Improved GC-MS/MS determination of pesticide residues making use of atmospheric pressure chemical ionization

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Abstract

The capabilities of a recently launched atmospheric pressure chemical ionization (APCI) source for mass spectrometry (MS) coupled to gas chromatograpy (GC) have been tested in order to evaluate its potential in pesticide residue analysis in fruits and vegetables. Twenty five pesticides were selected due to their high fragmentation under electron ionization (EI), making that the molecular ion ($M^{+\cdot}$) is practically absent in their spectra. The fragmentation of these pesticides under APCI conditions was studied, with the result that $M^{+\cdot}$ was not only present but also highly abundant for most compounds, with noticeable differences in the fragmentation patterns in comparison with EI. Moreover, the addition of water as modifier was tested to promote the formation of protonated molecules ($[M+H]^+$). Under these conditions, $[M+H]^+$ became the base peak of the spectrum for the majority of compounds, thus leading to an increase of sensitivity in the subsequent GC-MS/MS method developed using triple quadrupole analyzer (QqQ).

Highly satisfactory sensitivity and precision, in terms of repeatability, were reached and linearity was satisfactory in the range 0.01-100 ng/mL. The developed methodology was applied to apple, orange, tomato and carrot QuEChERS fortified extracts in order to evaluate the matrix effects.

In summary, the soft and reproducible ionization in the APCI source has greatly favored the formation of $[M+H]^+$ oppositely to EI where abundant fragmentation occurs and where the molecular ions have

low abundance or are even absent in the mass spectrum. In this way, the use of APCI has facilitated the development of tandem MS methods based on the selection of abundant $[M+H]^+$ as precursor ion.

Keywords

Atmospheric pressure chemical ionization; gas chromatography; tandem mass spectrometry; triple quadrupole; pesticides.

1. Introduction

Gas and liquid chromatography (GC and LC) coupled to mass spectrometry (MS) are the techniques of choice in pesticide residue analysis (PRA) for a wide variety of sample matrices. The availability of different types of analyzers as single (Q) or triple (QqQ) quadrupole, time-of-flight (TOF) and ion trap detector (ITD) allows performing both quantitative and qualitative analysis with satisfactory sensitivity and selectivity [1-8]. GC-MS is commonly applied for non-polar, volatile and thermostable compounds. In some cases, a derivatization step is required to make them GC-amenable, especially for transformation products, which are often more polar and less-volatile than their parent compounds. On the contrary, LC-MS is more appropriate for non-GC amenable compounds, i.e. thermolabile, polar and (semi) non-volatile analytes. Due to the large diversity in polarity, volatility and stability of pesticides, nowadays it is compulsory to use both techniques in a complementary way. MS ion sources play an important role in PRA. Notable differences can be found in the ionization processes occurring in LC-MS and GC-MS.

The most common sources in LC-MS are those based on atmospheric pressure ionization, specifically electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) [9]. Due to the low fragmentation achieved by these ionization techniques, the presence of the protonated $[M+H]^+$ or deprotonated $[M-H]^-$ molecule is common in the LC-MS spectra. On the contrary, ionization in GC-MS conventionally occurs under vacuum conditions: electron ionization (EI) and chemical ionization (CI). Undoubtedly, EI is the most commonly applied in the wide majority of GC-MS applications, including PRA. It is well known that this source produces high fragmentation of the molecules, leading in many cases to the absence of the molecular ion ($M^{+\cdot}$) in EI spectra. CI produces softer ionization. The reaction with a reagent gas occurs with an energy transfer that generally does not exceed 5 eV, so mass spectra exhibit less fragment ions than in EI. Better selectivity and sensitivity are achieved for some compounds under CI, as well as less matrix interferences, but this technique is quite restricted to specific analyte chemical classes [10-12] since it is not as universal ionization as EI. The interest in application of multiresidue methods including a wide variety and large number

of compounds makes EI mode the most commonly applied in PRA. This is favored by its robustness and good reproducibility. In addition, EI spectra in full scan mode (under 70 eV) are available in commercial libraries, facilitating the identification of compounds by an easy search matching.

Many methods have been reported in PRA based on GC-(EI)MS, using single quadrupole under Selected Ion Monitoring (SIM) mode, or ion trap operating under tandem mass spectrometry (MS/MS) [3, 13, 14] . Recently, triple quadrupole analyzer (QqQ) operating in selected reaction monitoring (SRM) mode has received much attention due to its better performance for quantitative multiresidue analysis [15-19]. The selection of adequate precursor and product ions and the subsequent application of SRM mode enhance selectivity and sensitivity, minimizing or even eliminating matrix interferences. In this way, very low detection limits can be achieved, favored by the low chemical background noise in the chromatograms.

However, the extensive fragmentation due to the high energy transferred to the molecules during the ionization process produces little or no molecular ions for many pesticides, as for example organochlorine (OCs) pesticides, organophosphorous (OPs), pyrethroids and chloroacetanilides [20-22]. Besides, compounds belonging to the same chemical family can show similar EI spectra; so the use of common ions/transitions can complicate the identification and quantification processes, especially if analytes are coeluting. When the molecular ion is absent or has very low abundance, it is necessary to select an (abundant) fragment ion as precursor. In addition to the loss of sensitivity, the specificity of the method can be also affected. Thus, the potential of tandem MS is lost.

Specific problematic cases, as the aforementioned, would require a soft and universal technique able to provide abundant molecular ions to be used as precursor ions in multiresidue GC-MS/MS analysis. Atmospheric pressure ionization in GC-MS was first introduced by Horning et al. in 1973 [23]. Subsequent modifications were described in the 80's [24, 25], but the technique has not been implemented yet as a common routine analysis because of the high costs of the specialized instrumentation and the unavailability of easy interchangeable ionization sources. Recently, a new APCI source using a nitrogen purge gas has been developed and commercialized [26, 27, 28].

Although it has not been widely applied, it offers attractive analytical capabilities in GC-MS analysis. From first applications in 2009 until now, GC-APCI-TOF MS has been used in different fields, like pesticide residue analysis [29], pharmaceuticals development [30], profiling of phenolic compounds in oil [31] and methabolic profiling [32].

The aim of this paper is to evaluate the potential of the APCI source in GC-MS/MS using triple quadrupole analyzer. For this purpose, 25 pesticides have been selected in order to study their ionization behavior under atmospheric pressure conditions and to evaluate the potential advantages in comparison with EI ionization. Spectra obtained with both sources have been compared paying special attention to the presence and abundance of the molecular ion and/or the protonated molecule. Experiments under product ion scan mode and SRM have been performed, and the most specific and/ or sensitive transitions have been selected in order to improve the performance of GC-MS/MS for some problematic pesticides, which suffer high fragmentation under EI. Different fruits and vegetable fortified extracts obtained after QuEChERS application (AOAC Official method) [33], and spiked with the 25 studied pesticides, have been analyzed in order to evaluate the applicability of the new ion source.

2. Experimental

2.1. Reagents

All pesticide standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Stock standard solutions (around 500 μg/mL) were prepared by dissolving reference standards in acetone and stored in a freezer at -20 ºC. Working standard mixtures were prepared by volume dilution of stock solutions in hexane.

Hexane, acetone, acetonitrile (MeCN), toluene, glacial acetic acid (HAc), anhydrous $MgSO_4$ and anhydrous sodium acetate (NaAc) were purchased from Scharlab (Barcelona, Spain). All solvents were for pesticide residue analysis or HPLC grade. Two types of 2 mL micro-centrifuge tubes for QuEChERS d-SPE containing 50 mg PSA and 150 mg anhydrous $MgSO_4$, or 50 mg PSA, 150 mg anhydrous $MgSO_4$ and 50 mg C_{18}, were obtained from Teknokroma (Barcelona, Spain).

2.2. Instrumentation

2.2.1. GC-(EI) MS/MS

A GC system (Agilent 6890N, Palo Alto, USA) equipped with an autosampler (Agilent 7693) was coupled to a triple quadrupole (QqQ) mass spectrometer (Quattro Micro GC, Waters, Boston, USA), equipped with an EI source. A fused silica HP-5MS capillary column (length 30 m x I.D. 0.25 mm x d_f 0.25 μm) (J&W Scientific, Folson, CA, USA) was used for GC separation. Injector was operated in splitless mode, injecting 1 μL at 280 ºC. The oven temperature was programmed as follows: 70 ºC (1 min), 25 ºC/min to 150 ºC and 10 ºC/min to 300 ºC (3 min). Helium was used as carrier gas at a constant flow rate of 1 mL/min (linear velocity of 40 cm/s). The interface and source temperatures were set at 260 ºC and 250 ºC, respectively. In order to prevent damage in the EI filament, a solvent delay of 4 min was selected. Targetlynx (a module of MassLynx) was used to process the data.

