JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Analytical Study of Trichlorfon Residues in Kaki Fruit and Cauliflower Samples by Liquid Chromatography−Electrospray Tandem Mass Spectrometry

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A detailed analytical study on trichlorfon residues in selected vegetables samples has been carried out, focused on the reliable quantification and confirmation of this compound, and on stability of residues under storage. As a consequence, a rapid and sensitive LC−ESI-MS/MS method has been developed for the determination of residues of this insecticide in kaki fruit (flesh and peel) and cauliflower samples. Extraction was performed with acetonitrile using a high-speed blender. After 4-fold dilution of the extract with water, 20 μL was directly injected in the LC−ESI-MS/MS system (triple quadrupole), using matrix-matched standards calibration for quantification. Under optimized MS/MS conditions, limit of detections between 0.006 and 0.013 mg/kg were reached, and a limit of quantification of 0.05 mg/kg was established, with a runtime of only 15 min. Recoveries from spiked blank samples at 0.05 and 0.5 mg/kg were in the range 83−101% with relative standard deviations lower than 10%. The method was applied to treated and untreated samples collected from field residues trials, using quality control samples analysis for the evaluation of the method. Despite the acquisition of two MS/MS transitions in selected reaction monitoring mode, the analysis of treated samples revealed the presence of a chromatographic peak close to the analyte that corresponded to a trichlorfon isobaric compound that shared the same MS/MS transitions. This unusual situation in LC−MS/MS-based procedures required the application of an efficient chromatographic separation to avoid this interference. All experiments have been made in compliance with the principles of Good Laboratory Practices (GLP) and following the European SANCO guidelines for pesticides residue analysis (PRA).

KEYWORDS: Trichlorfon; pesticide residues; vegetable samples; liquid chromatography; tandem mass spectrometry; triple quadrupole

INTRODUCTION

Kaki fruit (*Diospyros kaki* L.f.) and cauliflower (*Brassica oleracea convar. Botrytis* L.) are extensively consumed commodities and are important products for the economy and export trade of several countries particularly in the Mediterranean area where these crops are well adapted to climatic conditions. However, to obtain a high-quality and healthy crop at harvest, the use of pesticides is normally required, but always under strict regulations. Trichlorfon (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) is widely applied as an insecticide in these crops. This compound is an inhibitor of cholinesterase activity, possibly due to in vivo conversion to dichlorvos (*1*). In the European Community, the maximum residue limit (MRL) allowed for trichlorfon in brassica vegetables and miscellaneous fruits is 0.5 mg/kg (*2*). Analytical methods allowing the reliable determination of concentrations at least 5-fold lower than MRL are advisable to be confident about the reported data.

Trichlorfon residues in fruits and vegetables have been determined until now by gas chromatography (GC) with flame photometric detection (FPD) (*3*, *4*) and electron capture detection (ECD) (*5*). Recently, the use of GC coupled to mass spectrometry (MS) has been considered a suitable approach to improve selectivity and sensitivity (*6*, *7*). Although GC can be an adequate technique for the determination of trichlorfon residues, the trichlorfon instability at high temperature has been reported (*4*, *6*−*8*). The thermal degradation of trichlorfon in the injection port of the GC to dichlorvos can hinder a correct quantification, due to other amounts of dichlorvos that could come from the formulation manufacturing and degradation product from trichlorfon (*6*). To minimize these problems, liquid chromatog-

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10.1021/jf052737j CCC: $33.50
Published on Web 01/26/2006

raphy (LC) appears as a suitable alternative to GC. Recently, one method has been reported for trichlorfon in oranges using LC−APCI-MS. In this application, the determination of trichlorfon at 0.12 mg/kg, among other pesticides, is described, after performing the extraction by matrix solid-phase dispersion, obtaining recoveries between 68% and 74% (*9*).

An increased number of papers have been published in the last 5 years dealing with the use of LC−MS/MS in pesticide residue analysis, demonstrating the great potential of this technique in this field. The excellent sensitivity of LC−MS/MS allows in many cases one to reduce the sample pretreatment, even facilitating the direct injection of the extracts (*10*−*12*). However, one of the main drawbacks of LC−MS/MS methods is the matrix effect in the ionization source, producing an inhibition or enhancement of the MS signal (*13*−*15*). Different possibilities to compensate for this undesirable effect have been proposed as: (i) the use of labeled internal standard (*16*) being the most easy and convenient way although with the limitations of the commercially availability, price, and/or purity of reference standard; (ii) the simple dilution of the extract to give a signal comparable to standard solutions (*17*), with the drawback of the unavoidable loss in sensitivity; and (iii) the quantification with matrix-matched standard calibration (*18*−*20*), which compensates the matrix effect, with the drawback of finding a real representative blank sample and of assuming that all samples present similar matrix effects. In some cases, a combination of two of these approaches is applied, for example, dilution of sample extract and matrix-matched standard calibration (*21*−*23*).

Although LC−MS/MS presents high specificity, it is still possible to report false positive findings. The European Union (*24*) has established confirmation criteria using mass spectrometry based on the collection of identification point (IPs), where a minimum of 4 IPs is required for confirmation of banned compounds and 3 IPs for authorized compounds. Using triple quadrupole analyses (*Qq*Q) in selected reaction monitoring mode (SRM), a minimum of 4 IPs is reached acquiring two MS/MS transitions. Although this European decision is related to products of animal origin, nowadays several examples can be found in the literature making use of more than one transition for the confirmation of positive data, also in products of plant origin (*10*, *22*, *23*, *25*−*27*).

The aim of this work is to develop a rapid, sensitive, and selective method for the quantification and confirmation of trichlorfon residues in kaki fruit (flesh and peel) and cauliflower samples, minimizing the sample pretreatment and reducing in this way the risks of analytical errors associated with this step. A detailed study on potential interferents and on stability of samples under storage is performed as well.

EXPERIMENTAL PROCEDURE

Reagents and Chemicals. Trichlorfon reference standard was purchased from Dr. Ehrenstorfer (Augsburg, Germany). HPLC-grade acetonitrile and methanol were purchased from ScharLab (Barcelona, Spain). LC-grade water was obtained by purifying demineralized water in a Nanopure II system (Barnstead Newton, MA).

Stock standard solution of trichlorfon was prepared by dissolving 50 mg of powder, accurately weighted, in 100 mL of acetonitrile, obtaining a final concentration of 500 μg/mL, that was stored at −20 °C. Working solutions, used for LC−MS/MS analysis or for sample fortification, were obtained by diluting stock solution with acetonitrile.

