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Multi-residue determination of 130 multiclass pesticides in fruits and vegetables by gas chromatography coupled to triple quadrupole tandem mass spectrometry

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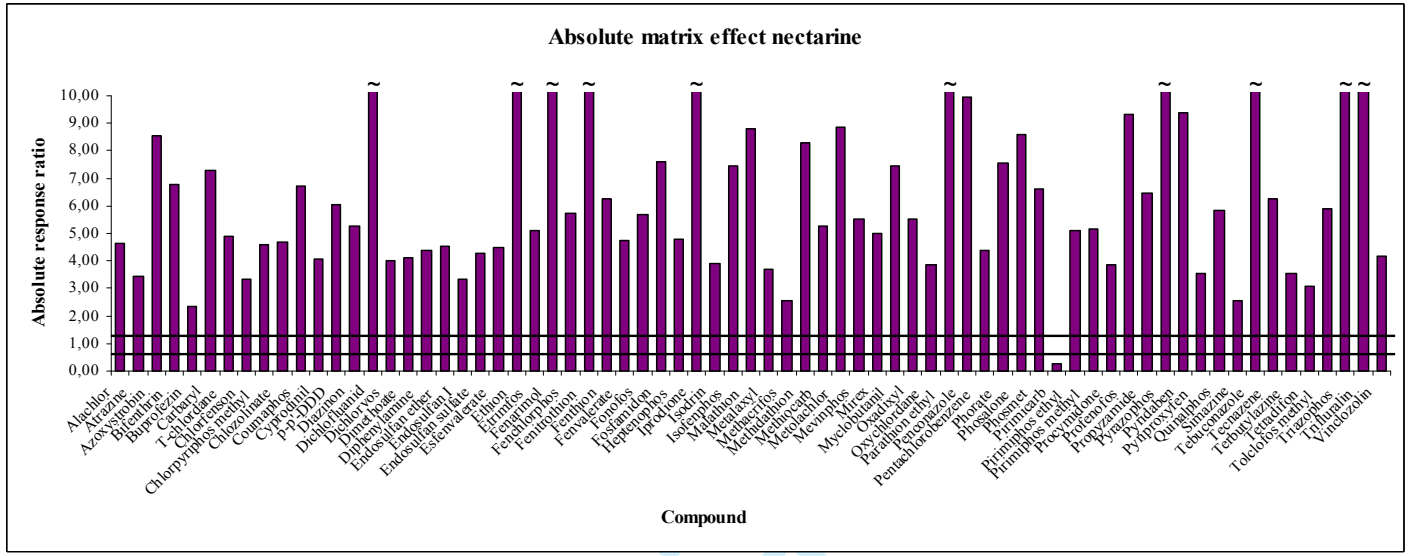
Keywords:	Pesticides , gas chromatography tandem mass spectrometry, fruits and vegetables, triple quadrupole, multiresidue analysis, matrix effect

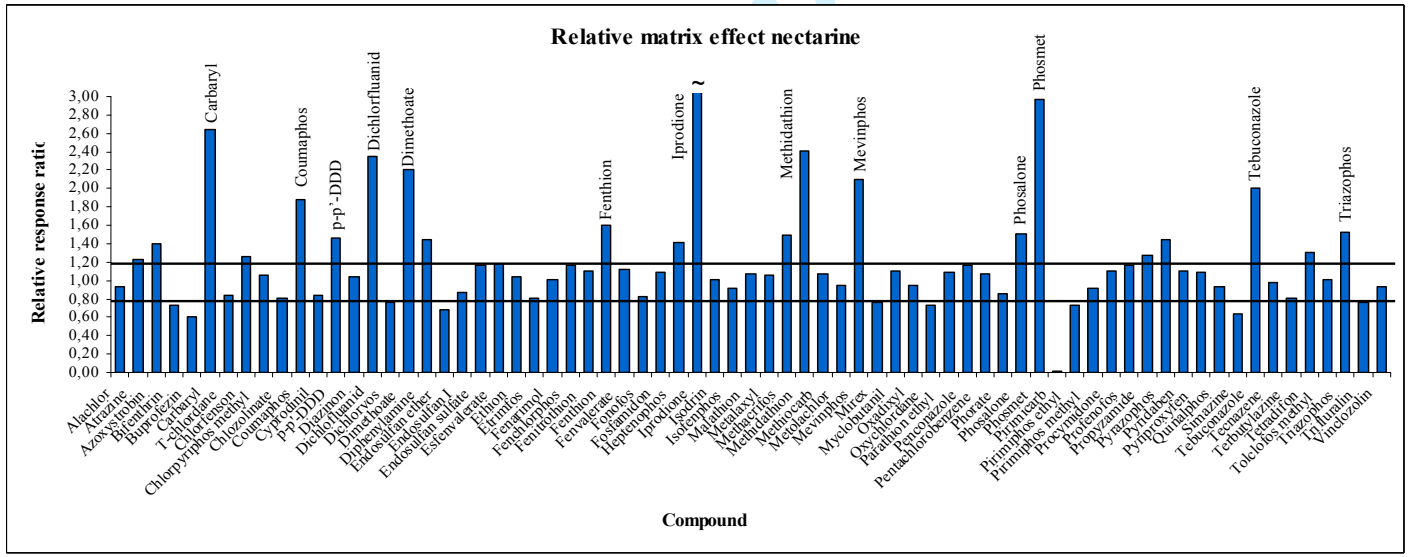
FIGURE CAPTIONS

Figure 1. (a) Absolute and relative matrix effect for nectarine samples.**(b)** Relative matrix effect for spinach samples in the GC-MS/MS determination of selected pesticides.

Figure 2. GC-MS/MS SRM chromatograms for selected pesticides (within a wide range of retention times) pesticides in orange, nectarine and spinach samples fortified at 0.01 mg/Kg. Only the quantification transition is shown.

Figure 3. GC-MS/MS SRM chromatograms for pesticides detected in a nectarine sample (nectarine 1, Table 3). (Q) quantification transition, (q) confirmative transition.


Absolute matrix effect nectarine
Absolute response ratio
10,00
9,00
8,00
7,00
6,00
5,00
4,00
3,00
2,00
1,00
0,00
Alachlor
Atrazine
Azoxystrobin
Bifenthrin
Buprofezin
Carbaryl
T-chlordane
Chlorfenson
Chlorpyriphos methyl
Chlozolinate
Coumaphos
Cyprodinil
p-p'-DDD
Diazinon
Dichlofluanid
Dichlorvos
Dimetoate
Diphenylamine
Endosulfan ether
Endosulfan sulfate
Esfenvalerate
Ethion
Etrimfos
Fenarimol
Fenchlorphos
Fenitrothion
Fenthion
Fenvalerate
Fonofos
Fosfamidon
Heptenophos
Iprodione
Isodrin
Isofenphos
Malathion
Metalaxyl
Methacrifos
Methidathion
Methiocarb
Metolachlor
Mevinphos
Mirex
Myclobutanil
Oxadixyl
Oxychlordane
Parathion ethyl
Pentachlorobenzene
Penconazole
Phorate
Phosalone
Phosmet
Pirimicarb
Pirimiphos ethyl
Pirimiphos methyl
Procymidone
Profenofos
Propyzamide
Pyrazophos
Pyridaben
Pyrimoxyfen
Quinalphos
Simazine
Tebuconazole
Tecnazene
Terbuthylazine
Tetradifon
Tolclofos methyl
Triazophos
Trifluralin
Vinclozolin
Compound