2.2.2. GC-(APCI) MS/MS

A GC system (Agilent 7890A, Palo Alto, CA, USA) equipped with an autosampler (Agilent 7693) and coupled to a triple quadrupole (QqQ) mass spectrometer (Xevo TQ-S, Waters Corporation, Manchester, UK), with an APCI source was used (the trade name is APGC). A fused silica DB-5MS capillary column (length 30 m x I.D. 0.25 mm x d_f 0.25 μm) (J&W Scientific, Folson, CA, USA) was used for GC separation. Injector was operated in splitless mode, injecting 1 μL at 280 ºC. The oven temperature was programmed as follows: 70 ºC (1 min), 25 ºC/min to 150 ºC and 10 ºC/min to 300 ºC (3 min). Helium was used as carrier gas at 2 mL/min, which corresponds to a linear velocity of 52 cm/ s. A pulsed splitless injection was carried out using an initial pressure of 240 kPa.

The interface temperature was set to 310 ºC using N_2 (from liquid N_2) as auxiliary gas at 250 L/hr and as cone gas at 170 L/hr, and N_2 (from gas cylinder quality 99.9990 %) as make-up gas at 320 mL/ min. The APCI corona pin was operated at 1.8 μA. The ionization process occurred within an closed ion volume, which enabled control over the protonation/charge transfer processes. The water, used as modifier when working under proton-transfer conditions, was placed in an uncapped vial, which was located within a specially designed holder placed in the source door. Targetlynx (a module of MassLynx) was used to process the data.

2.3. Sample treatment

Samples of apple, orange, tomato and carrot were purchased from a local market in Castellón (Spain). A sample treatment based on the well-known QuEChERS procedure was applied (AOAC official method 2007.01 [33]): 15 g of triturated sample were weighted in a 50 mL polypropylene centrifuge tube, mixed with 15 mL MeCN (with 1% HAc) and shaken by hand for 30 s. Then, 6 g anhydrous $MgSO_4$ and 1.5 g anhydrous NaAc were added and immediately shaken vigorously by hand to prevent formation of $MgSO_4$ aglomerates. Then, the tube was centrifuged at 3000 rpm for 2 min.

For the cleanup step, 1 mL of the upper MeCN extract was poured into a d-SPE tube containing 150 mg $MgSO_4$ and 50 mg PSA (exceptionally, tubes used for orange extract purification contained 150 mg $MgSO_4$, 50 mg PSA and 50 mg C_{18}). The tubes were shaken on a Vortex for 30 s and centrifuged

at 3000 rpm for 2 min. 500 μL of the extract were transferred into an evaporation graduated tube containing 1 mL of toluene and evaporated to approximately 300 μL under a gentle nitrogen stream at 50 ºC. The extracts were fortified with standard pesticides at 10 ng/mL and adjusted to a final volume of 500 μL with toluene.

3. Results and discussion

3.1. Selection of pesticides

In order to fully demonstrate the capabilities of the APCI source, 25 pesticides were selected on the basis of their mass spectral behavior in the EI source. The compounds selected can be problematic for the development of GC-MS/MS methods under EI conditions, due to the high fragmentation suffered and the subsequent difficulty to select appropriate MS-MS transitions. These pesticides were divided into three groups as a function of their EI mass spectra:

- Group 1: compounds for which the absence of the $M^{+\cdot}$ in electron ionization spectra forces the selection of an abundant fragment ion (i.e. with lower *m/z*) as the precursor ion in MS/MS, leading to a possible loss in specificity. Representative examples are found in pesticides as pyriproxifen and buprofezin. In these cases, the fragmentation of the molecular ion $M^{+\cdot}$ (321 for pyriproxifen and 305 for buprofezin) leads to a EI spectrum mostly "dominated" by the presence of fragment ions with low *m/z*. Consequently, the commonly selected transitions for them have M/2 or M/3 as precursor ions.

- Group 2: highly fragmented compounds, leading in EI spectra rich in fragment ions. Their mass spectra show many ions, all with poor intensity. Consequently, the transitions selected in MS/MS a method that may not be sensitive enough to perform trace analysis at the low levels expected in the samples. Pesticides belonging to the endosulfan family exhibit this trend, as well as the OC pesticides aldrin, endrin and their isomers.

- Group 3: compounds which EI spectra show strong similarity to other compounds, making then difficult to use selective and specific MS/MS transitions. This usually occurs with pesticides belonging to the same chemical family, as the OP pesticides mevinphos, dicrotophos, monocrotophos and phosphamidon. The same transitions, with *m/z* 127 as precursor ion, are commonly selected for these compounds since it is the base peak of the spectrum for all of them, which correspond to a part of the molecule common to OP

family. In these cases, only retention times allow distinguishing each analyte in a SRM method. However, this situation becomes critical if compounds are nearly eluting, or even coeluting, as may occur with heptachlor epoxide B and oxychlordane. These pesticides can not be included in the same GC-(EI)MS/MS method since they usually elute at the same chromatographic time and no specific transitions can be found for oxychlordane. This problem is even worse when trying to carry out a non-target screening analysis in scan mode, as the similarity in the mass spectra can lead to a doubtful identification.

3.2. Full scan experiments

Once the model analytes were selected, pesticide standards in solvent were used to study their ionization under APCI source. All APCI spectra were obtained, evaluated and compared with EI spectra. The behavior of the molecular ion of each analyte under both ionization conditions is summarized in **Table 1**. As it can be seen, these analytes either do not show $M^{+\cdot}$ under EI or its abundance is very low. For these reason, most transitions in an EI-based SRM method use a fragment as precursor ion. In some occasions, this precursor ion has a low *m/z* value, as in the transition 105>77 used for for buprofezin (M = 305). A clear improvement was observed when using APCI under charge-transfer conditions: several compounds (18 out of 25) exhibited $M^{+\cdot}$, although it was not the base peak of the spectrum in most cases. As an example, **Figure 1** shows buprofezin spectra in the EI and APCI sources. The $M^{+\cdot}$ (305) was practically absent in the EI spectrum (**Figure 1a**), while its abundance was around 30% in the APCI spectrum (**Figure 1b**). In addition, rather different fragmentation patterns were observed in EI and APCI.

Most analytes (17 out of 25) presented the $[M+H]^+$ ion in the charge-transfer APCI spectrum, even together with $M^{+\cdot}$ (**Figure 1b)**. This behavior can be understood considering the two possible ionization mechanisms in APCI, as explained in a recent work [29]: charge transfer, yielding typically $M^{+\cdot}$, or protonation, which promotes the formation of the protonated molecule $[M+H]^+$. In the primary ionization event, the nitrogen plasma (N_2^+ and N_4^+) created by the corona discharge needle is the responsible of the analyte molecules ionization. The second mechanism takes place due to the

presence of water vapor traces in the source, which reacts with the nitrogen plasma ions and leads to the generation of $H_3O^{+\cdot}$ that produces analyte $[M+H]^+$ ions by a proton transfer process.

Considering the observed tendency of pesticides to be protonated, an additional experiment was performed enhancing the proton-transfer conditions by introducing water as modifier in the APCI source. Better results than those obtained under charge-transfer conditions were obtained, since the $[M+H]^+$ was present for most compounds (21 out of 25). In addition, $[M+H]^+$ was commonly the peak base of a low fragmented spectrum. A representative example is shown in **Figure 1c** (buprofezin), which APCI spectrum using water as modifier showed high abundance of $[M+H]^+$, and also low fragmentation. Moreover, some pesticides such as terbufos, azinphos methyl, azinphos ethyl, aldrin, oxychlordane and heptachlor epoxide B, that did not show an abundant $M^{+\cdot}$ (or $[M+H]^+$) with charge-transfer conditions, increasing slightly their abundance under proton-transfer enhanced conditions.

It was concluded that the use of water as modifier improved the presence of the protonated molecule in the mass spectra of all the studied pesticides. The selection of $[M+H]^+$ as precursor ion would allow developing a SRM method with more specific transitions, solving problematic cases included in group 1. Thus, in the aforementioned example of buprofezin, the transition 105>77 could be replaced by another one using 306 ($[M+H]^+$) as precursor ion.

On the other hand, pesticides belonging to group 2 (e.g. endosulfan family), experimented a very different fragmentation behavior under both ionization modes. As it can be seen in the **Figure 2**, $M^{+\cdot}$ for endosulfan ether and endrin was practically absent, and numerous fragment ions constituted the EI spectrum. On the contrary, the APCI spectrum was characterized by high abundance of the $[M+H]^+$ cluster and by low fragmentation, with $[M+H]^+$ being the base peak of the spectrum.

In the last group of pesticides (group 3), common transitions have to be selected in EI. They would be spectrally resolved only if transitions coming from the molecular ion were used, as it makes the difference between the molecules. An example is shown in **Figure 3** for mevinphos, dicrotophos,

monocrotophos and phosphamidon. The EI spectra of these OP pesticides are characterized by the presence of the same fragment ion (*m/z* 127) as the base peak (**Figure 3a).** All transitions coming from 127 as parent ion are common to these four pesticides, as it corresponds to the same chemical structure. Selecting more specific ions (e.g. *m/z* 192, 193, 264) are possible and this would lead to more specific transitions but with a dramatic loss of sensitivity. However, in APCI (using water as modifier), all of them showed its corresponding $[M+H]^+$ with satisfactory sensitivity (**Figure 3b**) (it was the base peak of the spectrum for some of them); so specific transitions can be selected for each compound using the protonated molecule as precursor ion. Thus, pairs of compounds belonging to the group 3 could be also spectrally resolved by APCI selecting the $[M+H]^+$ as precursor ion.