Instrumentation. A HPLC system Waters Alliance 2795 (Waters, Milford, MA) was interfaced to a Quattro micro triple quadrupole mass spectrometer (Waters) using an orthogonal Z-spray-electrospray interface (ESI). The LC separation was performed injecting 20 μL and using a Discovery C_{18} column (50 × 2.1 mm i.d., 5 μm) (Supelco, Bellefonte, PA), at a flow rate of 300 μL/min. The mobile phase used was a water−methanol gradient where the percentage of methanol was changed linearly as follows: 0 min, 5%; 7 min, 5%; 12 min, 50%; 13 min, 50%; 15 min, 5%. Drying gas as well as nebulizing gas was nitrogen generated from pressurized air in a high-purity nitrogen generator NM30LA 230Vac Gas Station from Peak Scientific (Inchinnan, Scotland). The desolvation gas flow and cone gas flow were selected as 600 and 60 L/h, respectively. Infusion experiments were performed using the built-in syringe pump directly connected to the interface.

For operation in MS/MS mode, the collision gas was argon 99.995% (Carburos Metálicos, Valencia, Spain) with a pressure of 3×10^{-3} mbar in the collision cell. Capillary voltages of 3.5 kV were used in positive ionization mode. The interface temperature was set to 350 °C, and the source temperature was set to 120 °C. Dwell times of 0.5 s/scan were chosen for each transition. A solvent delay of 7.5 min was selected to give an additional cleanup using the built-in divert valve controlled by the Masslynx NT v.4.0 software. The quantification (*Q*) and confirmation (*q*) transitions are 257→109 and 259→109, respectively, both optimized at a cone voltage of 30 V and a collision energy of 15 eV. The application manager QuanLynx was used to process the quantitative data obtained from calibration standards and from crop samples.

Sample Preparation. Three types of samples were analyzed: cauliflower and peel and flesh of the kaki fruit. The samples were cut into small pieces without any pretreatment and were triturated with a chopper K55E (Dito Sama, Aubusson, France). An aliquot of homogenized sample (20 g for cauliflower and kaki flesh, and 10 g for kaki peel) was accurately weighted (precision 0.1 mg) and mixed with 60 mL of acetonitrile. After extraction for 2 min with a high-speed blender Ultra-Turrax T25 (Janke & Kunkel GmbH & Co., Staufen, Germany) at 8000 rpm, the entire extract was filtered through a 25−30 μm filter paper (Filtros ANOIA S. A., Barcelona, Spain) using a vacuum pump, washed with 10 mL of acetonitrile, and the volume was adjusted to 100 mL with acetonitrile. Finally, the extract was 4-fold diluted, taking an aliquot of 2.5 mL and diluting with LC-grade water up to 10 mL.

To remove solid particles, an aliquot of diluted extract was passed through a 0.45 μm Nylon syringe filter (Scharlab, Barcelona, Spain). Next, 20 μL of extract was directly injected in the LC−MS/MS system.

Fortification of samples was performed delivering 1 mL (0.5 mL for kaki peel) of trichlorfon standard solutions of 10 and 1 μg/mL in acetronitrile to 20 g of homogenized sample (10 g for kaki peel) to obtain 0.5 and 0.05 mg/kg, respectively. These samples were equilibrated, under room conditions, for 1 h prior to extraction.

Validation Study. Three validation data sets were obtained for each type of sample, kaki flesh, kaki peel, and cauliflower, following the spirit of the European Union SANCO working documents (*28*, *29*).

The calibration curve was obtained by analyzing nine matrix-matched standard solutions at concentrations between 0.5 and 250 ng/mL. Acceptance criteria were that the correlation coefficient was higher than 0.99 and the linearity residuals were lower than 30%.

The accuracy and the precision were obtained by analyzing trichlorfon in kaki and cauliflower blank samples spiked at two concentration levels (0.05 and 0.5 mg/Kg) and were evaluated within 1 day in quintuplicate at each concentration level. Acceptance criteria for accuracy were that recovery fit between 70 and 110% and for precision that relative standard deviation (RSD) was lower than 20% (*29*).

The limit of detection (LOD), defined as the lowest concentration that the analytical process can reliably differentiate from background levels, was estimated for a signal-to-noise ratio of 3 from the chromatograms at the lowest analyte concentration tested. The limit of quantitation (LOQ) was established as the lowest concentration tested, which gave acceptable recoveries and precision.

To evaluate the ability of the analytical procedure to give a selective measurement for the analyte, a specificity study was carried out including the analysis of a procedure blank, a sample blank, and a blank sample spiked at the LOQ level. The response should not exceed 30% of that of LOQ to consider the method to have satisfactory specificity.

Stability Study. Two different stability studies were performed to ensure the adequate preservation of samples and processed sample extracts.