Relative matrix effect nectarine
Relative response ratio
3,00
2,80
2,60
2,40
2,20
2,00
1,80
1,60
1,40
1,20
1,00
0,80
0,60
0,40
0,20
0,00
Carbaryl
Coumaphos
p-p'-DDD
Dichlorfluanid
Dimetoate
Fenthion
Iprodione
Methidathion
Mevinphos
Phosalone
Phosmet
Tebuconazole
Triazophos
Alachlor
Atrazine
Azoxystrobin
Bifenthrin
Buprofezin
Carbaryl
T-chlordane
Chlorfenson
Chlorpyriphos methyl
Chlozolinate
Coumaphos
Cyprodinil
p-p'-DDD
Diazinon
Dichlofluanid
Dichlorvos
Dimetoate
Diphenylamine
Endosulfan ether
Endosulfan I
Endosulfan sulfate
Esfenvalerate
Ethion
Etrimfos
Fenarimol
Fenchlorphos
Fenitrothion
Fenthion
Fenvalerate
Fonofos
Fosfamidon
Heptenophos
Iprodione
Isodrin
Isofenphos
Malathion
Metalaxyl
Methacrifos
Methidathion
Methiocarb
Metolachlor
Mevinphos
Mirex
Myclobutanil
Oxadixyl
Oxychlordane
Parathion ethyl
Pentachlorobenzene
Penconazole
Phorate
Phosalone
Phosmet
Pirimicarb
Pirimiphos ethyl
Pirimiphos methyl
Procymidone
Profenofos
Propyzamide
Pyrazophos
Pyridaben
Pyrimoxyfen
Quinalphos
Simazine
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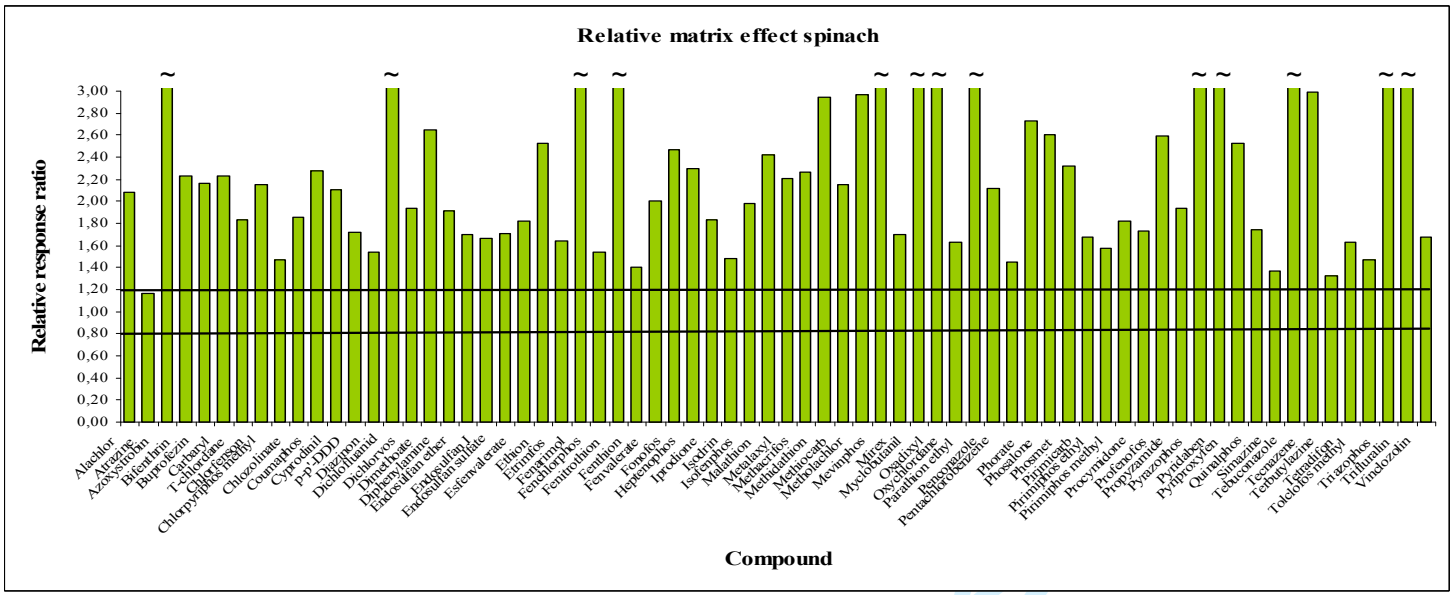
Terbuthylazine
Tetradifon
Tolclofos methyl
Triazophos
Trifluralin
Vinclozolin
Compound


Relative matrix effect spinach
Relative response ratio
3,00
2,80
2,60
2,40
2,20
2,00
1,80
1,60
1,40
1,20
1,00
0,80
0,60
0,40
0,20
0,00
Alachlor
Atrazine
Azoxystrobin
Bifenthrin
Bupirimate
Carbaryl
Cis-chlordane
Chlorfenson
Chlorpyrifos methyl
Chlozolinate
Coumaphos
Cyprodinil
pp-DDD
Diazinon
Dichlofluanid
Dichlorvos
Dimethoate
Diphenylamine
Endosulfan ether
Endosulfan I
Endosulfan sulfate
Esfenvalerate
Ethion
Etrimfos
Fenarimol
Fenchlorphos
Fenitrothion
Fenthion
Fenvalerate
Fonofos
Heptenophos
Iprodione
Isodrin
Isofenphos
Malathion
Metalaxyl
Methacrifos
Methidathion
Methiocarb
Methlachlor
Mevinphos
Mirex
Myclobutanil
Oxadixyl
Oxychlordane
Parathion ethyl
Pentachloroaniline
Pentachlorobenzene
Phorate
Phosalone
Phosmet
Pirimicarb
Pirimiphos ethyl
Pirimiphos methyl
Procymidone
Profenofos
Propyzamide
Pyrazophos
Pyridaben
Pyriproxyfen
Quinalphos
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Triazophos
Trifluralin
Vinclozolin
Compound

