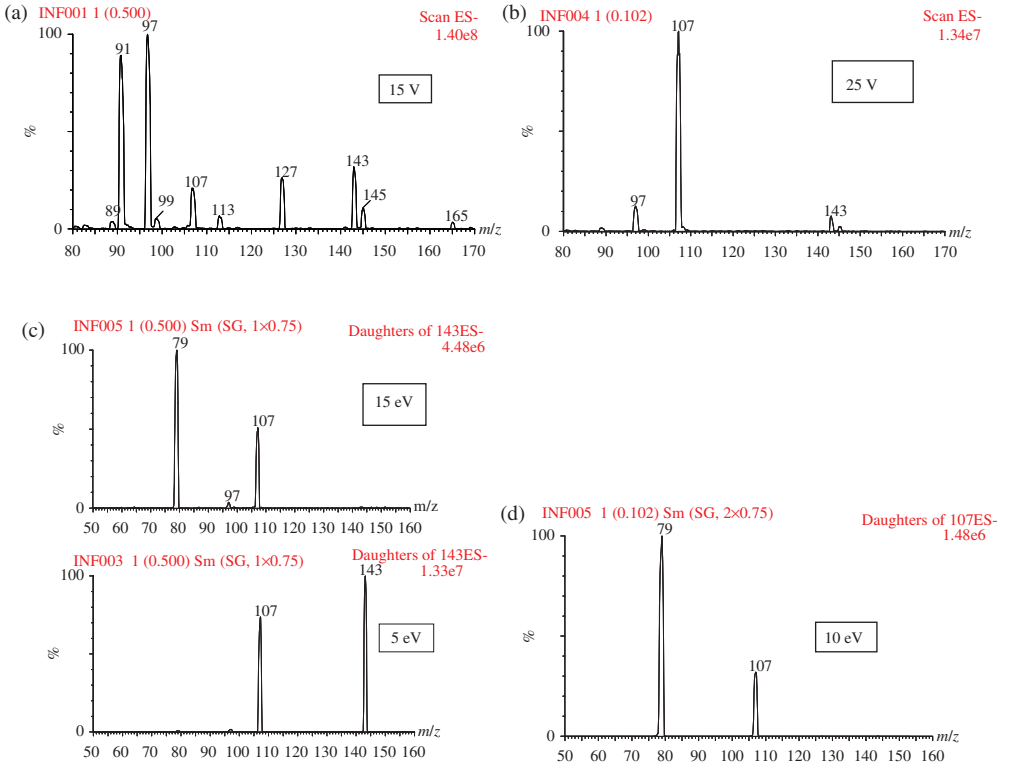


Figure 1. Negative ESI full-scan mass spectra of ethephon at cone voltages of (a) 15 V and (b) 25 V. Product ion spectra for (c) precursor ion m/z 143 at a collision energy of 5 eV (bottom) and 15 eV (top). Product ion spectrum for (d) precursor ion m/z 107 at 10 eV.

Table 1. MS optimised conditions for the LC/MS/MS determination of ethephon.

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

Notes: (Q) = Quantification transition; (q) = confirmation transition.

The optimised MS conditions are summarised in Table 1. According to the abundance of the different transitions obtained in the SRM mode, the transition m/z 107 > 79 was chosen for quantification, and the transitions m/z 143 > 107, m/z 143 > 79, m/z 145 > 107 and m/z 145 > 79 were all selected for confirmation purposes. Q/q ratios were obtained from injection in sextuplicate of an aqueous standard at a concentration of 0.5 μg L^{-1}. As expected from relative abundances of ^{35}Cl and ^{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 and q_4), with the result that lower values of Q/q ratios were obtained (Q/q ratio 1 means that Q and q intensities are similar).

3.2 *Direct injection*

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

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

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

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

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

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

3.3 *Pre-concentration step*

3.3.1 *On-line SPE/LC*

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

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

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

3.3.2 *Off-line SPE*

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

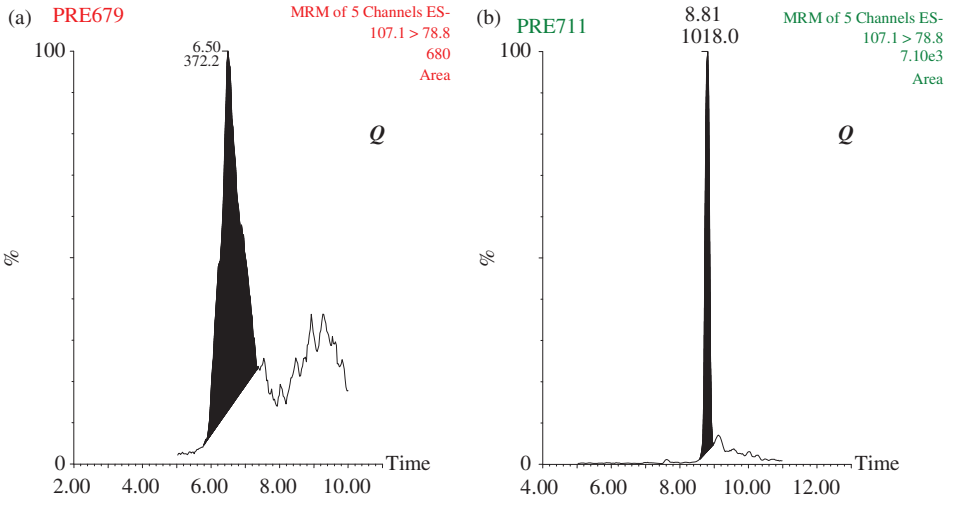


Figure 2. LC/MS/MS chromatograms of 10.0 μg L^{-1} ethephon standard using two different analytical columns: (a) Discovery C_{18} and (b) Mediterranea SEA_{18}.