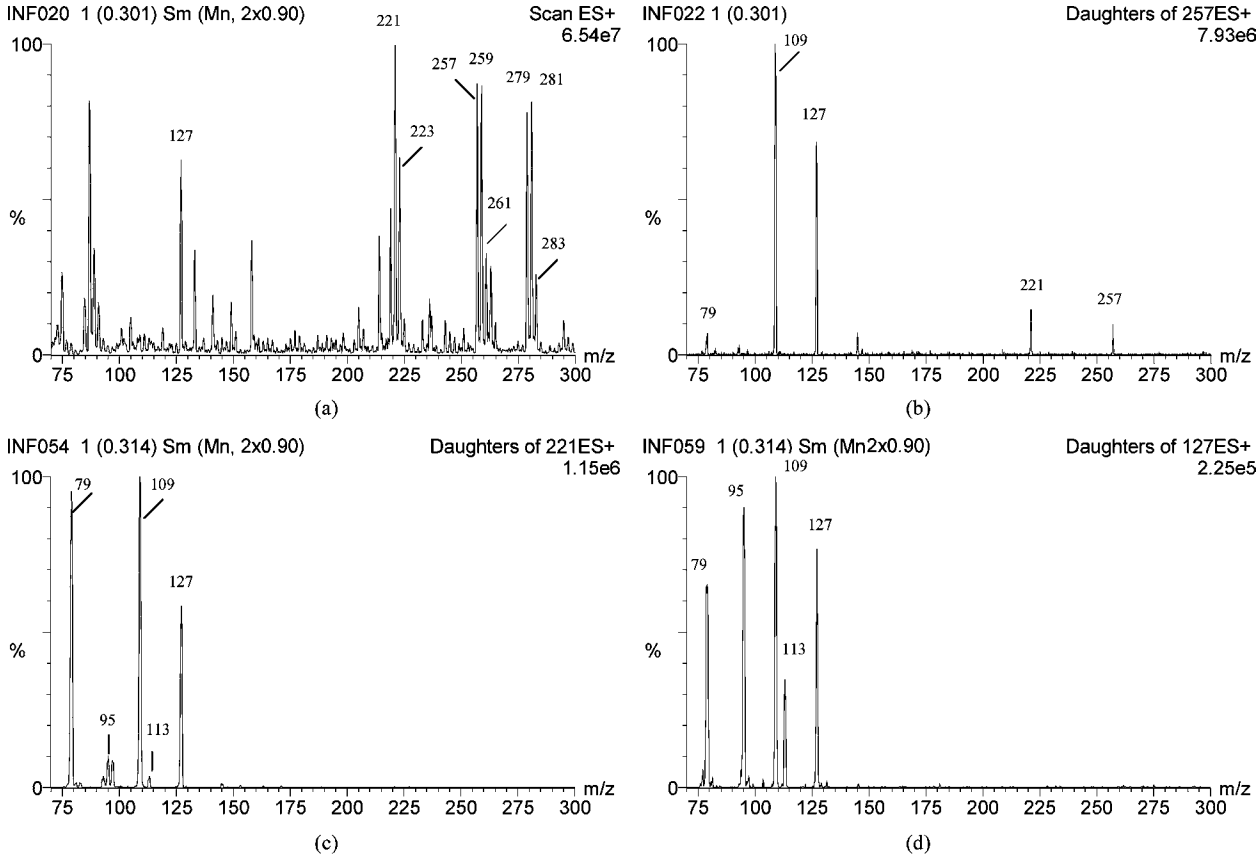

Figure 1. (**a**) Positive ion electrospray full scan mass spectrum of trichlorfon acquired by infusion of 5 μg/mL standard solution, at cone voltage of 30 V. (**b**) Product ion spectrum of $m/z = 257$ at cone voltage and collision energy of 30 V and 15 eV, respectively. (**c**) Product ion spectrum for in-source fragment $m/z = 221$ at cone voltage and collision energy of 40 V and 25 eV, respectively. (**d**) Product ion spectrum for in-source fragment $m/z = 127$ at cone voltage and collision energy of 50 V and 20 eV, respectively.

The stabilities of diluted sample extracts for the three type of matrixes, fortified at 0.1 μg/mL (0.5 mg/kg in sample) and stored at 4 and −18 °C, were evaluated for 2 months, by analyzing three replicates (injection in duplicate) at 0, 6, 15, and 60 days.

The stability of blank samples fortified at 0.5 mg/kg and stored 1 year at −18 °C was also studied, by analyzing four replicates (injection in duplicate).

During these experiments, a blank sample fortified at 0.5 mg/kg was used as quality control (QC) and analyzed together with the stability samples. Results were accepted if QC gave acceptable recoveries (70−110%) and the deviation between stability samples and QC concentrations was lower than 15%.

Data Evaluation. For quantification of trichlorfon in samples collected from field residue trials, an external matrix-matched standard calibration curve was used in every set of samples analyzed with, at least, three points within the calibration range. To ensure the quality of the analysis, at least two QC (0.5 and 0.05 mg/kg) values were also included in each batch of samples. The quantification of the sample list was considered acceptable if QC recoveries were between 70% and 110%. Every sample extract was injected in duplicate.

The use of MS/MS detection easily allows one to improve the specificity of the method, due to the possibility of confirmation of the analyte identity by acquiring a second transition, to obtain 4 or 5 IPs depending on the precursor and the product ions selected (*24*). According to this, confirmation was performed in positive samples by acquisition of two MS/MS transitions: the most intense used for quantification (Q) and the other used for confirmation (q). Apart from the retention time criteria, positive findings were confirmed by comparison of the Q/q intensity ratios. The average Q/q calculated for all standards injected in a sequence was taken as the theoretical ion ratio, and Q/q values calculated for positive findings in samples were compared to the theoretical one. Confirmation was considered reliable if the deviation of both standard and sample ion ratios was lower than 15% (*24*).

RESULTS

MS Optimization Experiments. The full-scan mass spectrum and selected MS/MS spectra of trichlorfon are shown in **Figure 1**. They were obtained from infusion of 5 μg/mL standard solutions (50:50 acetonitrile:water) at a flow rate of 10 μL/min. As a result of the basic character of the phosphonic ester group, trichlorfon shows positive ionization. The full scan mass spectrum presents three peaks at m/z 257, 259, and 261 corresponding to the protonated molecular ion $[M + H]^+$ with the characteristic isotopic pattern (3:3:1) that confirms the presence of three chlorine atoms (**Figure 1a**). The MS spectrum for protonated trichlorfon was optimized at a cone voltage of 30 V. **Figure 1a** also shows three peaks at m/z 279, 281, and 283 corresponding to the $[M + Na]^+$ adduct. Despite their similar sensitivity, the use of $[M + Na]^+$ as precursor ions was avoided due to their poor fragmentation, as has been proved for other pesticides (*26*).

Next, MS/MS spectra were optimized selecting the most abundant ions (m/z 257 and 259) as precursor ions, showing in both cases an important fragment at m/z 109 optimized at a collision energy of 15 eV (**Figure 1b**). Another abundant ion at m/z 221 (precursor ion 257) or m/z 223 (precursor ion 259) was also obtained; meanwhile, the less abundant fragments at m/z 127 and 79 were observed independently of the precursor ion selected. A possible explanation for the formation of these ions is illustrated in the fragmentation pathway shown in **Figure 2**. The ions 221/223 can be explained by a neutral loss of HCl; the presence of the remaining two chlorine atoms in the fragment leads to two different product ions depending on the precursor ion selected. The posterior neutral loss of $Cl_2C{=}C$: would