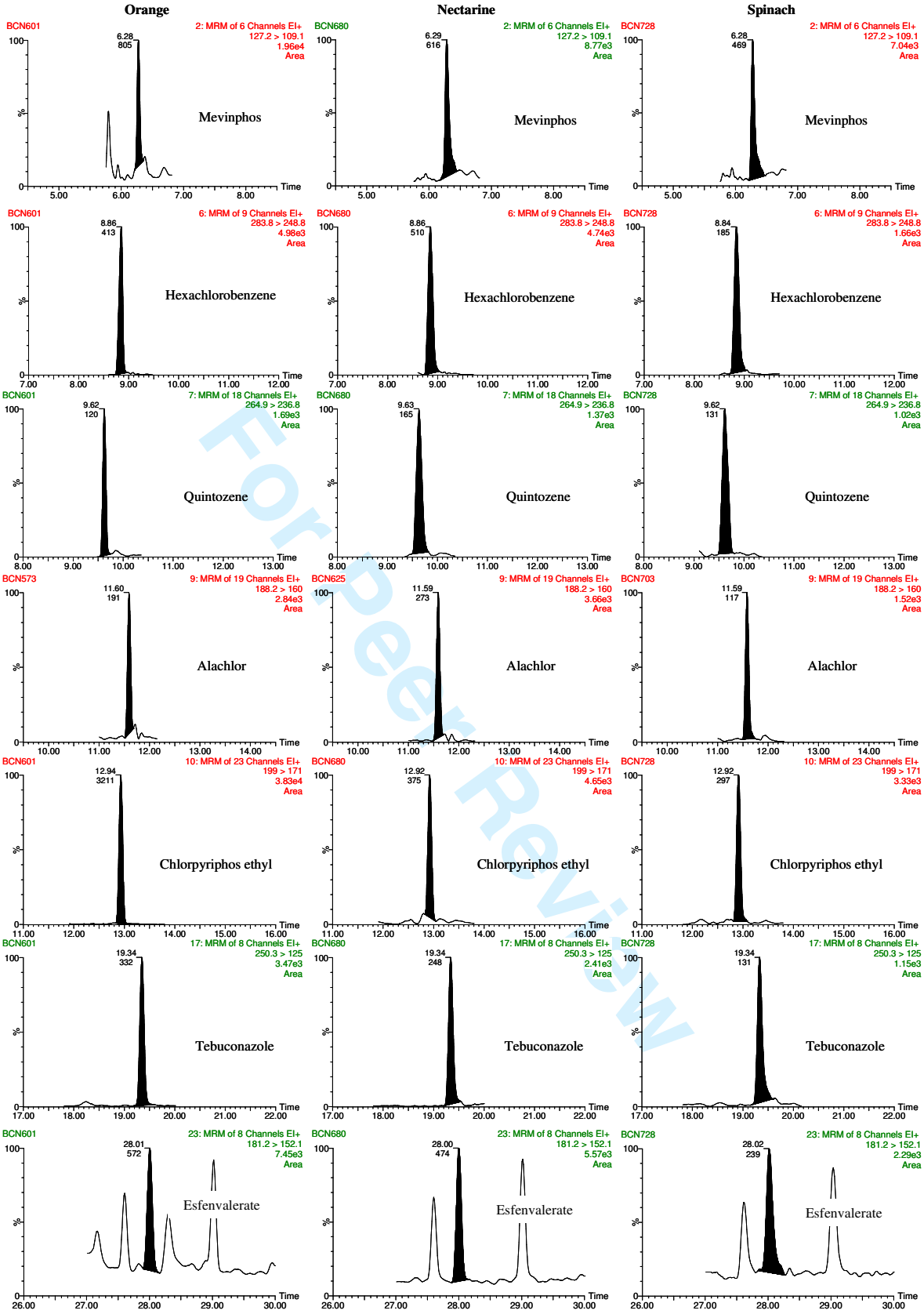

Figure 2

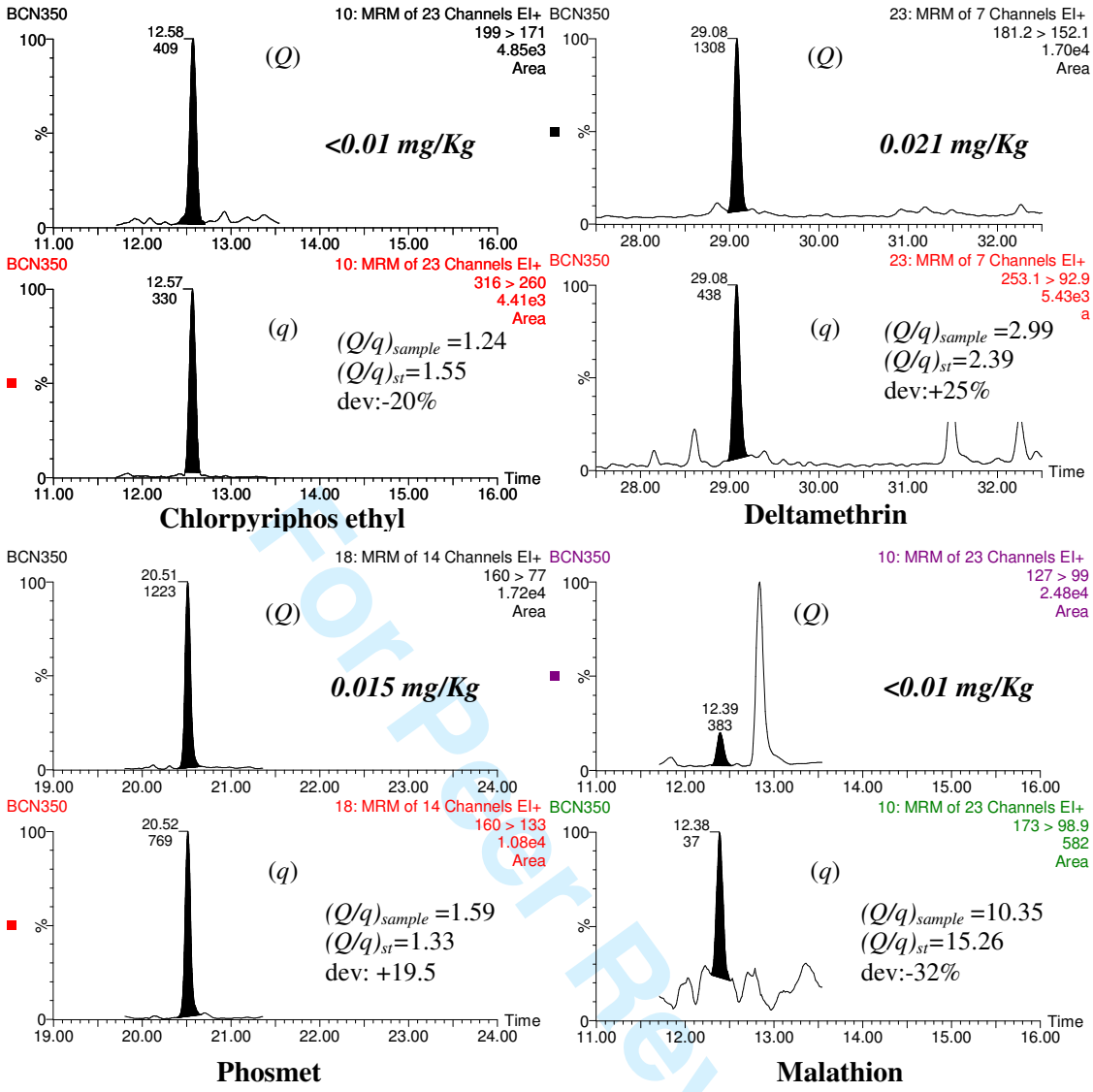

Figure 3

Original Paper

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Multi-residue determination of 130 multiclass pesticides in fruits and vegetables by gas chromatography coupled to triple quadrupole tandem mass spectrometry

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ABSTRACT

A multi-residue method has been developed and validated for the simultaneous quantification and confirmation of around 130 multiclass pesticides in orange, nectarine and spinach samples by GC-MS/MS with triple quadrupole analyzer. Compounds have been selected from different chemical families including insecticides, herbicides, fungicides and acaricides. Three isotopically labelled standards have been used as surrogates in order to improve accurate quantitation.