3.3. Optimization of the cone voltage

Once the ions are formed in the APCI source, they are sampled through the skimmer cone by generating a voltage, which can be optimized for each ion. Small voltages can lead to poor ion sampling, and too high voltages can lead to fragmentation before reaching the mass analyzer. In the case of our APCI source, the nozzle of the skimmer cone is big enough to reduce the influence of the cone voltage. The effect of the cone voltage was studied in the range 10-50 V and, in all cases, values over 40 V led to a loss in sensitivity. As it is shown in Table 2, for the pesticides studied optimum values ranged from 10 to 40 V, although differences in sensitivity were small.

3.4. Optimization of the flow rate of He

Carrier gas (He) flow rates of around 1 mL/min are typically applied in GC-(EI)MS since they provide a good compromise between column efficiency -according to the van Deemter equation for 0.25 mm internal diameter capillary columns used- and ionization efficiency of the EI source. However, when using an APCI source, higher carrier gas flow rates (2 mL/min) can be used without loss in ionization performance, thus allowing an improvement in detection response, enhancing resolution of critical pairs of components. APCI full scan chromatograms acquired at flow rates 1.2 and 2 mL/min, corresponding to linear velocities of 40 and 52 cm/s respectively, were evaluated and

compared. As can be seen in **Figure 4**, the reduction of peak width in the last eluting compounds using 2 mL/min led to an improvement of the resolution, as occurs with fenarimol and azinphos ethyl, which could not be chromatographically resolved using 1.2 mL/min as flow rate. Moreover, early eluting compounds as dichlorvos, dicrotophos and monocrotophos showed an improvement of the peak shape. No significant differences were observed on the chromatographic peaks for the rest of compounds. Thus, 2 mL/min was selected as carrier gas flow rate.

3.5. Product ion scan experiments

Product ion scan experiments were performed in order to find selective transitions based on the use of $[M+H]^+$ as precursor ion. Moreover, as $[M+H]^+$ was the base peak of the mass spectrum for most compounds, satisfactory sensitivity was achieved in most cases. Product ion scan was performed at different collision energies (10, 20 and 30 eV) and the most sensitive transitions were selected for the development of the subsequent SRM method.

For those pesticides which $[M+H]^+$ peak showed low abundance, as azinphos methyl or azinphos ethyl, an alternative precursor ion was also selected. This pair of pesticides represents another example of compounds included in group 3; so the selection of specific transitions had to be carefully studied when a precursor ion different than $[M+H]^+$ had to be selected. **Figure S1a** (supplementary data) shows that the EI spectra of both pesticides are practically identical, with the absence of $M^{+\cdot}$; so the same transitions (normally coming from the fragment ion *m/z* 160) have to be used in conventional SRM methods for both compounds. On the contrary, different fragmentation patterns were observed in APCI spectra (**Figure 1Sb,** supplementary data), so that the selection of different precursor ions is possible. In the case of azinphos ethyl, *m/z* 289 was selected as precursor ion since it was the most abundant non-common peak. However, the best precursor ion for azinphos methyl (expectedly m/z 261) was also present in the APCI spectrum of azinphos ethyl, so its selection could not ensure the desirable specificity in single MS. In order to study whether tandem MS improve specificity, product ion scan spectra from m/z 261 were compared for both pesticides with the result that different product ions were present (see **Figure S2,** supplementary data), allowing an adequate

GC-MS/MS determination of both compounds. Thus, it is feasible using m/z 261 as precursor ion for azinphos methyl determination without losing specificity due to the presence of azinphos ethyl.

3.6. SRM method

Once selected the best combinations for precursor and product ions and established the optimum collision energies, a SRM method was developed. Experimental MS/MS parameters for each compound are shown in **Table 2**. Three transitions were chosen for each compound using $[M+H]^+$ as precursor ion for all of them with the exception of terbufos, endosulfan sulfate, azinphos methyl and azinphos ethyl, in which the selection of an alternative precursor ion improved method sensitivity, without compromising specificity.

In the new generations of triple quadrupole instruments (as the one used in this work), sensitivity and repeatability of response are not affected by the dwell time used during acquisition. In this work, dwell times as low as 10 ms were used without resolution and sensitivity losses. This value was automatically calculated by the software depending on the peak width, number of points per peak desired and number of transitions acquired simultaneously. Values from 10 to 250 ms were used to obtain 12 points per peak.

SRM chromatograms acquired under EI and APCI sources were compared to corroborate the potential of APCI to solve problematic cases. The pair oxychlordane and heptachlor epoxide B represents another case included in the group 3 since both pesticides have most transitions in common. Moreover, the coincidence in the retention times makes their simultaneous determination troublesome in a SRM method. As **Figure 5a** shows, when transition 235 > 141 (typical for oxychlordane) was acquired, a peak due to heptachlor epoxide B was also present using the EI source. However, the use of 421 > 151 (421 corresponds to the $[M+H]^+$ ion of oxychlordane) in APCI allows the determination of oxychlordane without interference from heptachlor epoxide B (**Figure 5b**).

A similar case was observed for chlorpyriphos methyl and chlorpyriphos ethyl. Transitions selected in EI for both pesticides are not specific. In contrast, transitions coming from $[M+H]^+$ in APCI

source allowed their spectral differentiation, although this case was less critical since they were chromatographically resolved.

3.7. Instrumental analytical characteristics

The developed SRM method was applied to evaluate instrumental linearity and precision under APCI conditions using water as modifier. Linearity was studied by injecting standards in solvent (n=2) in the range 0.1-100 ng/mL (corresponding to 0.1-100 µg/kg in sample). Most compounds showed a linear tendency from 0.1 to 100 ng/mL with $r^2 > 0.99$ and residuals lower than 30%.

Precision of the method was estimated from area responses obtained after repeated injections of a 0.5 ng/mL standard (n=10) and expressed as repeatability in terms of relative standard deviation (R.S.D., %). Results were satisfactory since R.S.D. were lower than 20% for all compounds.

In order to study the applicability of the developed chromatographic method, apple, orange, tomato and carrot blank samples were extracted applying the QuEChERS method, and the extracts obtained were spiked at 10 ng/mL (corresponding to 10 µg/kg in sample) with all studied pesticides. Responses for spiked samples were compared to those of standards in solvent. Product ion spectra were evaluated in all the matrices and no significant differences in the fragmentation pattern were observed with respect to standards in solvent. Thus, it seemed that matrix effects did not affect to the fragmentation in the collision cell.

SRM chromatograms of all pesticides/matrix combinations were evaluated. No significant matrix effects were observed, although a little enhancement of the response in the sample extracts occurred. **Figure 6** illustrates representative examples for ethion and fenarimol in tomato and carrot samples, respectively. Satisfactory chromatographic peaks were obtained for all compounds at 10 ng/mL, as well as satisfactory sensitivity that led to an estimation of LOQ (calculated as 10 times S/N) between 0.02-2 µg/kg depending on the analyte and matrix under study. The good selectivity achieved in this case contrast with the results obtained in a previous work [34], in which ethion and fenarimol could not be adequately determined in these samples due to the heavy matrix interferences observed when

applying a GC-MS SIM method (using a single quadrupole). Therefore, in the present work the use of GC-MS/MS combined with an APCI source provided a notable increase in sensitivity and selectivity.

4. Conclusions

Spectral fragmentation of 25 selected pesticides has been evaluated using a GC(APCI)-MS/MS system with triple quadrupole analyzer. The soft ionization process in the APCI source has favored the presence of the $M^{+\cdot}$ and/or $[M+H]^+$ ion in the spectrum of most pesticides, inversely to the pattern fragmentation typically observed under EI ionization mode. Moreover, the use of water as modifier has enhanced the sensitivity for $[M+H]^+$, which was the base peak of the spectrum in most cases. Thus, further experiments were performed under these conditions.

Pesticides under study were sorted into three model groups as a function of their fragmentation pattern under EI source. For the wide majority, APCI demonstrated a strong potential to improve the tandem MS determination in comparison with EI mode because of the possibility to select a more abundant and specific precursor ion in SRM mode. This occurred for highly fragmented pesticides, for which a fragment ion with low *m/z* should be used as precursor ion (group 1), as well as for those compounds which transitions showed poor sensitivity (group 2). The use of APCI allowed in these cases to select more specific and sensitive transitions using $[M+H]^+$ as precursor ion. Some pesticides (group 3) presented spectral fragmentation similar to other compounds, normally belonging to the same chemical family, and APCI offered the possibility to spectrally resolve them selecting their respective $[M+H]^+$ as precursor ion.