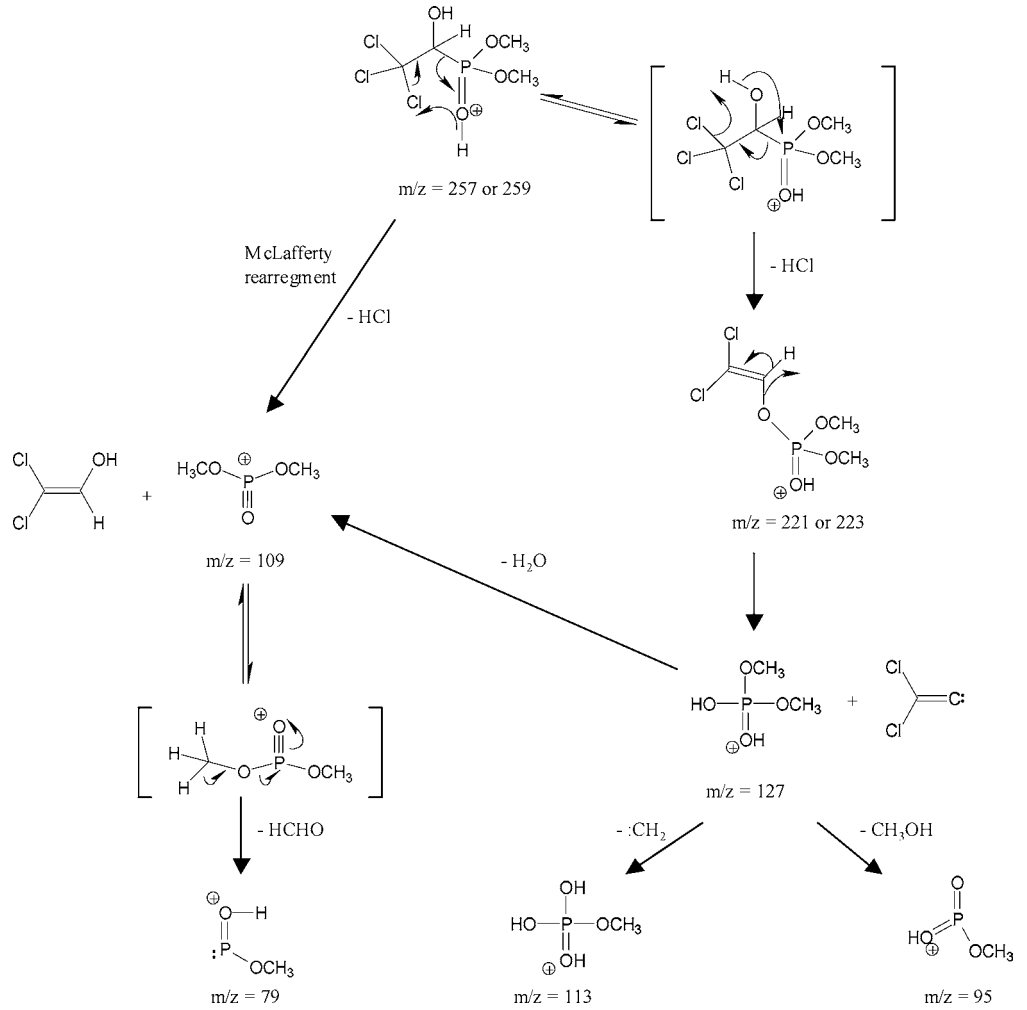

Figure 2. Fragmentation pathway proposed for trichlorfon.

explain the fragment at *m*/*z* 127, which is independent of the precursor ion selected due to the absence of chlorine atoms in its structure. The main product ion at *m*/*z* 109 may be obtained from any of the precursor ions, after a McLafferty rearrangement or after a neutral loss of water from the ion at *m*/*z* 127. Finally, the product ion at *m*/*z* 79 would be obtained by a neutral loss of acetaldehyde from the *m*/*z* 109 ion. To support this fragmentation pathway, different MS spectra of trichlorfon were acquired modifying the cone voltage to obtain optimum in-source fragmentation for the most abundant product ions. Thus, by increasing the cone voltage up to 60 V, abundant in-source fragment ions at *m*/*z* 221 and 127 were obtained. The MS/MS spectra of these in-source fragment ions were also acquired (**Figure 1c** and **d**, respectively), obtaining the same ions (*m*/*z* 127, 109, 79), and supporting the fragmentation pathway suggested in **Figure 2**. Additionally, two ions at *m*/*z* 113 and 95 were observed in the product ion spectra of 127 and with less abundance in that of 221. These ions might be explained from the ion at *m*/*z* 127 after losses of :CH_2 and methanol, respectively (see **Figure 2**).

With all of this information, up to 18 MS/MS transitions could be selected for the confirmation of trichlorfon if necessary, using *m*/*z* 257, 259, 221, 223, and 127 as precursor ions.

Method Optimization. Although the ultimate confirmation of trichlorfon could be achieved by the acquisition of up to 18 different transitions, only two are, in principle, sufficient to obtain a safe confirmation. Thus, the two most sensitive transitions 257→109 and 259→109 were selected for quantification (*Q*) and confirmation (*q*) purposes, respectively. Both transitions were optimized at a cone voltage of 30 V and 15 eV of collision energy. The simultaneous acquisition of these two transitions in the SRM mode allowed one to quantify and to confirm the presence of trichlorfon in one unique injection. As stated in the previous section, due to the presence of 3 chlorine atoms in the molecule, both selected transitions presented approximately the same sensitivity; therefore, the ion intensity ratio was around 1, and this allowed the confirmation of positive samples even at the LOD level.

Regarding sample procedure, one of the goals of the method was to simplify the sample pretreatment, and therefore the direct injection of sample extracts was evaluated. As trichlorfon is very soluble in polar organic solvents, an extraction with acetonitrile or methanol was checked. Matrix effect was evaluated for both extractants and was higher for methanol than acetonitrile, which is in accordance with results previously reported for pesticide determination in fruits (*12*). Next, extraction with acetonitrile was selected, although some matrix effects still remained. To compensate this matrix effect, and because of the absence of commercial available isotopic labeled trichlorfon, a matrix-matched calibration was performed. The application of this approach was feasible because of the availability of nontreated samples (blank samples) coming from the same orchard as treated samples. However, the direct injection of 20 μL of acetonitrile sample extracts produced a poor peak shape, and a 4-fold dilution with water was necessary to improve the chromatographic behavior. Because of the high sensitivity of