Samples were extracted by using accelerated solvent extraction (ASE) with ethyl acetate. In the case of spinach, an additional clean-up step by gel permeation chromatography was applied. Determination was performed by GC-MS/MS in electron ionization mode adquiring two MS/MS transitions for each analyte. The intensity ratio between quantitation transition (Q) and identification transition (q) was used as confirmatory parameter (Q/q ratio). Accuracy and precision were evaluated by means of recovery experiments in orange, nectarine and spinach samples spiked at two concentration levels (0.01 and 0.05 mg/Kg). Recoveries were in most cases between 70-120 % and RSD were below 20 %. The limits of quantification objective for which the method was satisfactorily validated in the three samples matrices were for most pesticides 0.01 mg/Kg.

Matrix effects over the GC-MS/MS determination were tested by comparison of reference standards in pure solvent with matrix-matched standards of each matrix. Data obtained showed enhancement of signal for the majority of analytes in the three matrices investigated. Consequently, in order to reduce the systematic error due to this effect, quantification was performed using matrix-matched standard calibration curves. The matrix effect study was extended to other food matrices such as raisin, paprika, cabbage, pear, rice, legume and gherkin, showing in all cases a similar signal enhancement effect.

Key words

Pesticides; gas chromatography tandem mass spectrometry; triple quadrupole; fruits and vegetables; matrix effect; acceleration solvent extraction; multi-residue analysis

1. INTRODUCTION

Pesticides are used to protect crops before and after harvest from infestation by pests and plant diseases. A consequence of their use may be the presence of pesticide residues in treated products, fruits, vegetables, grains and other commodities. Even after being washed, stored, processed and prepared, some residues may remain in both, fresh products and processed foods. The European Commission has set harmonized Maximum Residue Levels (MRL) in the Regulation (EC) Nº 396/2005 [1] in order to avoid that different Member States gave different MRL values for the same pesticide in the same crop, a situation which gave rise to questions from consumers, farmers and traders [2, 3] .

Nowadays, the control of pesticide residues in food commodities has become a requirement for compliance with the legislation, ensuring safety of the population and international and national trade. Therefore, multi-residual methodologies capable to determine a large number of pesticides simultaneously with satisfactory sensitivity and selectivity are highly required. However the different physicochemical properties presented by the different pesticide chemical classes increases the difficulty when developing a unique analytical method for multi-residue pesticide determination in food commodities.

Typically, the determination of pesticides in complex matrices, such as fruits and vegetables, involves a sample treatment using different techniques as Soxhlet extraction [4], solid phase extraction (SPE) [5, 6], supercritical fluid extraction (SFE) [7], microwave-assisted extraction (MAE) [8], matrix solid phase dispersion (MSPD) [9] and accelerated solvent extraction (ASE) [10-12]. Some of the procedures reported for fruits and vegetables require the application of additional clean-up steps to remove interferences (such as chlorophyll or fat) and also to improve detection limits. Gel permeation chromatography (GPC) and solid phase extraction (SPE) have been commonly applied for this purpose [13].

The QuEChERS (quick, easy, cheap, effective, rugged and safe) method developed in 2005 by Lehotay and co-workers [14] could be referenced as an example of a sample preparation technique (extraction and clean-up) applied for the multi-residue determination of pesticides in food and agricultural products. The key of this approach is the development of a rapid extraction procedure called dispersive solid-phase extraction which quickly removes water and non-target compounds with magnesium sulphate and a primary-secondary amine sorbent. Several advantages have been reported for this method compared to traditional sample preparation methods of pesticide residue analysis, like high recoveries for a wide volatility range of pesticides, accurate results, quick treatment, reduced use of solvent and reactives and in addition being robust and reliable. In combination with gas chromatography/mass spectrometry, with ion trap analyzer, and with liquid chromatography/tandem mass spectrometry, with triple quadrupole analyzer, this approach has been successfully validated for a large number of pesticides in lettuce and orange [14]. This method was subjected to improvements, using buffering during the extraction to improve the recoveries of problematic pesticides (e.g. folpet, dichlofluanid, chlorothalonil and pymetrozine), without sacrificing recoveries of other pesticides in fruits and vegetables samples [15]. It has been applied in a collaborative study to determine multiple pesticides residues in fruits and vegetables for twenty representative pesticides in three matrices (grapes, lettuces and oranges) with satisfactory results [16].

The determination of GC-amenable pesticides in food samples has been traditionally carried out by gas chromatography (GC) coupled to mass spectrometry (MS), due to the excellent resolution of capillary GC and satisfactory sensitivity and confirmation power of GC-MS based on electron ionization (EI) full scan mass spectra. Several applications of multi-residue GC-MS methods have been described in the literature in different food commodities including vegetables (potato, cabbage, carrot, cucumber and beans), fruits (apple and orange), rice, baby

food and other products, some of them reaching more than 100 compounds [17-21]. Most of them use single quadrupole MS analyzer working in Selected Ion Monitoring (SIM) mode with one target and some qualifier ions for quantitative analysis of pesticides. However, in recent years, the application of tandem mass spectrometry (MS/MS) has emerged as a more valuable approach, which allows higher selectivity and sensitivity, minimizing or even removing many chromatographic interferences.

The use of tandem mass spectrometry (MS/MS) with triple quadrupole (QqQ) analyzer takes advantage of adequate precursor and product ions selection and offers the possibility of applying selected reaction monitoring (SRM), one of the most selective and sensitive approaches for simultaneous quantification and confirmation. In this way, matrix interferences are minimized, even eliminated, improving the selectivity and the sensitivity, reaching very low detection limits, due to the lower chemical noise in the chromatograms. In addition, acquiring two SRM transitions and evaluating their Q/q ratio (quantification transition (Q), confirmation transition (q)) leads to a reliable confirmation of the compound detected in sample [22, 23].

Several authors have reported the application of GC-MS/MS using QqQ analyzer for the determination of pesticide residues in different food commodities, such as meat [24-26], cereals and dry animal feed [27, 28], eggs [29] and vegetables and fruits [30-35].

In this paper, a wide-scope multi-residue method has been developed based on GC-MS/MS with QqQ analyzer for the determination of a large number of pesticides in fruits and vegetables. The procedure has been applied for the screening, quantification and confirmation of around 130 pesticides in three matrices (orange, nectarine and spinach). Sample treatment is based on the standard operative procedures already applied in the Chemistry Laboratory of Public Health Agency of Barcelona (ASPB) and consists on a efficient ASE with ethyl

acetate, an interesting alternative to the use of acetonitrile, which is specially needed at present due to the difficulties to get commercial acetonitrile available at low prices.