Product ion scan experiments have been also performed to evaluate the fragmentation of the correspondent $[M+H]^+$. Abundant product ions were normally achieved, and this facilitated the application of tandem MS methods selecting $[M+H]^+$ as precursor ion. GC-MS/MS making use of APCI offers interesting features in comparison to EI source for quantitative multiresidue analysis, since transitions selected can be more specific and sensitive. Preliminary experiments on linearity and precision of the GC-(APCI)-MS/MS method have been performed with satisfactory results. The applicability of the method has been also tested in real samples comparing responses of fortified blank QuEChERS extracts and standards in solvent. Matrix effects have not been observed in terms of fragmentation behavior and no interferences have been found in SRM transitions.

Results obtained in this work are optimistic for future improvements of multiresidue quantitative analysis, although further experiments are still required to fully evaluate the potential of this approach in environmental applications and/or in food safety.

Acknowledgements

This work has been developed under financial support of Bancaixa (P1-1B2009-25). The authors acknowledge the financial support of Generalitat Valenciana, as research group of excellence PROMETEO/2009/054, and are very grateful to the Serveis Centrals d'Instrumentació Científica (SCIC) of the University Jaume I for the use of GC-(APCI)(QqQ) MS/MS Xevo TQ-S. T. Portolés is very pleased to Conselleria de Educación, Formación y Empleo for her postdoctoral grant.

References

[1] L. Alder, K. Greulich, G. Kempe, B. Vieth, Mass Spectrom. Rev. 25 (2006) 838.

[2] S.J. Lehotay, K. Mastovska, A. Amirav, A.B. Fialkov, P.A. Martos, A.d. Kok, A.R. Fernández-Alba, TrAC Trends Anal. Chem. 27 (2008) 1070.

[3] F.J. Santos, M.T. Galceran, J. Chromatogr. A 1000 (2003) 125.

[4] S.D. Richardson, Anal. Chem. 84 (2012) 747.

[5] T. Pihlström, G. Blomkvist, P. Friman, U. Pagard, B.-. Österdahl, Anal. Bioanal. Chem. 389 (2007) 1773.

[6] C. Soler, J. Mañes, Y. Picó, Crit. Rev. Anal. Chem. 38 (2008) 93.

[7] S. Grimalt, J. V.Sancho, O.J. Pozoa, F.E. Hernándeza, J. Mass Spectrom. 45 (2010) 421.

[8] F. Hernández, T. Portolés, E. Pitarch, F.J. López, J. Mass Spectrom. 44 (2009) 1.

[9] T.R. Covey, B.A. Thomson, B.B. Schneider, Mass Spectrom. Rev. 28 (2009) 870.

[10] L. Amendola, F. Botrè, A.S. Carollo, D. Longo, L. Zoccolillo, Anal. Chim. Acta 461 (2002) 97.

[11] C.-. Shen, X.-. Cao, W.-. Shen, Y. Jiang, Z.-. Zhao, B. Wu, K.-. Yu, H. Liu, H.-. Lian, Talanta 84 (2011) 141.

[12] Q. Guo, M. Deng, B. Yu, L. Tan, J. AOAC Int. 93 (2010) 295.

[13] L. Cherta, J. Beltran, T. Portolés, F. Hernández, Anal. Bioanal. Chem. 402 (2012) 2301.

[14] D. Steiniger, G. Lu, J. Butler, E. Phillips, Y. Fintschenko, J. AOAC Int. 93 (2010) 1169.

[15] M.I. Cervera, C. Medina, T. Portolés, E. Pitarch, J. Beltrán, E. Serrahima, L. Pineda, G. Muñoz, F. Centrich, F. Hernández, Anal. Bioanal. Chem. 397 (2010) 2873.

[16] F.J. Camino-Sánchez, A. Zafra-Gómez, J. Ruiz-García, R. Bermúdez-Peinado, O. Ballesteros, A. Navalon, J.L. Vílchez, J. Food Compos. Anal. 24 (2011) 427.

[17] J.L. Martínez Vidal, F.J. Arrebola Liébanas, M.J. González Rodríguez, A. Garrido Frenich, J.L. Fernández Moreno, Rapid Commun. Mass Spectrom. 20 (2006) 365.

[18] J.W. Wong, K. Zhang, K. Tech, D.G. Hayward, C.M. Makovi, A.J. Krynitsky, F.J. Schenck, K. Banerjee, S. Dasgupta, D. Brown, J. Agric. Food Chem. 58 (2010) 5868.

[19] U. Koesukwiwat, S.J. Lehotay, N. Leepipatpiboon, J. Chromatogr. A 1218 (2011) 7039.

[20] R. Húšková, E. Matisová, S. Hrouzková, L. Švorc, J. Chromatogr. A 1216 (2009) 6326.

[21] J. Dong, Y.-. Pan, J.-. Lv, J. Sun, X.-. Gong, K. Li, Chromatographia 74 (2011) 109.

[22] E. Jover, J. Maria Bayona, J. Chromatogr. A 950 (2002) 213.

[23] E.C. Horning, D.I. Carroll, I. Dzidic, Clin. Chem. 23 (1977) 13.

[24] W.A. Korfmacher, L.G. Rushing, J. Arey, B. Zielinska, J.N. Pitts Jr., HRC & CC J High Resolut Chromatogr Chromatogr Commun 10 (1987) 641.

[25] W.A. Korfmacher, L.G. Rushing, R.J. Engelbach, J.P. Freeman, Z. Djuric, E.K. Fifer, F.A. Beland, HRC & CC J High Resolut Chromatogr Chromatogr Commun 10 (1987) 43.

[26] R. Schiewek, M. Lorenz, R. Giese, K. Brockmann, T. Benter, S. Gäb, O.J. Schmitz, Anal. Bioanal. Chem.(2008) 1.

[27] C.N. McEwen, Int. J. Mass Spectrom. 259 (2007) 57.

[28] C.N. McEwen, R.G. McKay, J. Am. Soc. Mass Spectrom., 16 (2005) 1730.

[29] T. Portolés, J.V. Sancho, F. Hernández, A. Newton, P. Hancock, J. Mass Spectrom. 45 (2010) 926.

[30] T. Bristow, M. Harrison, M. Sims, Rapid Commun. Mass Spectrom. 24 (2010) 1673.

[31] R. García-Villalba, T. Pacchiarotta, A. Carrasco-Pancorbo, A. Segura-Carretero, A. Fernández-Gutiérrez, A.M. Deelder, O.A. Mayboroda, J. Chromatogr. A 1218 (2011) 959.

[32] A. Carrasco-Pancorbo, E. Nevedomskaya, T. Arthen-Engeland, T. Zey, G. Zurek, C. Baessmann, A.M. Deelder, O.A. Mayboroda, Anal. Chem. 81 (2009) 10071.

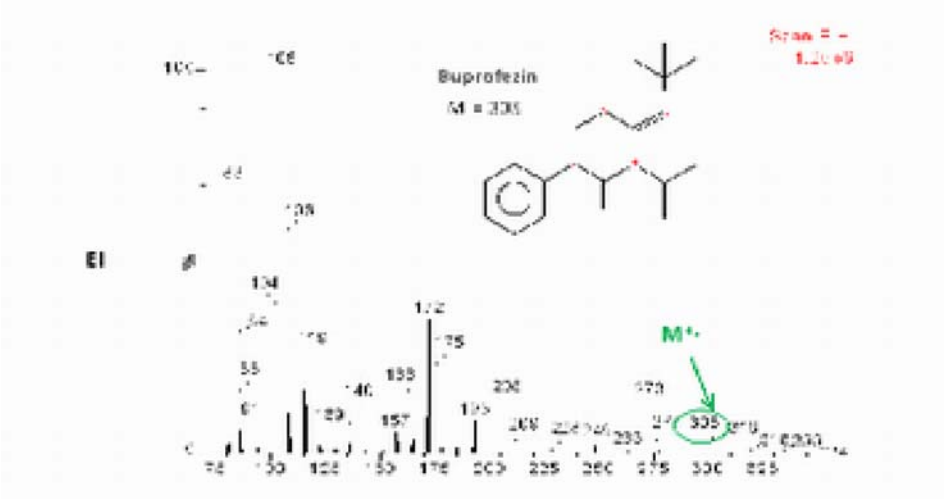
[33] S.J. Lehotay, K. Maštovská, A.R. Lightfield, J. AOAC Int. 88 (2005) 615.

[34] L. Cherta, J. Betran, F. López, F. Hernández. Application of fast GC MS in combination to quechers method for the determination of pesticide residues in fruits and vegetables. Article submitted.