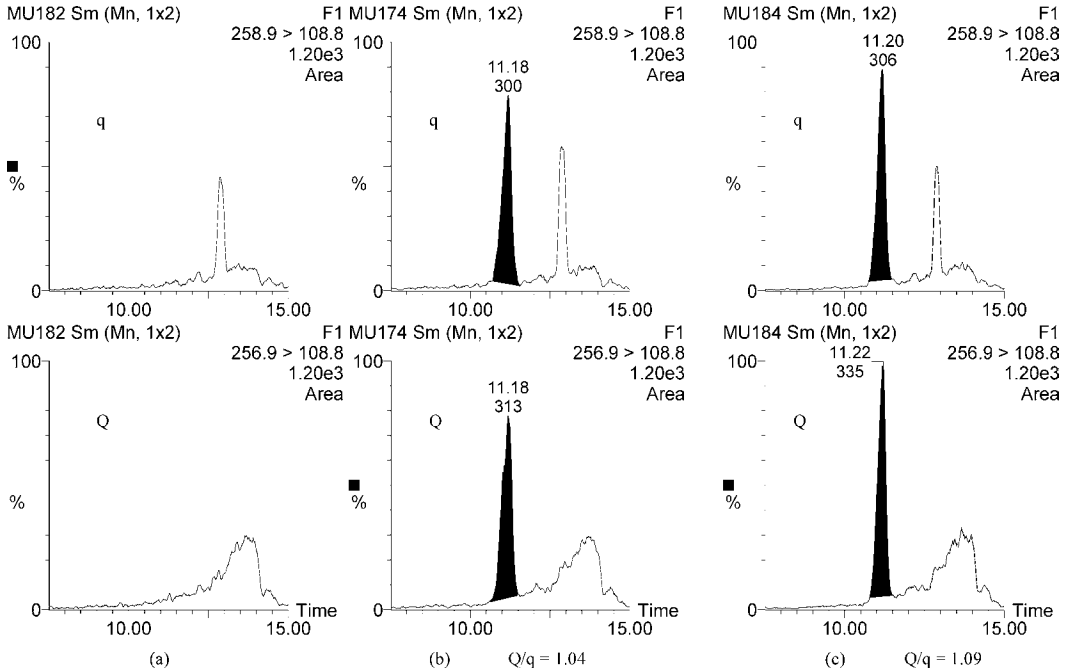

Figure 3. LC–ESI-MS/MS chromatograms for (**a**) blank cauliflower sample, (**b**) standard (2.5 μg/L), and (**c**) cauliflower sample fortified at 0.05 mg/kg with trichlorfon. Top, confirmation channel (*q*, 259→109); bottom, quantification channel (*Q*, 257→109).

Table 1. Recoveries and Relative Standard Deviations (RSD) at Two Concentration Levels for Trichlorfon in Spiked Peel and Flesh Kaki and Cauliflower ($n = 5$)

	level of fortification (mg/kg)		
matrix	0.05 (RSD)	0.5 (RSD)	LOD[a] (mg/kg)
cauliflower	83 (3)	84 (2)	0.013
kaki peel	98 (4)	101 (6)	0.013
kaki flesh	98 (7)	97 (3)	0.006

[a] Limits of detection (LOD).

the method, the LOQ objective (0.05 mg/kg) was still achievable despite the 4-fold dilution. The sample dilution presents additional advantages such as the lower amount of matrix loaded in the column, the decrease of ionization suppression, and the compensation of possible variability between samples from different origin or variety. This last advantage can be of great importance when no ideal blank samples are available, as reported for fosetyl-aluminum in lettuce from different origin and variety (*17*).

Finally, mobile phase additives such as formic acid or ammonium acetate were tested to improve the trichlorfon response, but not increase was observed. So, a water/methanol gradient was optimized using a short Discovery C_{18} column. The retention time of trichlorfon under these conditions was around 11.2 min, which allowed one to select a solvent delay of 7.5 min, giving an additional cleanup step that avoided the overload of the interface with early-eluting interferences that could affect the analyte ionization.

Validation Results. Calibration curves showed a good adjustment to a second-order equation between 0.5 and 250 ng/mL, with a correlation coefficient higher than 0.995 in all cases, and residuals lower than 28%.

The method presented satisfactory recoveries and excellent precision (**Table 1**) with average recoveries between 83% and 101%, for the two levels assayed in peel and flesh kaki fruit and cauliflower, and RSD always lower than 7%.

LODs of 0.013 mg/kg for peel kaki and cauliflower and 0.006 mg/kg for flesh kaki were estimated from the chromatograms at the 0.05 mg/kg level. As expected, higher LODs were obtained for peel kaki because of the lower sample amount processed in this method. However, despite using the same amount of sample (20 g) for cauliflower and flesh kaki, the LOD for cauliflower was higher, which indicates an upper ionization suppression produced by cauliflower matrix. Despite this, the use of matrix-matched calibration compensated this matrix effect, obtaining satisfactory results for both recoveries and precision.

The LOQ for the three studied matrixes was fixed to the lowest fortification level tested, that is, 0.05 mg/kg, because satisfactory recovery and adequate precision were obtained. In this way, the reliable quantification at the 0.05 mg/kg level is clearly demonstrated, according to the European SANCO guidelines (*28*, *29*). The specificity of the procedure was also evaluated, and no responses were detected for neither the procedure blank nor the peel and flesh kaki fruit or cauliflower sample blank. Therefore, the method was formally specific.

Typical LC−MS/MS chromatograms of standard solutions and sample extracts (blank and fortified at 0.05 mg/kg) are shown in **Figure 3**. They were obtained after direct injection of the 4-fold diluted sample extract. As can be seen, the total chromatographic run was 15 min; the high sensitivity of the method using the selected transitions allows the quantification and confirmation of trichlorfon at the LOQ level with *Q*/*q* ratios close to 1. The absence of any peak in the blank sample at the expected retention time shows the specificity of the method.

Stability of Samples. The stability study (see **Table 2**) in fortified diluted extracts was satisfactory at the temperatures tested, obtaining recovery values between 84% and 120% (4 °C) and 89% and 131% (−18 °C) over 2 months. On the other hand, the stability study in homogenized (triturated) fortified samples stored at −18 °C was also successful, with recovery values between 95% and 107% after 1 year of storage. Thus, the stability of trichlorfon in frozen samples and in refrigerated extracts was demonstrated, during at least 1 year and 2 months, respectively.

Application of the Method to Field Samples. The method developed was applied, over 2 years, to 216 samples: 72 from

Table 2. Sample Stability under Storage

	fortified extract 0.5 mg/kg ($n = 3$)						fortified sample 0.5 mg/kg ($n = 4$)
temperature storage:	4 °C			−18 °C			−18 °C
analysis time (days):	6	15	60	6	15	60	365
cauliflower	99 (7)[a]	97 (8)	88 (7)	99 (6)	104 (5)	108 (6)	96 (6)
kaki peel	84 (6)	93 (9)	99 (3)	89 (6)	95 (8)	102 (7)	95 (8)
kaki flesh	101 (12)	120 (11)	106 (1)	113 (6)	119 (3)	131 (7)	107 (9)

[a] Mean recovery (%) and RSD (%) in brackets.