2. EXPERIMENTAL

2.1. Reagents

Reference standards were purchased from Dr Ehrenstorfer (Augsburg, Germany). Stock standard solutions (around 500 µg/mL) were prepared by dissolving reference standards in acetone and were stored in a freezer at -20ºC. Working pesticide standard mixtures were prepared by dilution of stock solutions in hexane (for GC-MS/MS optimization) or in ethyl acetate (for sample fortification and for matrix effect study).

Three isotopically labelled compounds, purchased from Dr. Erhenstorfer, were used as surrogates: *p,p'*-DDE D_8 (100 µg/mL), hexachlorobenzene (HCB) $^{13}C_6$ (100 µg/mL) and terbutylazine D_5 (100 µg/mL). Individual stock solutions of 10 µg/mL were prepared by volume dilution in acetone. A mixture solution of labelled standards (2 µg/mL) was prepared by volume dilution of individual stock solutions in ethyl acetate. Further dilutions of this mixture were prepared in ethyl acetate.

In order to simplify chromatographic determination during optimization, analytes were divided in two groups. Two matrix-matched calibration curves containing the two pesticides mixtures were prepared from standards diluted in blank extracts for every matrix, orange, nectarine and spinach in order to perform sample quantification. The preparation was performed differently, for orange and nectarine, and for spinach. For the first group, 5 mL of sample extract was evaporated to dryness under a gentle nitrogen stream. Then, it was redissolved with 100 µL of the isotopically labelled compounds solution of 500 µg/L and 150 µL of the pesticide mixture at adequate concentration. For spinach, 250 µL of sample extract was evaporated to dryness under a nitrogen stream, and it was redissolved with 100 µL of the internal standard mixture of 625 µg/L and 150 µL of the pesticide mixture at adequate concentration.

Acetone (pesticide residue analysis quality), ethyl acetate, hexane (ultra trace quality) were purchased from Scharlab (Barcelona, Spain) and cyclohexane (for GC, Suprasolv) were purchased from Merck (Barcelona, Spain). Inert diatomaceous earth (high purity quality) Hydromatrix and anhydrous sodium sulphate were purchased from Varian (Middelburg, The Netherlands) and from Scharlab, respectively.

2.2. Apparatus

Accelerated solvent extraction (ASE) was performed using a Dionex (Sunnyvale, USA) ASE 200 system equipped with solvent controller that allowed automated delivery of up to four solvents. The volume of the extraction cell used was 33 mL and the bottom was covered with two cellulose filters (19.8 mm I.D). Ethyl acetate was selected as extraction solvent and the extraction temperature and pressure were set at 70 ºC and 10.34 MPa (1500 psi), respectively. The pre-heating and static times were set at 2 and 3 min, respectively. The contact solvent time was 5 min, with a flush volume of 60 % and executing 2 cycles.

Gel Permeation Chromatography (GPC) clean up step was performed with a GPC system Agilent 1100 (Palo Alto, USA) equipped with a fraction collector, adapted to inject large sample volumes and with two connected Envirogel GPC clean-up columns from Waters (Milford, MA, USA). Both columns were packed with high-performance, fully-porous, highly cross-linked, styrene divinylbenzene copolymer particles: 15mm x 19 mm (pre-column) and 300 mm x 19 mm, respectively.

2.3. GC instrumentation

A GC system (Agilent 6890N, Palo Alto, USA) equipped with an autosampler (Agilent 7683) was coupled to a triple quadrupole (QqQ) mass spectrometer Quattro Micro GC (Waters, Boston, USA), operating in EI mode. The GC separation was performed using a fused silica HP-5MS capillary column with a length of 30 m, an internal diameter of 0.25 mm and a film

thickness of 0.25 μm (J&W Scientific, Folson, CA, USA). The oven was programmed as follows: 70ºC (1.5 min); 25 ºC/min to 180ºC (3 min); 5 ºC/min to 300ºC (5.1 min). Splitless injections of 1 μL of the sample extracts were carried out with an injector temperature of 240°C and a splitless time of 1 min. Helium 99.999% (Carburos metálicos, Valencia, Spain) was used as a carrier gas at a flow of 1 mL/min. The interface temperature was set to 260ºC.

The ionization mode selected was EI (with a solvent delay of 4 min), setting the source temperature at 250ºC. The MS/MS procedure was designed as Selected Reaction Monitoring (SRM) mode using Argon 99.995% (Carburos metálicos, Valencia, Spain) as the collision gas at a pressure of $2.5x10^{-3}$ mbar in the collision cell. A dwell time per channel between 0.01-0.05 s was chosen, depending on the number of transitions recorded in each window and on the peak width of each compound, in order to get a minimum of 16 points per peak.

Heptacosa (Perfluorotri-n-butylamine), used for the daily mass calibration, was injected using a syringe in the reference reservoir for this purpose. The Quanlynx application manager was used to process the data obtained from calibration standards and from fruit and vegetable sample extracts.

2.4. Sample preparation

Orange, nectarine and spinach samples were purchased directly from a local market, in the city of Barcelona (Spain). Samples were chopped, homogenised and then stored in a freezer at – 20ºC until analysis.

The extraction of samples was performed as follows: 7 g of diatomaceous earth was added to 10 g of triturated sample and then homogenised in a mortar. The content was transferred to a 33 mL-extraction cell; a volume of 0.5 mL of the isotopically labelled internal standard mixture (1 μg/mL) was added, and then it was subjected to the ASE procedure with ethyl acetate as described before. The ethyl acetate extract (around 50 mL) was concentrated to

approximately 35 mL in TurboVap at 35ºC under a nitrogen stream. Then, approximately 2 g of anhydrous sodium sulphate was added to eliminate the existing water. At this point, the need of applying clean up step has to be considered according to sample type. In the case of orange and nectarine, this step was not required. The organic extract obtained from ASE was collected into a volumetric flask and the final volume was adjusted to 50 mL with ethyl acetate. An aliquot of 10 mL was evaporated to dryness in TurboVap and the residue was redissolved with 0.5 mL of ethyl acetate and directly injected into the GC-MS/MS system.

For spinach samples, a GPC clean-up step was necessary. For this purpose, the organic extract obtained from ASE, was evaporated to approximately 1 mL in TurboVap. Then, volume was adjusted to 2 mL with ethyl acetate, and filtered through a 0.45 µm, 25 mm Millex filter. A 1 mL aliquot of the filtered extract was injected into the GPC system and eluted with cyclohexane:ethyl acetate (1:1, v/v) at a flow rate of 5 mL/min (collect time 14.5-21.0 min). The total volume collected was evaporated to dryness in a TurboVap at 35ºC under a nitrogen stream. The residue was redissolved with 1 mL of ethyl acetate and injected into the GC-MS/MS system.