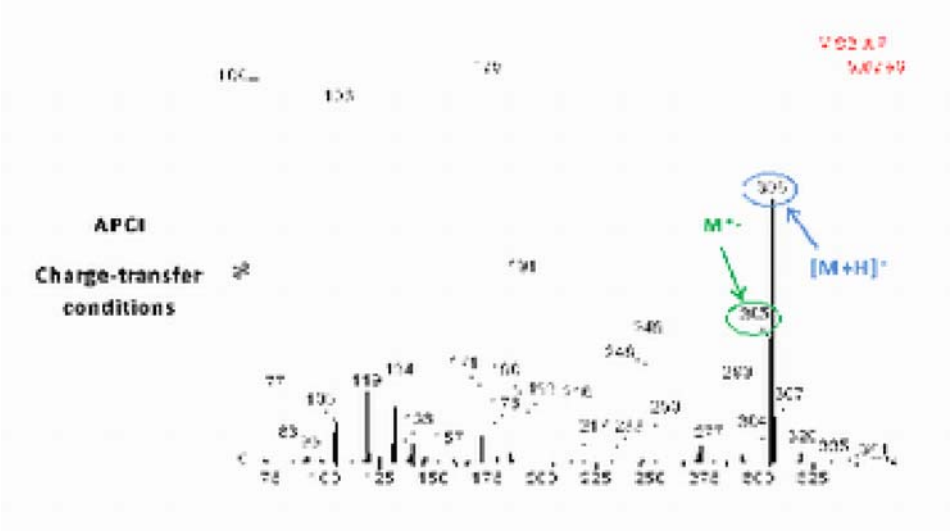
Figures.

Figure 1. Comparison of buprofezin spectrum using: a) EI source, b) APCI source under charge-transfer conditions and c) APCI under proton-transfer conditions.

a)



b)



c)

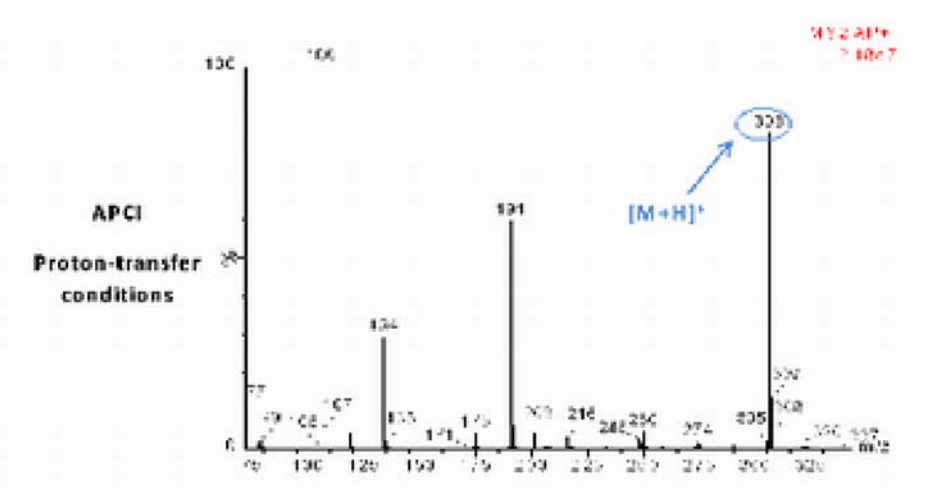
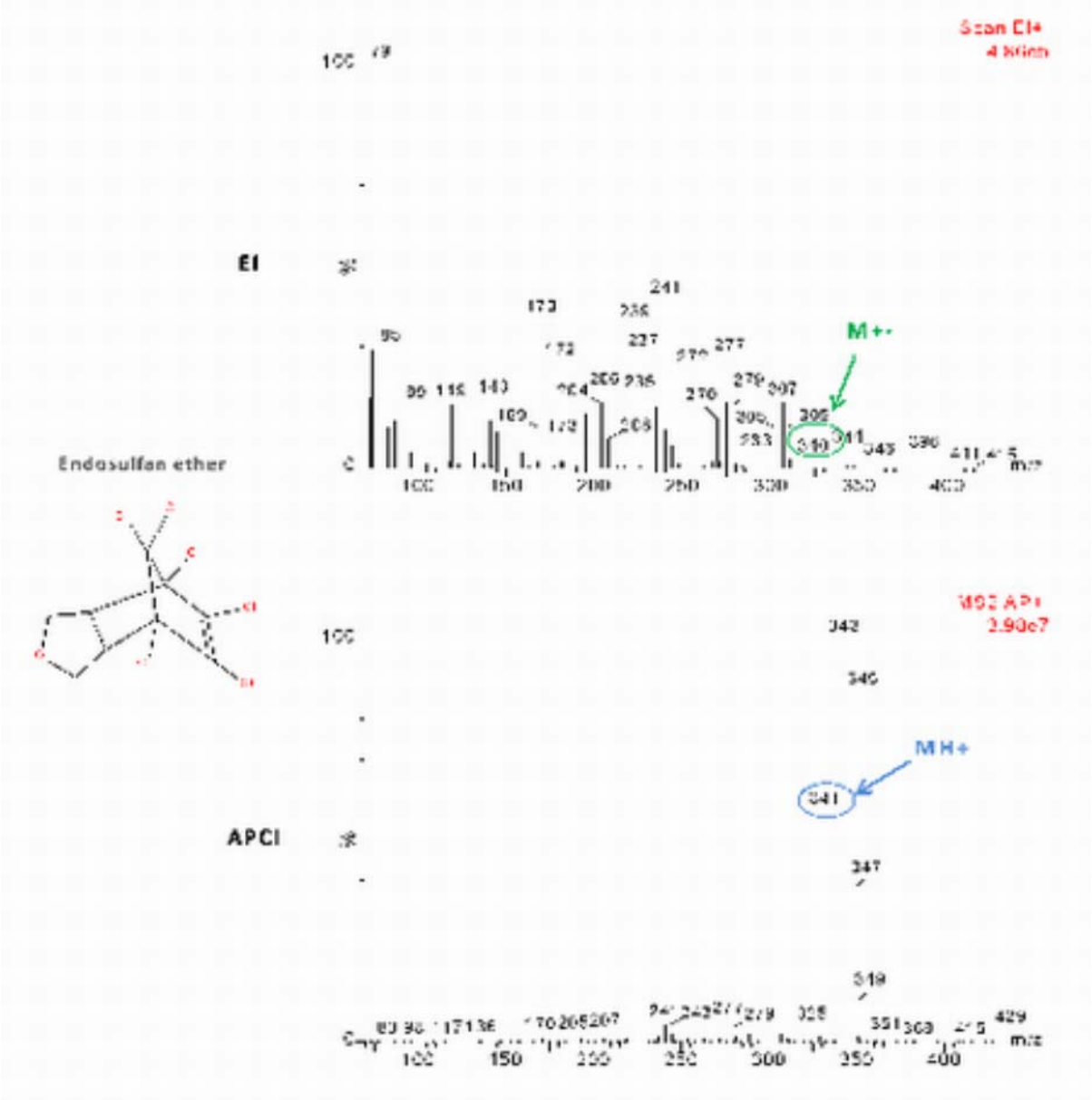


Figure 2. Full scan spectra obtained under EI (top) and APCI using water as modifier (bottom) for: a) endosulfan ether and b) endrin.

a)



b)