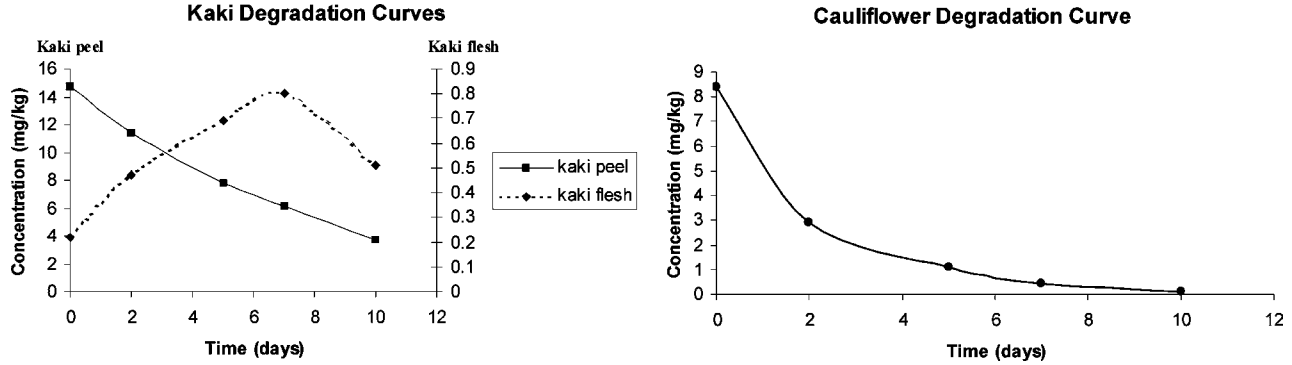

Figure 4. Degradation curves for trichlorfon in kaki fruit and cauliflower.

Table 3. Robustness and Reproducibility of the Method over 2 Years of Analysis[a]

matrix	QC (0.05 mg/kg)	*n*	QC (0.5 mg/kg)	*n*
cauliflower	84 (8)	24	84 (3)	24
kaki peel	106 (20)	14	93 (7)	18
kaki flesh	82 (18)	17	89 (12)	18

[a] Recoveries (%) and relative standard deviations (RSD %) for quality control samples (QC) analyzed in every type of sample matrix.

each matrix (peel and flesh of kaki fruit and cauliflower). In every set of samples analyzed (typically 6−8 samples), two quality controls (QCs) were included (one at the LOQ level and the other at the 10×LOQ level), obtaining average recoveries between 82% and 106% (**Table 3**). Up to 115 QCs were analyzed during the 2 years period, obtaining a RSD ≤ 20% (0.05 mg/kg) and RSD ≤ 12% (0.5 mg/kg). These data demonstrate the robustness of the method and the reproducibility over a wide range of time.

Trichlorfon was not detected in any blank sample, while treated samples presented different concentration levels according to the collection day after last application (DALA) and sample type. As **Figure 4** shows, 10 days after application the trichlorfon level in kaki peel decreased down to 30% of the initial concentration at day 0, while kaki flesh showed a smooth increase in trichlorfon concentration, and a subsequent decrease after 7 days. The cauliflower degradation curve shows a notable decrease in pesticide concentration after 2−5 days of the application. These data show the behavior of trichlorfon in treated field samples under Mediterranean conditions. Obviously, pesticide residue levels in fruit depend on many variables such as application rate and collection day after last application, but also climate, variety of fruit, environmental conditions, and fertilization regime. **Figure 5** shows the chromatograms obtained for several samples of peel kaki collected at different times and illustrates the decrease in the concentration of trichlorfon over time.

Regarding confirmation of positive samples, in all analyses performed, both standards in solvent and spiked blank samples showed *Q*/*q* ratios within the expected tolerance (ion ratio around 1 with deviation lower than 15%), using the transition 257→109 for quantification and 259→109 for confirmation. All positive real samples were confirmed, obtaining ion ratio deviations lower than 6%, as can be seen in **Figure 5** where the *Q*/*q* ratios obtained were 1.04 and 1.05. No false positives were found in any of the samples. As a summary, **Table 4** shows the average deviations for *Q*/*q* ratios in the samples and QC from each matrix type.

It is interesting to point out the occurrence of an additional peak at a retention time close to the analyte (**Figure 5**). This peak might reveal either the existence of other compounds in the test substance applied or a trichlorfon transformation product, as it was present in all treated sample analyzed. However, this peak was not detected either in blank samples or in the QCs. This compound has to be isobaric with trichlorfon and possibly structural isomeric, as it shares both selected transitions with similar ion ratios. Thus, the similar intensity of both selected transitions suggested the presence of three chlorine atoms in the molecule. Different potential structures for this compound are proposed in **Figure 6**. Jimenez et al. (*6*) also found an interference in trichlorfon analysis, which was elucidated as compound C or D of **Figure 6** according to its GC−MS behavior. In the present work, the concentration of this interference increased over time; meanwhile, trichlorfon concentration decreased according to DALA. This fact supports the hypothesis that the unknown compound is an isomeric transformation product from trichlorfon. The detection of a small peak in the treated samples at 0 DALA suggests that this compound could be also present at small concentration in the test substance applied in the residue trials.

The presence of this isobaric interference reveals an important fact that, when using LC coupled to tandem MS, is normally underestimated: the possibility of finding an interference that