2.5. Validation study

The *linearity* of the method was studied by analyzing matrix-matched standards (5 concentration levels, in duplicate) ranging by one side from 12 to 120 µg/L (which corresponded to 0.003-0.03 mg/Kg in orange and nectarine and to 0.0024-0.024 mg/Kg in spinach) and by the other side from 60 to 600 µg/L (which corresponded to 0.015-0.15 mg/Kg in orange and nectarine and 0.012-0.12 mg/Kg in spinach). Linearity was assumed when regression coefficient was >0.99 with residuals lower than 30%.

The *accuracy* was estimated by means of recovery experiments, analyzing orange, nectarine and spinach samples (n=5) spiked at two concentrations levels (0.01 mg/Kg and 0.05 mg/Kg).

Spiked samples were prepared by adding the adequate volume of standard mixtures over the triturated sample (10 g), and left to stand for 1 hour. Then they were subjected to extraction procedure as described in 2.4.

According to the Regulation (EC) 396/2005 [1], values of MRL (or the lower limit of analytical determination) for pesticides selected are equal or higher than 0.01 mg/Kg in orange, nectarine and spinach. So, validating the method to 0.01 mg/Kg should be appropiate for regulatory purposes.

Precision was determined from the above mentioned recovery experiments, carried out at two fortification levels. It was expressed as repeatibility in terms of relative standard deviation (RSD) (n=5) at each fortification level.

S*electivity* of the method was estimated considering the absence of interfering peaks at the retention time of each compound and based on the acquisiton of two MS/MS transitions for each analyte by selecting adequate precursor and product ions.

The *limit of quantification* (LOQ) objective was established as the lowest concentration level validated with satisfactory values of recovery (70-120%) and precision (RSD $\leq$ 20%), i.e. 0.01 mg/Kg for most of analyte matrix combinations tested.

The *limit of detection* (LOD) was estimated as the analyte concentration that produced a peak signal of three times the background noise in the chromatogram of the sample spiked at the lowest level studied. The LOD was obtained using a software option for estimating the S/N ratio and referring/recounting this value to a S/N value of three.

As *confirmation criteria* of positives in samples, the Q/q ratio was considered, defined as the ratio between the intensity of the quantification transition (Q) and the intensity of the confirmation transition (q). The Q/q reference value for each compound in each sample matrix was calculated as the mean value obtained from matrix matched standards at different

concentration levels in the range of 60 – 600 µg/L. For the reliable confirmation of positive findings, a maximum ratio tolerance ±20% (when Q/q ratio value is lower than 2), ±25% (Q/q ratio between 2-5), ±30% (Q/q ratio between 5-10) or ±50% (Q/q ratio higher than 10) were accepted, in the line of the European Union Decision 2002/657/EC [23]. Obviously, agreement in the retention time between reference standard and sample was also required to give a detection as positive.

3. RESULTS AND DISCUSSION

The analytical procedures presented in this work were based on the methodology already established as standard operating procedures in the Chemistry Laboratory of ASPB (Spain) for the determination of pesticides in fruits and vegetables. These procedures have been satisfactorily applied in this laboratory but using GC-MS with single quadrupole analyzer for the measurement. Our purpose was to improve those methodologies by changing the analytical determination using GC-MS/MS with QqQ analyzer, in order to improve sensitivity and selectivity taking advantage of the possibility of applying selected reaction monitoring (SRM) adquiring two MS/MS transitions for each compound

3.1. GC-MS/MS optimization

Optimization of the MS/MS method was performed for all pesticides using hexane standard solutions injected in the EI ionization mode. After obtaining the full scan spectra for each compound, the base peak of the spectrum was selected as precursor ion. Once the precursor ion was selected, different values of collision energy (between 5-40 eV) were tested to study the fragmentation. The final purpose was to develop a SRM method with two MS/MS transitions (with the exceptions of surrogates with only one transition), normally the most sensitive ones, for each compound in order to have a reliable confirmation of the pesticide detected in samples.

Table 1 shows the precursor and the product ions corresponding to the quantitative and confirmative transitions monitored. Optimum values of collision energy for most compounds were found to be between 10-30 eV. The dwell time parameter was modified between 0.01 and 0.05 s in order to obtain a good chromatographic peak (with at least 16 points/peak) still maintaining satisfactory sensitivity for each compound.

The Q/q intensity ratios are also shown in **Table 1** for each matrix studied. Average Q/q ratios were calculated as the mean values obtained after injection of matrix matched standards at four concentration levels (60, 150, 300 and 600 μg/L), obtaining RSD tipically below 15%. As Q/q ratio values, similarly to retention times, might suffer slight variation along the time, they might be corrected with the matrix-matched standard calibration included in every sample analysis batch, if necessary.

3.2. Sample preparation optimization

Sample extraction was performed with ASE using ethyl acetate as extraction solvent. In the case of the more complex matrices, such as spinach, a purification step by GPC was required. As indicated above, these sample preparation procedures were already being applied in the Chemistry Laboratory of ASPB. Consequently, they were not really subjected to a complete optimization study in the present paper, as, before introduction in the routine work, the ASE procedure was already optimized by ASPB on the basis of the commercial information and application notes, and testing different times (pre-heat, static and heat). Ethyl acetate was chosen as extraction solvent because of its low cost and low toxicity. Moreover, ethyl acetate avoided problems of miscibility with subsequent solvent mixtures. ASE presented the advantages of higher efficiency, low toxicity of extraction solvent and short extraction time, as well as the simplicity of an automated extraction.

In order to purify extracts, GPC clean-up was considered highly recommendable for a wide range of matrices. A mixture of cyclohexane:ethyl acetate was selected as elution solvent. Different proportions of this mixture were tested, and finally cyclohexane:ethyl acetate (1:1, v/v) was selected as an adequate elution solvent. The extract collect time was set from 14.5 to 21 min, as a compromise between clean-up efficiency and sensitivity.

3.3. Matrix effect

Matrix effects for orange, nectarine and spinach samples were evaluated. The study was performed by comparing the response of reference standards prepared in pure solvent with the response of matrix matched standards (prepared as described in section 2.1.). The ratio between response in matrix and response in pure solvent was taken as absolute matrix effect. Moreover, due to the fact that labelled internal standards may correct signal suppressions or enhancements resulting from matrix interference, the ratio between relative responses of standard in matrix and standard in solvent was also studied. This ratio was taken as relative matrix effect. In both cases, a ratio value of 0.8-1.2 was established as acceptable; this means that no severe matrix effects affected in this case the response of the analytes after application of the overall analytical procedure.

Concentration levels tested for matrix effects were 150, 300 and 600 µg/L obtaining the average absolute response or relative response of analytes at these three levels. In the case of oranges, nearly 70% of pesticides suffered significative matrix effect, with response ratio out of the range 0.8-1.2. Most of them showed an evident signal enhancement in the presence of matrix. A similar behaviour was observed for nectarine and spinach matrices, although the degree of signal enhacement was higher, specially in spinach. When using responses relative to the internal standards, a notable correction was observed in all matrices. In spite of this, a considerable number of pesticides still gave a response out of the 0.8-1.2 range, as **Figure 1 a** illustrates for nectarine. The strong matrix effect could not be corrected with I.S. for spinach, as depicted **Figure 1 b**, where relative matrix effect for spinach reveals that most of pesticides suffered signal enhancement with responses out of the 0.8-1.2 range. In such a case, a high number of I.S. would be surely necessary to properly correct matrix effect for each analyte.