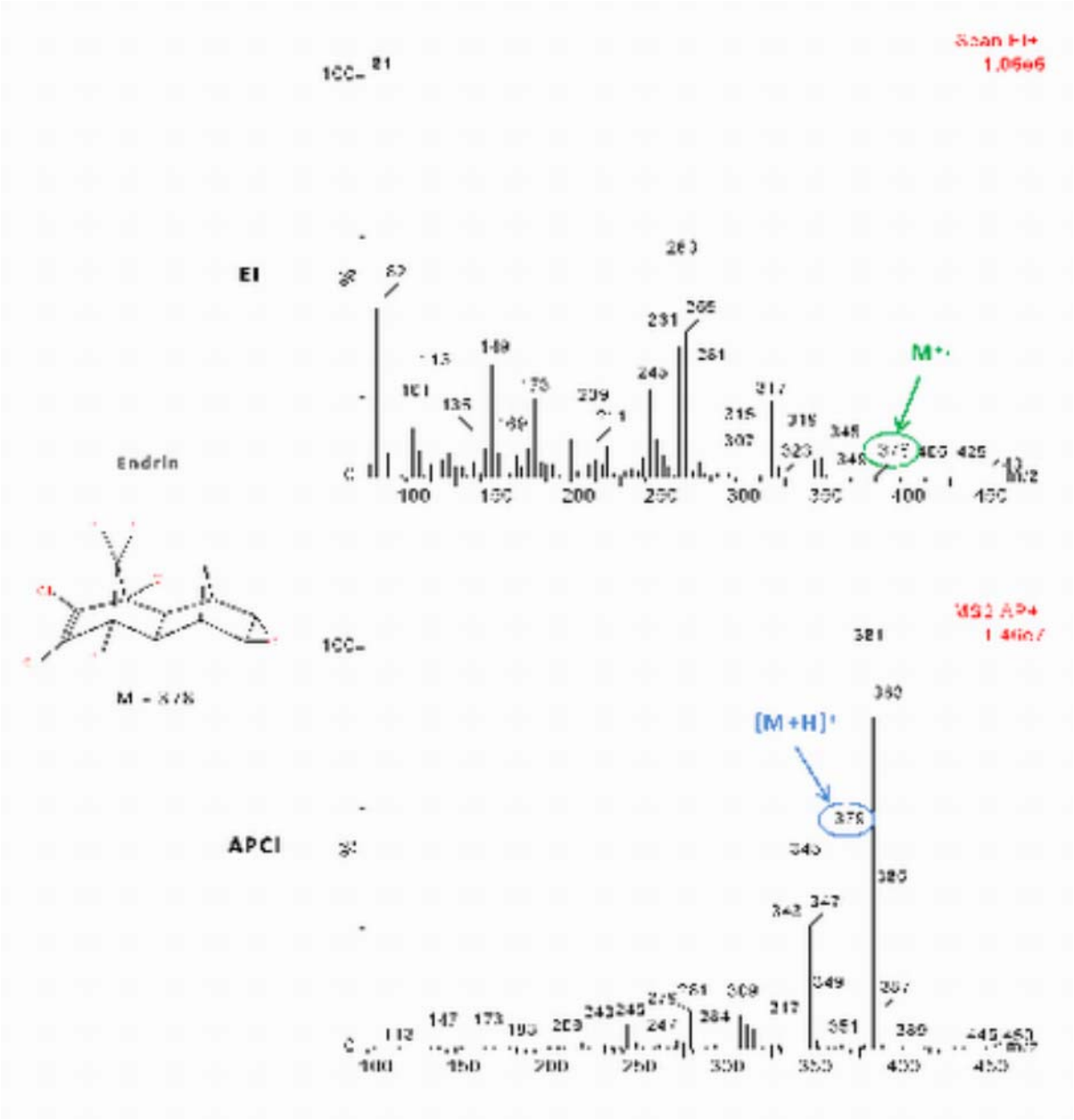


Figure 3. Comparison of full scan spectra obtained under a) EI and b) APCI using water as modifier for the OP pesticides mevinphos, dicrotophos, monocrotophos and phosphamidon.

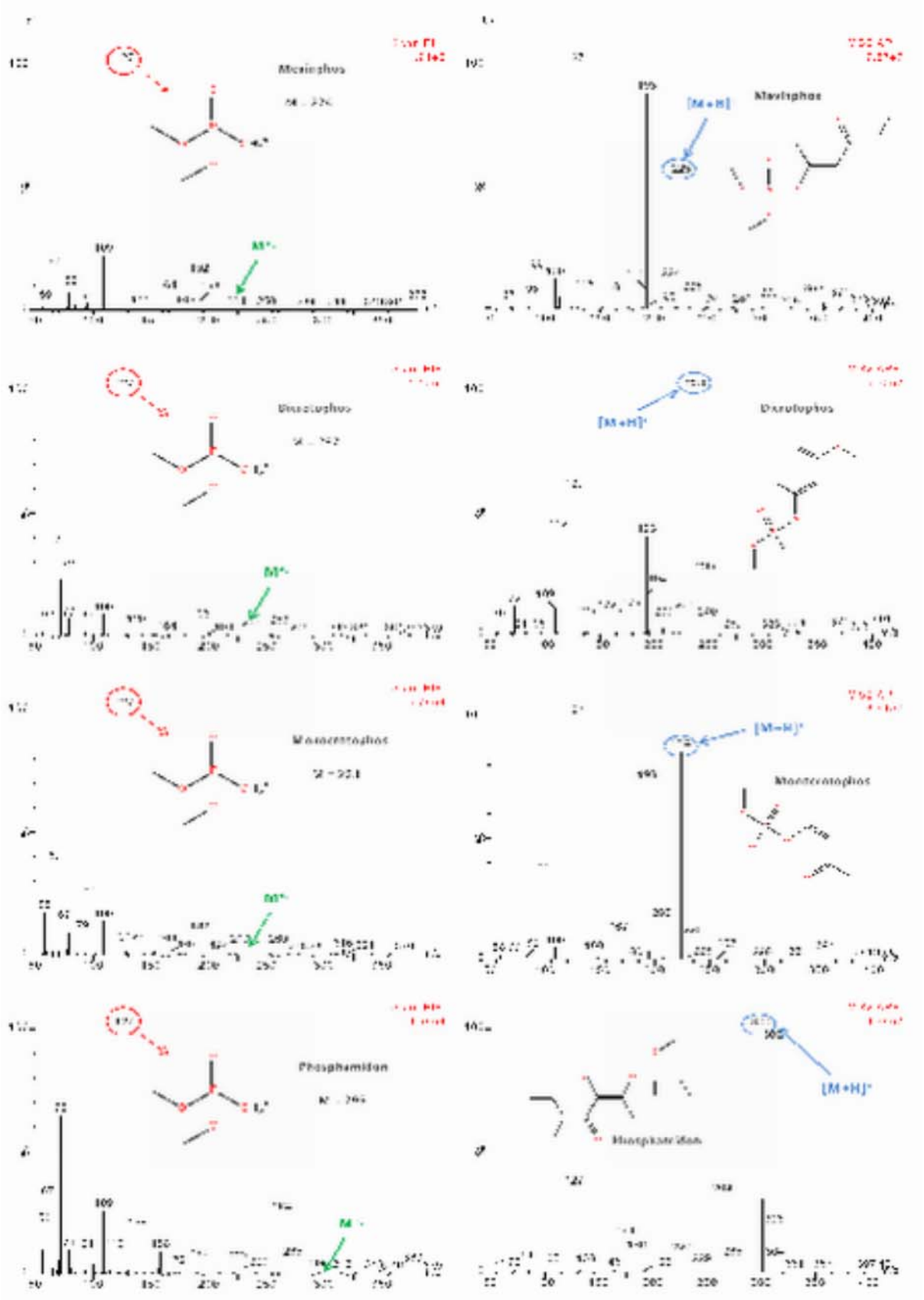

Mevinphos
[M+H]
Dicrotophos
[M+H]
Monocrotophos
[M+H]
Phosphamidon
[M+H]

Figure 4. Total ion GC-MS chromatogram of fenarimol and azinphos ethyl using 1.2 mL/min and 2 mL/min as flow rate.

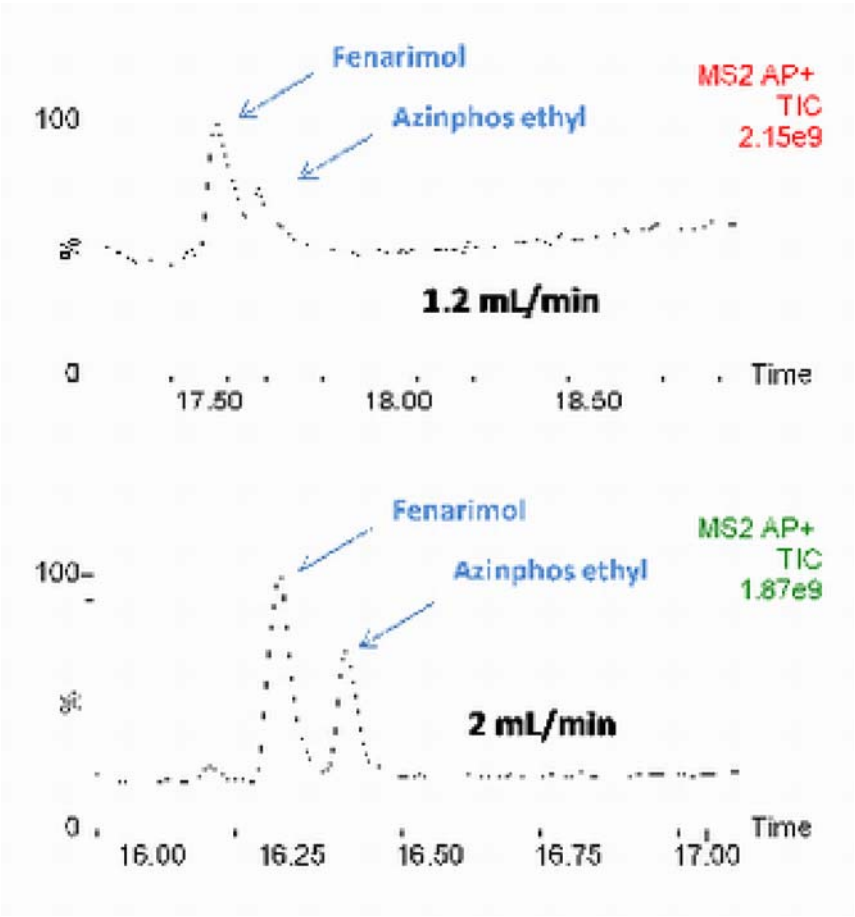


Figure 5. Chromatograms obtained for the selected SRM transitions acquired for heptachlor epoxide B (top) and oxychlordane (bottom) under a) EI (100 ng/mL) and b) APCI (10 ng/mL) sources.