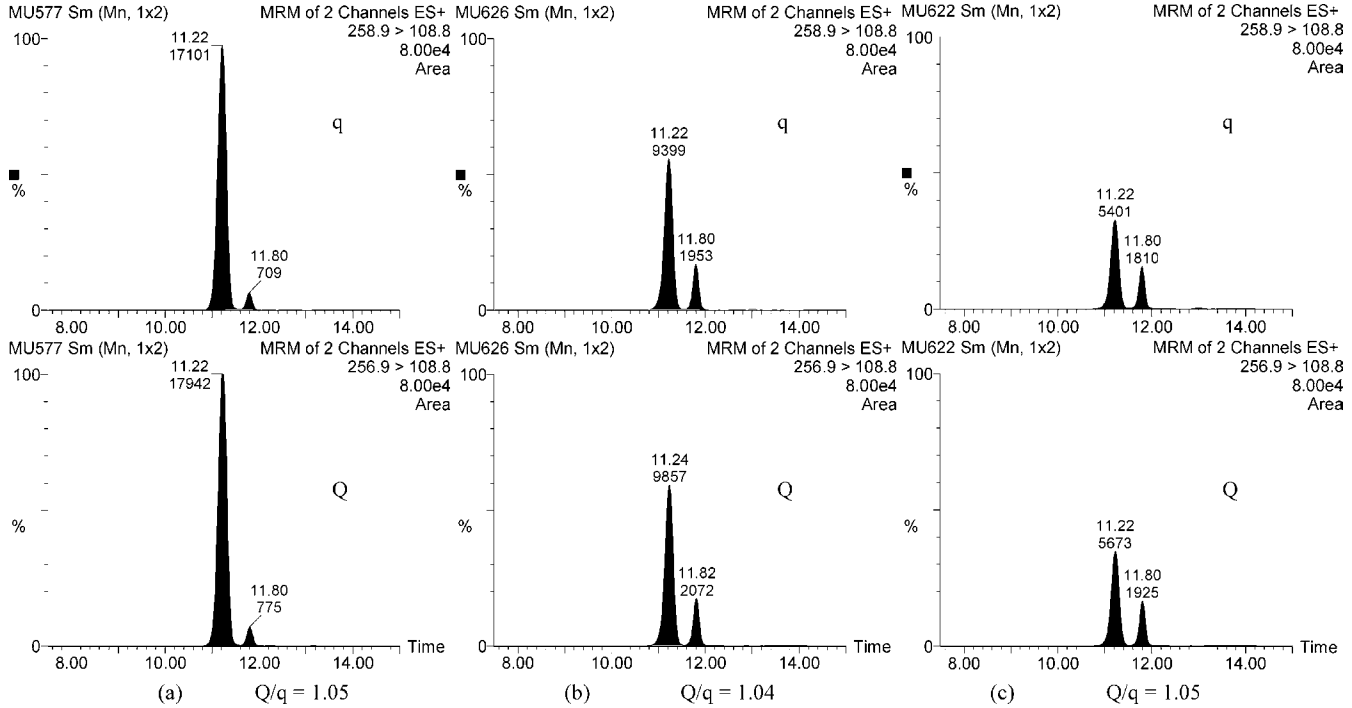

Figure 5. LC–ESI-MS/MS chromatograms for peel kaki samples: (**a**) sample collected at 2 DALA (11.6 mg/kg), (**b**) sample collected at 5 DALA (5.5 mg/kg), and (**c**) sample collected at 10 DALA (3.0 mg/kg). Top, confirmation channel (*q*, 259→109); bottom, quantification channel (*Q*, 257→109).

Table 4. Confirmation of Trichlorfon in Samples[a]

matrix	QC (0.05 mg/kg)	QC (0.5 mg/kg)	samples (0.05–0.5 mg/kg)	samples (0.5 mg/kg)
cauliflower	4.9	3.1	3.7	3.3
kaki peel	6.2	2.8	2.9	3.8
kaki flesh	4.5	2.6	3.4	1.5

[a] Average *Q*/*q* ratio deviations (%) for QC and samples analyzed that contained trichlorfon at different ranges of concentrations.

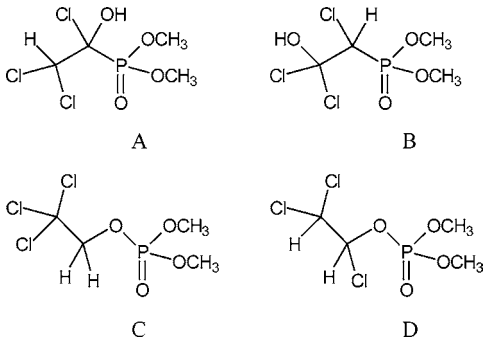

Figure 6. Proposed structures for the interference peak observed in the determination of trichlorfon.

shares with the analyte both the quantification and the confirmation transitions, with the same ion ratio. This fact is not frequent at all, but certainly illustrates the importance of an efficient chromatographic separation to avoid the reporting of false positives. In the case that both peaks were not correctly resolved, the interference would be reported as trichlorfon as all of the confirmation parameters (retention time and ion ratio for two selected transitions) would fit with those previously established as confirmation criteria.

Conclusion. This work has shown that LC−ESI-MS/MS is a powerful analytical technique that allows the rapid and reliable determination of trichlorfon residues in kaki and cauliflower samples. The high selectivity and sensitivity of LC−MS/MS allows the direct injection of the diluted sample extract, achieving a detection limit 50 times (cauliflower and peel kaki) and 100 times (flesh kaki) lower than the MRL in these crops. A methanol:water gradient separation using a Discovery C18 column permits a correct separation between the analyte and one isobaric interference that shared the same MS/MS transitions. Matrix-matched standard calibration together with a 4-fold dilution of the extracts compensated and minimized the matrix effect observed that produced ionization suppression. The method was validated at the 0.05 and 0.5 mg/kg levels, and its applicability was checked by analysis of a large number of field samples over 2 years, demonstrating its speediness and robustness, and suitability for routine analysis of trichlorfon in vegetable samples.

LITERATURE CITED

(1) *The Pesticide Manual*, 13th ed.; British Crop Protection Council, 2004.

(2) Teruel Muñoz, V. Limites Máximos de Residuos de Productos Fitosanitarios. http://www.mapya.es/agricultura/pags/fitos/registro/lmrs/pdf/LMRS.pdf, July, 2005.

(3) Lee, W. O.; Law, M. L. M.; Wong, S. K. Determination of Methamidophos Residues in Food Remnants. *Food Addit. Contam.* **1996**, *13*, 687−693.

(4) Podhorniak, L. V.; Negron, J. F.; Griffith, F. D. Gas Chromatography with Pulsed Flame Photometric Detection Multiresidue Method for Organophosphate Pesticides and Metabolite Residues at the Parts-Per-Billion Level in Representative Commodities of Fruit and Vegetables Crop Groups. *J. AOAC Int.* **2001**, *84*, 873−890.

(5) Brito, N. M.; Navickiene, S.; Polese, L.; Jardim, E. F. G.; Abakerli, R. B.; Ribeiro, M. L. Determination of Pesticide Residues in Coconut Water by Liquid−Liquid Extraction and Gas Chromatography with Electron-capture plus Thermionic Specific Detection and Solid-Phase Extraction and High-Performance Liquid Chromatography with Ultraviolet Detection. *J. Chromatogr., A* **2002**, *957*, 201−209.

(6) Jiménez, J. J.; Bernal, J. L.; del Nozal, M. J.; Toribio, L.; Bernal, J. Determination of Impurities in Pesticides and their Degradation Products formed during the Wine-making Process by Solid-phase Extraction and Gas Chromatography with Detection by Electron Ionization Mass Spectrometry. II. Bromopropylate, Trichlorphon, Parathion-methyl and Tebuconazole. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 2629−2636.

(7) Garrido, A.; González-Rodríguez, M. J.; Arrebola, F. J.; Martínez, J. L. Potentiality of Gas Chromatography-Triple Quadrupole Mass Spectrometry in Vanguard and Rearguard Methods of Pesticides Residues in Vegetables. *Anal. Chem.* **2005**, *77*, 4640−4648.