When using Oasis HLB cartridges, pre-formation of the ion-pair previously to SPE was required to favour the ethephon retention onto the cartridge. The general procedure applied was as follow: pre-conditioning of the cartridge by passing methanol, acetone, methanol and TBA 50 mM in HPLC water (3 mL of each one); loading 10 mL of water sample containing TBA (50 mM); air-drying under vacuum; and elution with 2 mL acetone. Several experiments, under different conditions, were carried out in order to evaporate the eluate and to change the solvent before injection into the LC/MS/MS system. Results were not satisfactory, proving that losses of ethephon took place along the evaporation process. The best results were obtained when the SPE eluate was 5-fold diluted with HPLC water and injected (after addition of TBA into the vial), but recoveries were always lower than 50% and poorly reproducible.

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

This optimised procedure led to satisfactory results when it was applied to HPLC water spiked with ethephon at 0.1 μg L^{-1} level, obtaining satisfactory recovery (average value for five replicates was 93%), with a relative standard deviation (RSD) of 12%. Linearity was studied by injecting aqueous standards at seven concentrations in the range 0.5–50 μg L^{-1}, obtaining correlation coefficients higher than 0.999. It corresponded to a linear range of 0.025–2.5 μg L^{-1} in water samples. The instrumental limit of detection (LOD), calculated for a signal-to-noise ratio of three from the chromatograms corresponding to the lowest standard analysed, was found to be 0.4 μg L^{-1}, which corresponds to a LOD of 0.02 μg L^{-1} in the water sample.

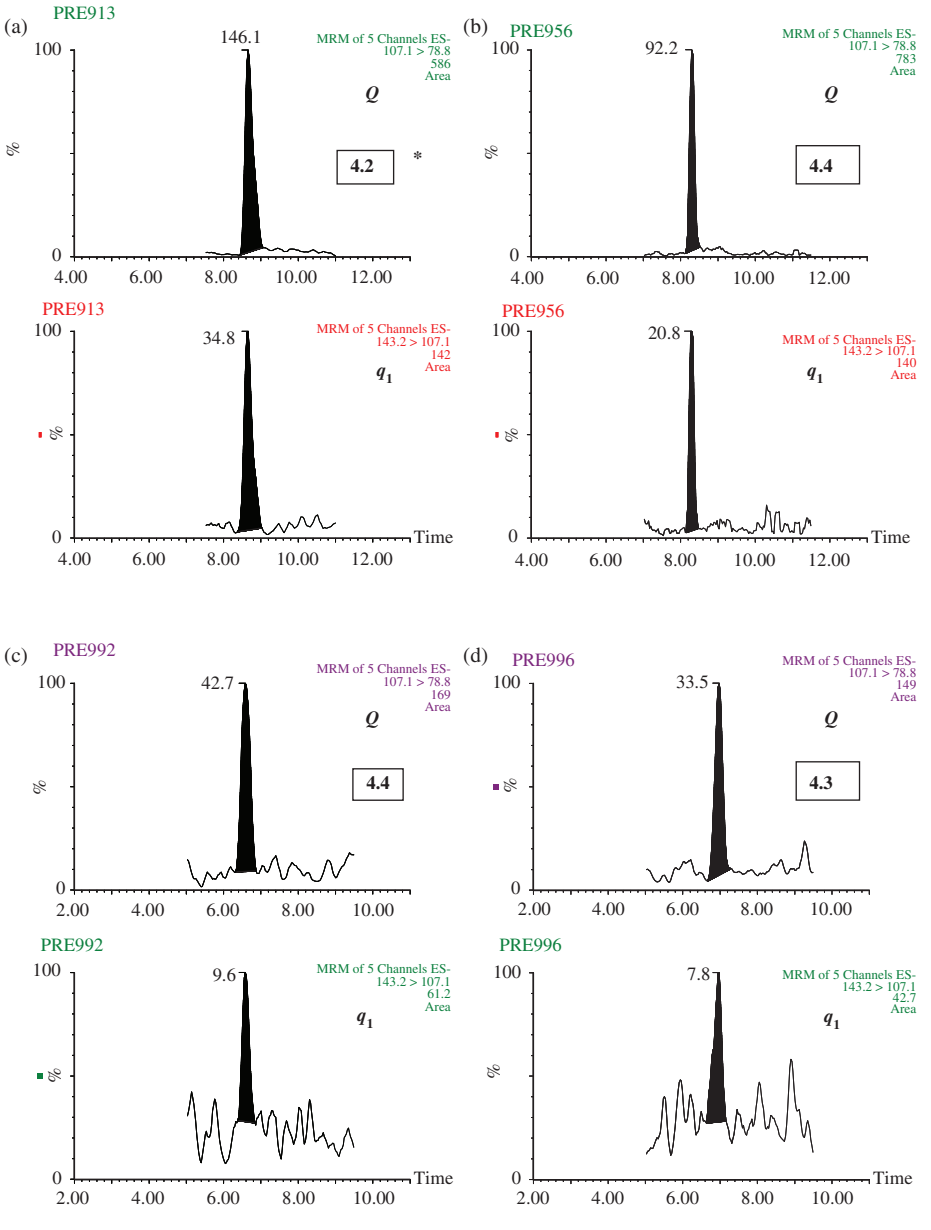
When the method was applied to the analysis of groundwater, mineral and surface water samples, fortified at 0.1 μg L^{-1} level, recoveries obtained were not satisfactory, varying between 30 and 40%. The reason might be that the amount of major anions present in the samples prevented ethephon to be retained into the MAX cartridges. At this point, we considered including a de-ionisation step prior to SPE, as reported Royer *et al.* [9], in order to remove major anions. De-ionisation was carried out by stirring the sample with an anion/cation-exchange mixed bed resin (AG 501-X8), which must be added in an amount that ensure partial de-ionisation only. At the typical pH values of natural waters, ethephon is mainly found as its deprotonated anionic forms $ClCH_2–CH_2–PO_2(OH)^-$ and $ClCH_2–CH_2–PO_3^{2-}$, which should not be removed from the samples when mixing with the resin. An optimisation of the amount of resin used was required for each type of water sample in order to remove anions with highest affinity for the anion-exchange sites, while anions with lower affinity, as ethephon, remain in the sample. We found this step critical and one of the key aspects to be solved in ethephon residues determination.