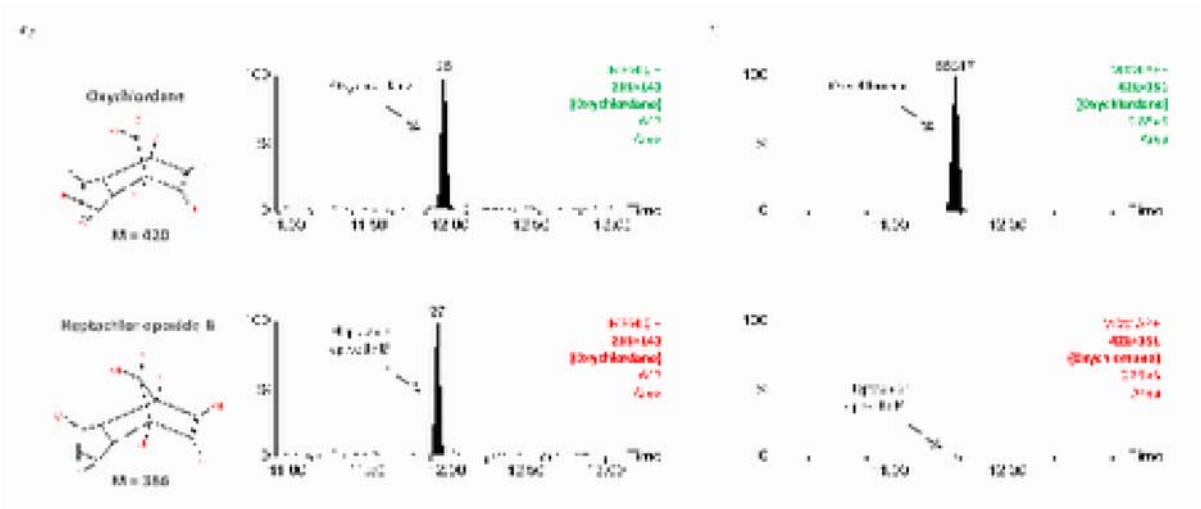
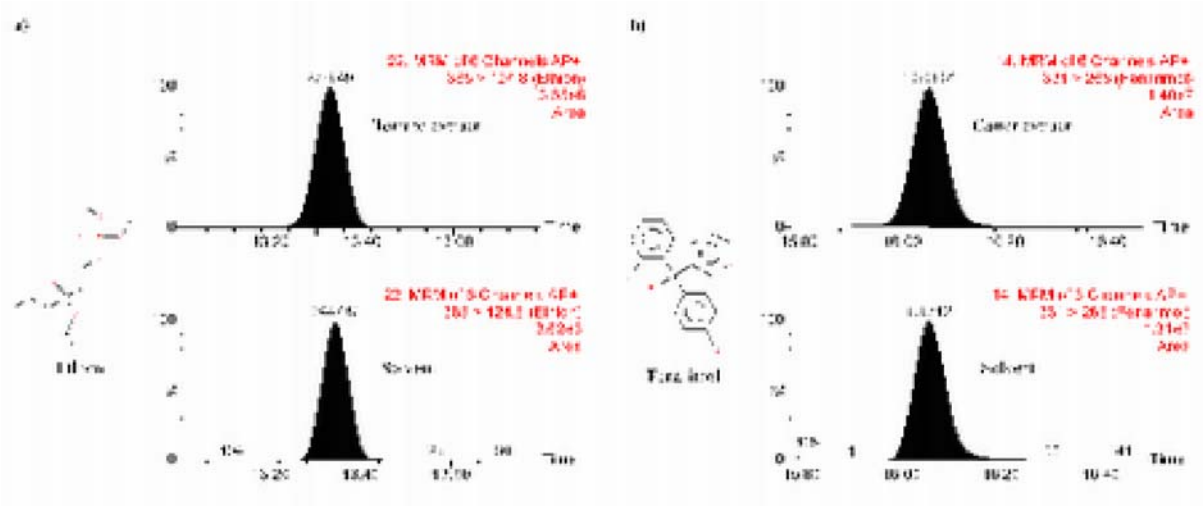


Figure 6. SRM chromatograms for a) ethion in spiked tomato extract (0.01 ng/mL) and in solvent standard (0.01 ng/mL) and b) fenarimol in spiked carrot extract (0.01 ng/mL) and in solvent standard (0.01 ng/mL).



Supplementary data

Figure S1. Comparison of full scan spectra obtained under a) EI and b) APCI using water as modifier for azinphos methyl and azinphos ethyl.

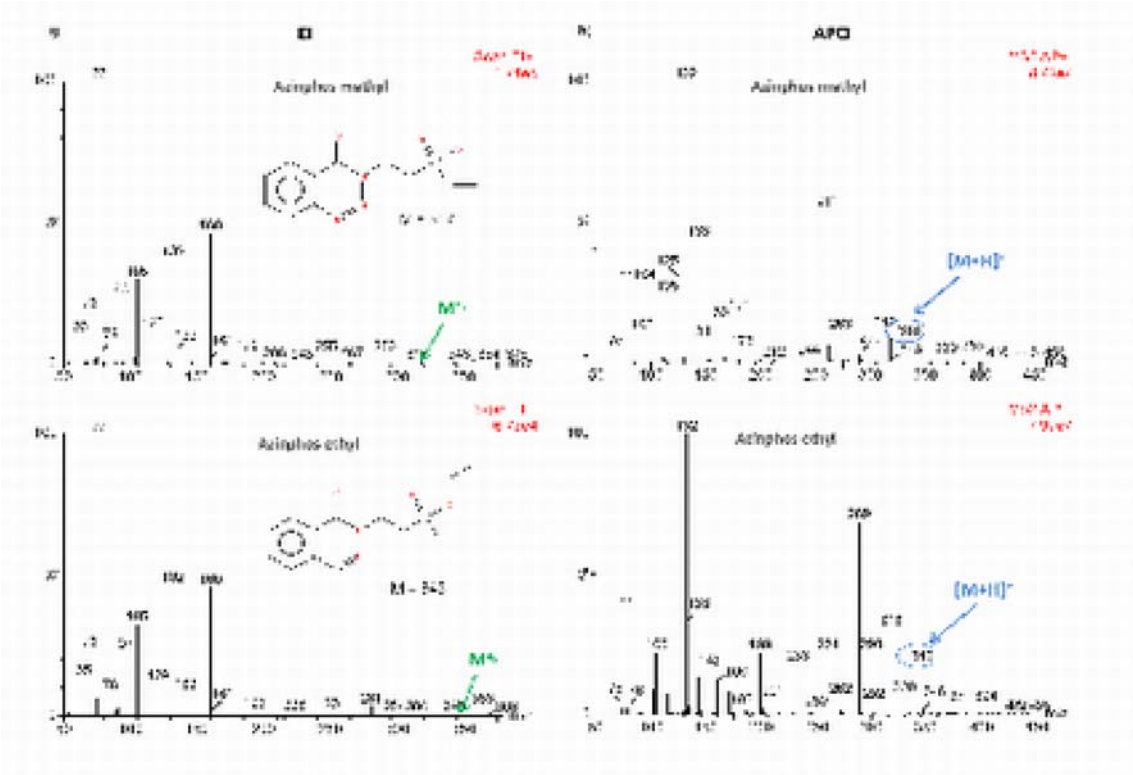


Figure S2. a) Product ion scan spectra of 261 at different collision energies for: a) azinphos methyl and b) azinphos ethyl.