(8) López-Avila, V.; Benedicto, J.; Bauer, K. M. Stability of Organochlorine and Organophosphorus Pesticides when Extracted from Solid Matrixes with Microwave Energy. *J. AOAC Int.* **1998**, *81*, 1224−1232.

(9) Blasco, C.; Font, G.; Picó, Y. Comparison of Microextraction Procedures to Determine Pesticides in Oranges by Liquid Chromatography−Mass Spectrometry. *J. Chromatogr., A* **2002**, *970*, 201−212.

(10) Sancho, J. V.; Pozo, O. J.; Zamora, T.; Grimalt, S.; Hernández, F. Direct Determination of Paclobutrazol Residues in Pear Samples by Liquid Chromatography-Electrospray Tandem Mass Spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 4202−4206.

(11) Hogenboom, A. C.; Hofman, M. P.; Kok, S. J.; Niessen, W. M. A.; Brinkman, U. A. Th. Determination of Pesticides in Vegetables using Large-Volume Injection Column Liquid Chromatography-Electrospray Tandem Mass Spectrometry. *J. Chromatogr., A* **2000**, *892*, 379−390.

(12) Pozo, O. J.; Marín, J. M.; Sancho, J. V.; Hernández, F. Determination of Abamectin and Azadirachtin Resiudes in Orange Samples by Liquid Chromatography-Electrospray Tandem Mass Spectrometry. *J. Chromatogr., A* **2003**, *992*, 133−140.

(13) Matuszewski, B. K.; Constanzer, M. L.; Chavez-Eng, C. M. Strategies for the Assessment of Matrix Effect in Quantitative Bioanalytical Methods Based on HPLC−MS/MS. *Anal. Chem.* **2003**, *75*, 3019−3030.

(14) Antignac, J. P.; de Wasch, K.; Monteau, F.; De Brabander, H.; Andre, F.; le Bizec, B. The Ion Suppression Phenomenon in Liquid Chromatography−Mass Spectrometry an its Consequences in the Field of Residue Analysis. *Anal. Chim. Acta* **2005**, *529*, 129−136.

(15) Hernández, F.; Sancho, J. V.; Pozo, O. J. Critical Review of the Application of Liquid Chromatography/Mass Spectrometry to the Determination of Pesticide Residues in Biological Samples. *Anal. Bioanal. Chem.* **2005**, *382*, 934−946.

(16) Choi, B. K.; Gusev, A. I.; Hercules D. M. Quantitative LC−ESI-MS analysis for pesticides in complex environmental matrix using external and internal standards. *Int. J. Environ. Anal. Chem.* **2000**, *77*, 305−322.

(17) Hernández, F.; Sancho, J. V.; Pozo, O. J.; Villaplana, C.; Ibañez, M.; Grimalt, S. Rapid Determination of Fosetyl-Aluminium Residues in Lettuce by Liquid Chromatography/Electrospray Tandem Mass Spectrometry. *J. AOAC Int.* **2003**, *86*, 832−838.

(18) Jansson, C.; Pihlström, T.; Österdahl, B. G.; Markides, K. E. A new Multi-Residue Method for Analysis of Pesticide Residues in Fruit and Vegetables using Liquid Chromatography with Tandem Mass Spectrometric Detection. *J. Chromatogr., A* **2004**, *1023*, 93−104.

(19) Sannino, A.; Bolzoni, L.; Bandini, M. Application of Liquid Chromatography with Electrospray Tandem Mass Spectrometry to the Determination of a new Generation of Pesticides in Processed Fruits and Vegetables. *J. Chromatogr., A* **2004**, *1036*, 161−169.

(20) Agüera, A.; López, S.; Fernández-Alba, R.; Contreras, M.; Crespo, J.; Piedra, L. One-year Routine Application of a new Method Based on Liquid Chromatography-Tandem Mass Spectrometry to the Analisis of 16 Multiclass Pesticidas in Vegetable Samples. *J. Chromatogr., A* **2004**, *1045*, 125−135.

(21) Taylor, J. C.; Hird, S. J.; Sykes, M. D.; Startin, J. R. Determination of Residues of Propamocarb in Wine by Liquid Chromatography-Electrospray Mass Spectrometry with Direct Injection. *Food Addit. Contam.* **2004**, *21*, 572−577.

(22) Sancho, J. V.; Ibañez, M.; Grimalt, S.; Pozo, O. J.; Hernández, F. Residue Determination of Cyromazine and its Metabolite Melamine in Chard Samples by Ion-Pair Liquid Chromatography Couples to Electrospray Tandem Mass Spectrometry. *Anal. Chim. Acta* **2005**, *530*, 237−243.

(23) Hetherton, C. L.; Sykes, M. D.; Fussell, R. J.; Goodall, D. M. A Multi-Residue Screening Method for the Determination of 73 Pesticides and Metabolites in Fruit and Vegetables using High-Performance Liquid Chromatography/Tandem Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 2443−2450.

(24) Commission Decision 2002/657/EC of 12 August 2002, implementing Council Directive 96/23/EEC concerning the performance of analytical methods and the interpretation of results. Official Journal of the European Communities L221, C(2002)-3044, pp 0008−0036.

(25) Ortelli, D.; Edder, P.; Corvi, C. Multiresidue Analysis of 74 Pesticides in Fruits and Vegetables by Liquid Chromatography−Electrospray-Tandem Mass Spectrometry. *Anal. Chim. Acta* **2004**, *520*, 33−45.

(26) Grimalt, S.; Pozo, O. J.; Marín, J. M.; Sancho, J. V.; Hernández, F. Evaluation of Different Quantitative Approaches for the Determination of Noneasily Ionizable Molecules by Different atmospheric Pressure Interfaces Used in Liquid chromatography Tandem Mass Spectrometry: Abamectine as Case of Study. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 1619−1630.

(27) Zamora, T.; Pozo, O. J.; López, F. J.; Hernández, F. Determination of Tridemorph and other Fungicide Residues in Fruit Samples by Liquid Chromatography-Electrospray Tandem Mass Spectrometry. *J. Chromatogr., A* **2004**, *1045*, 137−143.

(28) European Commission, Directorate General Health and Consumer Protection, Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of Directive 91/414. Document SANCO/3029/99 rev.4 of July 2000.

(29) European Commission, Directorate General Health and Consumer Protection, Quality Control Procedures for pesticide residues analysis. Document SANCO/10476/2003 of February 2004.

Received for review November 4, 2005. Revised manuscript received December 19, 2005. Accepted December 19, 2005. The Quattro Micro LC−MS/MS was funded by the European Union (Fondos Feder-Reino de España, Ministerio de Ciencia y Tecnología, Ref. UNJM-E004).

JF052737J