It can be concluded that for correct quantification of pesticides in orange, nectarine and spinach samples, matrix-matched standards calibration using relative responses as regards internal standards would had to be used.

In order to further study the applicability of developed procedures to other food matrices, matrix effect was also evaluated in other matrices such as mango, raisin, paprika, cabbage, pear, rice, legume and gherkin. The study was performed at a single concentration level of 100 µg/L. Typically more than 80% of pesticides investigated showed enhancement of signal in matrix when mango, raisin, paprika, pear and rice were studied. In the rest of matrices, the percentage of pesticides showing signal enhancement was lower (around 40-50%). So, although the degree of signal enhacement may vary from one vegetable matrix to other, it seems that in all matrices studied it would be necessary the use of matrix matched calibration using relative responses to internal standard for correct quantification of pesticides.

3.4. Validation results

Validation of the multi-residue method in orange, nectarine and spinach was carried out in terms of accuracy, precision, selectivity, limits of detection and limits of quantification. Three labelled internal standards were added as surrogates to improve quantitation**.** The use of the different surrogates was established considering the chemical families studied and the retention times of the analytes. Thus, the surrogates used for insecticides were: HCB $^{13}C_6$ or *p,p'*-DDE D_8 for OCs; HCB $^{13}C_6$ or terbutylazine D_5 for OPs; terbutylazine D_5 for pyretroids and carbamates and HCB $^{13}C_6$ for the rest of insecticides. The surrogates used for herbicides were: terbutylazine D_5 for triazines and HCB $^{13}C_6$ for the rest of herbicides. The surrogate used for acaricides and fungicides was HCB $^{13}C_6$.

Linearity of the chromatographic method using matrix-matched standards was satisfactory in the range of concentrations between 12-600 µg/L (0.003-0.15 mg/Kg in orange and nectarine and 0.0024-0.012 mg/Kg in spinach) with correlation coefficients higher than 0.99 and residuals lower than ± 30%.

Accuracy and precision were estimated by means of recovery experiments (n=5) at two concentration levels (0.01 mg/Kg and 0.05 mg/Kg) for each sample matrix studied. **Table 2** shows the results obtained for orange, nectarine and spinach samples. As it can be seen, most compounds presented satisfactory recoveries in orange and nectarine with values between 70-120% at both spiked levels. Several exceptions were found with recoveries between 60 and 70%, especially at the lowest fortification level assayed, although normally with satisfactory RSD. Dicofol, heptachlor epoxide and methoxychlor were poorly recovered at the lowest level in both matrices. Omethoate and pentachlorobenzene showed in general recoveries below 70% in the three sample matrices tested. Captan was specially problematic in all samples due to the well-known difficulties associated to its determination [36]. The low recoveries for azinphos methyl at the 0.05 mg/Kg in both, orange and nectarine, did not fit with those at the 0.01 mg/Kg, and further experiments would be necessary to get a better knowledge about this fact. Apart from this exception, only four recovery values were sligthly lower than 50% and always corresponded at 0.01 mg/Kg level (dicofol and methoxychlor in orange; methoxychlor and pentachlorobenzene in nectarine) but maintaining good precision (RSD ≤ 15%).

Data of spinach reveal that it was the most difficult matrix among the three studied, and thus 9 pesticides (acrinathrin, captan, *lambda*-cyhalothrin I and II, disulfoton, *tau*-fluvalinate I and II, folpet and tefluthrin) could not be detected, probably due to their behaviour during the GPC clean-up step. Improvement of these results might be achieved by further optimization of the GPC procedure. Additionally, acephate, dicofol and triflumizole could not be determined in spinach samples as they did not show any response, even in matrix matched standards, as shown in **Table 1**. Some other compounds could not be determined at the 0.01 mg/Kg level in none of the matrices due to their low sensitivity, being heptachlor epoxide an example of this behaviour in the three sample matrices studied.

Precision was satisfactory as the majority of pesticides showed values of RSD lower than 20%. The poorest RSD values were observed for dichlofluanid in spinach at 0.01 mg/Kg and endrin and pyrimethanil in nectarine at 0.05 mg/Kg. The lowest level validated, i.e. 0.01 mg/Kg, could be established as the LOQ objective for most of compounds investigated in orange, nectarine and spinach samples, with the few exception where unsatisfactory data were obtained. LOD, estimated as the analyte concentration giving a peak of three times the background noise in the chromatograms corresponding at the LOQ level, were generally in the range of 0.0001 to 0.01 mg/Kg. LOD values were obtained from the quantification transition (Q), i.e. the most sensitive one of the two transitions acquired.

In the procedure proposed, three internal standards have been used in combination with matrix matched calibration in order to correct the demonstrated matrix effects over recoveries. This approach has been found satisfactory for most analyte/matrix combinations in view of the recoveries obtained. Obviously, the use of higher number of labelled internal standards should improve the recovery for some of the 130 compounds studied, especially for those with higher differences in chemical structure related to the internal standard used.

As an example, **Figure 2** shows GC-MS/MS chromatograms for several pesticides in orange, nectarine and spinach samples fortified at 0.01 mg/Kg. Pesticides have been chosen within a wide range of retention times (between 6 min to 29 min) to better illustrate the performance of the method.The selectivity of the method was satisfactory and came from the acquisition of two specific SRM transitions for each pesticide. GC-MS/MS chromatograms did not show the presence of interfering peaks at the analyte retention time for none of the pesticides investigated in this work.

As regards Q/q ratios (see **Table 1**), they were, in general, rather similar in the matrices investigated for a given pesticide, with some exceptions, normally in spinach matrix (tefluthrin, metribuzin, carbaryl, fenitrothion, parathion ethyl, tolyfluanid, tetrachlorvinphos,

bupirimate, bromopropylate, cyfluthrin, cypermethrin, fluvalinate and azoxystrobin). This would make necessary to use the Q/q ratios of standards in matrix for an adequate confirmation of positives in samples instead of standards, in solvent or in any other food matrix. In many pesticides, favorable Q/q ratios (around 1-2) were obtained, what indicates that confirmation transition had similar sensitivity to quantification transition, wich would allow confirmation of positives at very low concentration levels. In a few compounds, confirmation would be problematic at low levels, due to unfavourable Q/q ratios (e.g. diphenylamine, parathion ethyl, buprofezin and azoxystrobin).

3.5. Application to real samples

In order to study the applicability of the methodology developed, several samples collected from a local market in Barcelona (Spain) were analyzed (six samples, two of each matrix).