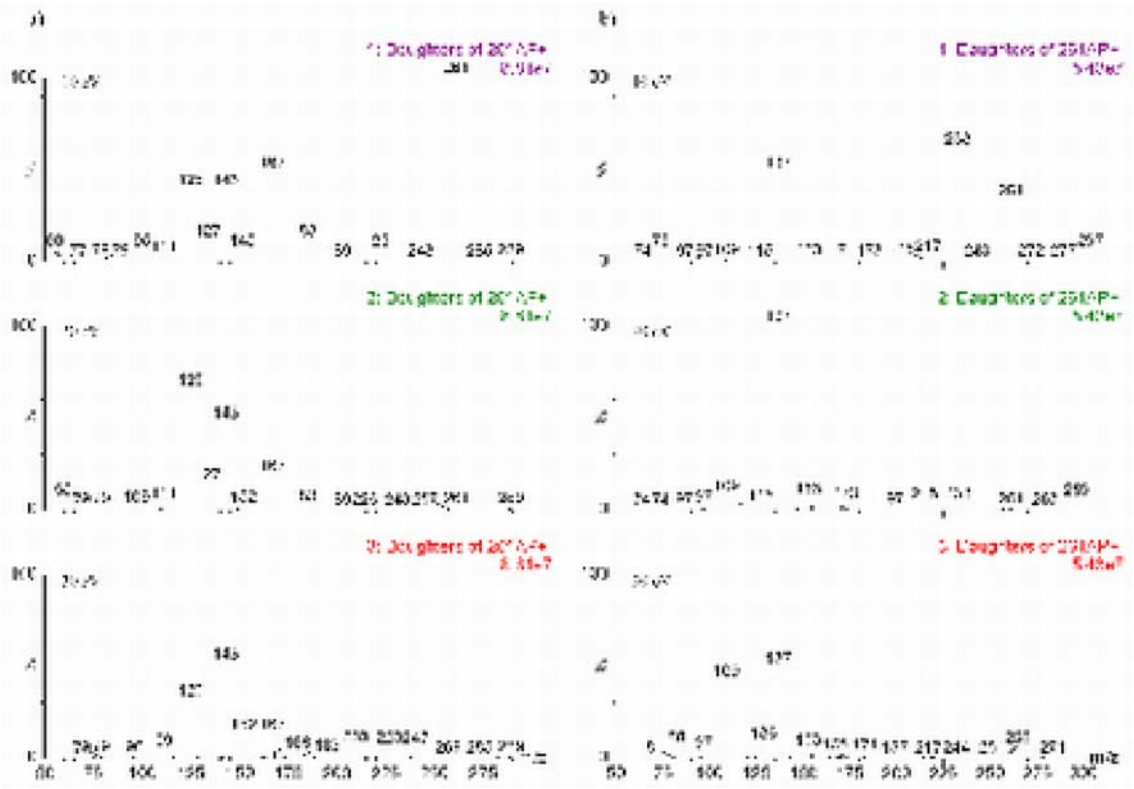


Table 1. Behavior of the molecular ion of the selected pesticides under EI and APCI ionization modes and single quadrupole mass analysis. MS/MS transitions commonly used under EI mode are shown.

t_R	Compounds	Molecular formula	M	EI	APCI		APCI + H_20	EI transitions	
				M+·	M+·	MH+	MH+		
4.70	Dichlorvos	$C_4H_7Cl_2O_4P$	**220**			+++	+++	185>93	109>79
5.97	Mevinphos	$C_7H_{13}O_6P$	**224**		++	+	++	127>109	192>127
6.96	Molinate	$C_9H_{17}NOS$	**187**	+		+++	+++	187>126	126>55
8.00	Dicrotophos	$C_8H_{16}NO_5P$	**237**	+	++	++	+++	127>109	127>95
8.24	Monocrotophos	$C_7H_{14}NO_5P$	**223**	+	++	++	+++	192>127	192>164
8.95	Terbufos	$C_9H_{21}O_2PS_3$	**288**				+	231>129	231>175
9.80	Phosphamidon	$C_{10}H_{19}ClNO_5P$	**299**		+	++	+++	127>109	264>127
9.76	Endosulfan ether	$C_9H_6Cl_6O$	**340**	+	+	++	++	272>237	239>204
9.94	Chlorpyriphos methyl	$C_7H_7Cl_3NO_3PS$	**321**			++	+++	288>93	197>169
10.77	Chlorpyriphos	$C_9H_{11}Cl_3NO_3PS$	**349**		+	+++	+++	199>171	316>260
10.85	Aldrin	$C_{12}H_8Cl_6$	**362**		+	+	++	261>191	263>193
11.39	Isodrin	$C_{12}H_8Cl_6$	**362**		++	++	++	193>157	195>123
11.56	Chlorfenvinphos	$C_{12}H_{14}Cl_3O_4P$	**358**			++	+++	267>159	323>267
11.56	Oxychlordane	$C_{10}H_4Cl_8O$	**420**		+	+	++	185>121	235>141
11.56	Heptachlor epoxide B	$C_{10}H_5Cl_7O$	**386**		+	+	++	355>265	351>261
12.23	Endosulfan I	$C_9H_6Cl_6O_3S$	**404**			++	++	239>204	272>237
12.72	Buprofezin	$C_{16}H_{23}N_3OS$	**305**	+	++	+++	+++	105>77	172>115
12.73	Dieldrin	$C_{12}H_8Cl_6O$	**378**	+	++	++	++	263>193	261>191
13.10	Endrin	$C_{12}H_8Cl_6O$	**378**		++	++	++	263>193	261>191
13.36	Ethion	$C_9H_{22}O_4P_2S_4$	**384**		+	+++	+++	231>129	231>175
14.01	Endosulfan sulfate	$C_9H_6Cl_6O_4S$	**420**		++		++	274>239	272>237
15.63	Azinphos methyl	$C_{10}H_{12}N_3O_3PS_2$	**317**				+	160>77	160>132
15.66	Pyriproxyfen	$C_{20}H_{19}NO_3$	**321**			++	+++	136>96	136>78
16.04	Fenarimol	$C_{17}H_{12}Cl_2N_2O$	**330**	+	++	+++	+++	251>139	219>107
16.17	Azinphos ethyl	$C_{12}H_{16}N_3O_3PS_2$	**345**				+	160>132	160>77

+ → very small peak
++ → clearly identificable peak (>20%)
+++ → base peak (or >80%)

Table 2. Experimental conditions of the optimized GC-(APCI)MS/MS method using water as modifier. Quantifier (Q) and qualifier (qi) transitions.

t_R (min)	Window (min)	Compounds	Cone voltage (v)	MS/MS Transitions		Collision energy (eV)
4.70	3.0-5.0	Dichlorvos	10	Q	221 > 145	10
				q_1	221 > 113	30
				q_2	221 > 127	20
5.97	5.0-6.5	Mevinphos	30	Q	225 > 127	10
				q_1	225 > 113	30
				q_2	225 > 193	10
6.96	6.5-7.5	Molinate	20	Q	188 > 126	10
				q_1	188 > 98	20
				q_2	188 > 160	10
8.00	7.5-9.7	Dicrotophos	40	Q	238 > 112	10
				q_1	238 > 127	20
				q_2	238 > 193	10
8.24		Monocrotophos	20	Q	224 > 127	10
				q_1	224 > 113	30
				q_2	224 > 193	10
8.95		Terbufos	10	Q	187 > 131	10
				q_1	187 > 97	20
				q_2	187 > 113	20
9.80	9.7-10.5	Phosphamidon	40	Q	300 > 127	20
				q_1	300 > 174	10
				q_2	300 > 227	10
9.76		Endosulfan ether	30	Q	341 > 217	30
				q_1	341 > 170	30
				q_2	341 > 205	20
9.94		Chlorpyriphos methyl	40	Q	322 > 125	30
				q_1	322 > 212	30
				q_2	322 > 290	20
10.77	10.5-11.3	Chlorpyriphos	20	Q	350 > 198	20
				q_1	350 > 294	10
				q_2	350 > 322	10
10.85		Aldrin	30	Q	363 > 159	20
				q_1	363 > 215	20
				q_2	363 > 327	10
11.39	11.3-12.0	Isodrin	30	Q	363 > 159	20
				q_1	363 > 215	20
				q_2	363 > 327	10
11.56		Chlorfenvinphos	30	Q	359 > 170	30
				q_1	359 > 99	10
				q_2	359 > 205	20

Table 2 (cont). Experimental conditions of the optimized GC-(APCI)MS/MS method using water as modifier. Quantifier (Q) and qualifier (qi) transitions.

t_R (min)	Window (min)	Compounds	Cone voltage (v)	MS/MS Transitions		Collision energy (eV)
11.56		Oxychlordane	10	Q	421 >151	20
				q_1	421 > 115	20
				q_2	421 > 285	30
11.56		Heptachlor epox B	20	Q	387 > 217	30
				q_1	387 > 251	20
				q_2	387 > 252	10
12.23	12.0-12.6	Endosulfan I	10	Q	405 > 323	10
				q_1	405 > 205	20
				q_2	405 > 217	30
12.72	12.6-13.0	Buprofezin	30	Q	306 > 106	20
				q_1	306 > 203	10
				q_2	306 > 250	10
12.73		Dieldrin	20	Q	379 > 325	10
				q_1	379 > 147	20
				q_2	379 > 261	20
13.10	13.0-13.8	Endrin	30	Q	379 > 343	10
				q_1	379 > 243	20
				q_2	379 > 244	20
13.36		Ethion	10	Q	385 > 125	20
				q_1	385 > 97	10
				q_2	385 > 143	30
14.01	13.8-14.6	Endosulfan sulfate	10	Q	323 > 217	30
				q_1	323 > 252	20
				q_2	323 > 287	10
15.63	14.6-21.0	Azinphos methyl	20	Q	261 > 125	20
				q_1	261 > 167	10
				q_2	261 > 183	10
15.66		Pyriproxyfen	10	Q	322 > 185	20
				q_1	322 > 129	30
				q_2	322 > 227	10
16.04		Fenarimol	40	Q	331 > 268	20
				q_1	331 > 139	30
				q_2	331 > 259	20
16.17		Azinphos ethyl	20	Q	289 > 137	20
				q_1	289 > 233	10
				q_2	289 > 261	10