The optimisation of this de-ionisation step was carried out for low conductivity mineral water samples (<500 μS cm^{-1}). The optimal amount of resin for 100 mL of sample was found to be 0.1 g, with a stirring time of 10 minutes. LC/MS/MS chromatograms corresponding to a mineral water sample spiked with ethephon at 0.1 μg L^{-1} after applying the de-ionisation step is depicted in Figure 3(b). Average recovery ($n = 5$) in mineral water was 77% with 18% RSD.

The high amount of TBA injected in comparison to other previous ion-pair LC/MS/MS based methods [20,22–24] (injection volume 100 μL of 60 mM TBA in the present work compared to 10–20 μL of 20–40 mM in previous work) led to a deterioration in the LC/MS/MS chromatograms when increasing the number of injections. This fact might affect the limit of detection of the procedure. However, despite this deterioration, both the quantitative (Q) and confirmative (q_1) transitions could be observed and Q/q ratios were accomplished after 30 injections in the same LC-column, allowing the confirmation of ethephon in the sample at 0.1 μg L^{-1} level (Figure 3 (c,d)). Present research is focused on the analysis of more saline water samples, in order to optimise the previous de-ionisation step and to establish the adequate amount of resin to remove most anions but remaining ethephon in the sample. Sample treatment for this kind of matrices, e.g. surface water, saline groundwater, or wastewater, seems to be the most problematic step, once the LC/MS/MS analysis has been optimised.

4. Conclusions

Determination of ethephon in water at sub-ppb levels is a difficult task due to its highly acid and polar character together with small molecular size. As a result, very few analytical



*Q/q ratios.

Figure 3. LC/MS/MS chromatograms of (a) ethephon standard of 2.5 µg L^{-1} (b) mineral water spiked with ethephon at 0.1 µg L^{-1} (corresponding to 2 µg L^{-1} in the final extract) (c) and (d) correspond to (a) and (b) after 30 injections in the LC system.

methods have been reported for this pesticide in water samples. Despite the efforts made, the result is that the analytical methodology developed until now is mostly low specific, not very sensitive and particularly time-consuming, with laborious sample treatments. In this work, we have performed a detailed study on the potential of ion-pairing liquid

chromatography coupled to tandem MS for determining residue levels of ethephon in water. In addition, several approaches have been tested for the extraction/pre-concentration step, selecting finally off-line SPE with Oasis MAX cartridges as the most efficient system. A partial de-ionisation of the sample using an anion/cation-exchange mixed bed resin was required in order to remove major anions in water [9] that would negatively affect the LC/MS/MS ethephon determination as an ion-pair.

Ion-pairing LC/MS/MS has been proven a useful approach for the sensitive determination of ethephon in water, allowing the determination of this compound in low-conductivity water at $0.1\,\mu g\,L^{-1}$ level. Sample treatment for high-salinity complex water matrices was found to be the most critical step, in order to get the partial de-ionisation of the sample, once the LC/MS/MS analysis has been optimised in the present work.

Acknowledgements

This work has been developed under financial support of the Ministry of Education and Science, Spain (CTM2006-06417). The authors acknowledge the financial support of Generalitat Valenciana, as research group of excellence PROMETEO/2009/054. C. Ripollés is very grateful to the Ministry of Education and Science for her pre-doctoral grant.

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