The results obtained are shown in **Table 3**. The OC insecticide mirex was detected in 50% of the samples analyzed but at concentrations below 0.01 mg/Kg. Persistent OC insecticides, like DDT and its metabolites DDD and DDE, or endosulfan sulfate were detected in some samples but at very low levels, very close to the LODs. Only three positive findings could be quantified, as they were above 0.01 mg/Kg: chlorpyrifos in orange 2 (0.016 mg/Kg) and, deltamethrin and phosmet in nectarine 1 (0.021 and 0.015 mg/Kg, respectively). In these cases, the concentration were lower than the MRL established for three insecticdes in the sample matrices analyzed.

As regards confirmation of positive findings, all pesticides detected were confirmed by the use of the two transitions monitored and the compliance of the Q/q intensity ratios. The acquisition of two transitions allows the simultaneous quantification and confirmation of pesticides in only one injection, as an alternative approach to the proposed elsewhere [30, 31] where one injection with only one transition is used as a screening method and a second

injection, of only potentially positive samples, is required for confirmation and quantification purposes. Anyway, in the case of exceeding MRLs, a second independent analysis would be required to confirm the presence of the pesticide in the sample as well as its concentration to be above the MRL. All Q/q ratios were within the range of the tolerance accepted [23] around the experimental Q/q value obtained from reference standards in matrix injected in the same analysis sequence. **Figure 3** shows GC-MS/MS chromatograms corresponding to the positive findings detected in one of the nectarine samples. A reliable identification of analytes in this sample was feasible by means of the experimental Q/q intensity ratios, even at concentrations lower than 0.01 mg/Kg.

4. CONCLUSIONS

A multi-residue method has been developed and validated for the simultaneous quantification and confirmation of around 130 pesticides in fruits and vegetables, selecting orange, nectarine and spinach as matrices under study. The potential of GC-MS/MS with triple quadrupole analyzer has shown to be a key tool for the quantitative determination of this high number of pesticides. The selection of two SRM transitions, one for quantification and one for confirmation, gives excellent selectivity and sensitivity and the possibility of safe identification, using Q/q intensity ratio as a confirmatory parameter.

Extraction of samples was made by ASE using ethyl acetate as solvent. The overall multi-residual method has been fully validated at 0.01 and 0.05 mg/Kg in the three types of samples, obtaining satisfactory accuracy and precision in most cases. The methodology developed in this work was applied to the analysis of market samples, where some pesticides were detected and identified at low concentration levels, even below 0.01 mg/Kg.

The study of matrix effect in orange, nectarine and spinach samples showed an evident enhancement of signal produced by matrix components for the majority of pesticides investigated, specially in spinach. A similar behaviour was observed for other food matrices investigated (mango, raisin, paprika, cabbage, pear, rice, legume and gherkin). The use of labelled I.S. helped to minimize matrix effects for some pesticide/matrix combinations, although did not always assure appropriate correction. Therefore, matrix-matched standard calibration was required in order to perform a correct quantification in samples.

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Table 1. Experimental conditions of the optimized GC-MS/MS method.

t_R (min)	Window (min)	Compounds	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell time (s)	Collision energy (eV)	Orange Q/q ratio [a]	Nectarine Q/q ratio [a]	Spinach Q/q ratio [a]
5,2	4,0-5,7	Dichlorvos	185	93	0,02	10	1,64 (11)	1,41 (11)	1,31 (2)
			109	79	0,02	10			
6,3		Mevinphos	127	109	0,01	10	1,65 (8)	1,53 (4)	1,69 (3)
			192	127	0,01	10			
6,4		Acephate	136	94	0,01	10	13,4 (15)	9,68 (5)	No response
			136	112	0,01	10			
6,7	6,4-7,1	Methacrifos	208	180	0,05	10	1,35 (14)	1,49 (9)	3,07 (13)
			240	180	0,05	10			
7,0		Pentachlorobenzene	250	142	0,05	30	1,70 (6)	1,66 (6)	1,40 (3)
			248	142	0,05	30			
7,3	6,9-8,3	Heptenophos	124	89	0,01	10	13,2 (19)	14,8 (11)	15,26 (9)
			109	79	0,01	10			
7,6		Omethoate	156	110	0,01	10	1,39 (7)	1,11 (13)	1,20 (7)
			110	79	0,01	10			
7,6		Tecnazene	178	143	0,01	10	1,25 (9)	1,23 (3)	1,19 (7)
			213	142	0,01	20			
7,8		Diphenylamine	168	167	0,01	10	107 (17)	114 (16)	108 (15)
			169	143	0,01	10			
7,8		Ethoprophos	158	97	0,01	10	1,79 (5)	2,25 (16)	2,21 (6)
			158	114	0,01	10			
8,0		Chlorpropham	127	65	0,01	20	16,5 (11)	9,82 (7)	17,7 (3)
			153	90	0,01	20			
8,2		Trifluralin	306	264	0,01	10	7,54 (15)	6,76 (12)	7,31 (13)
			264	160	0,01	20			
8,5	8,1-8,7	Phorate	121	65	0,05	5	2,07 (8)	2,53 (11)	2,91 (3)
			260	75	0,05	10			
8,6		α–HCH	217	181	0,05	10	1,08 (2)	1,13 (6)	1,04 (4)
			219	183	0,05	10			

[a] Average value calculated from matrix-matched standard calibration (four concentration levels) and RSD in parenthesis.
For every compound, the first transition corresponds to quantification (Q) and the second transition to confirmation (q)

Table 1 (cont.). Experimental conditions of the optimized GC-MS/MS method

t_R (min)	Window (min)	Compounds	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell Time (s)	Collision energy (eV)	Orange Q/q ratio [a]	Nectarine Q/q ratio [a]	Spinach Q/q ratio [a]
8,8	8,4-9,3	HCB	284	249	0,02	20	2,48 (4)	2,61 (9)	1,08 (12)
			284	214	0,02	20			
8,8		*HCB-$^{13}C_6$*	*292*	*257*	*0,02*	*20*	-	-	-
9,0		Dimethoate	93	63	0,02	10	1,89 (3)	1,89 (11)	2,02 (9)
			125	79	0,02	10			
9,1		Simazine	201	173	0,02	10	1,14 (13)	1,21 (3)	1,02 (8)
			186	91	0,02	10			
9,2		Atrazine	200	122	0,02	10	1,54 (12)	1,56 (10)	1,44 (13)
			200	132	0,02	10			
9,4	8,8-10,2	γ–HCH	217	181	0,01	10	1,08 (11)	1,02 (6)	1,08 (6)
			219	183	0,01	10			
9,5		β–HCH	217	181	0,01	10	1,04 (3)	1,07 (6)	1,09 (8)
			219	183	0,01	10			
9,6		*Terbutylazine D_6*	*234*	*178*	*0,01*	*10*	-	-	-
9,6		Terbufos	231	129	0,01	20	1,32 (4)	1,44 (12)	1,89 (3)
			231	175	0,01	10			
9,6		Quintozene	265	237	0,01	10	1,99 (14)	1,85 (18)	1,00 (4)
			237	119	0,01	20			
9,6		Terbutylazine	214	132	0,01	10	1,36 (4)	1,30 (16)	1,32 (13)
			229	173	0,01	10			
9,7		Fonofos	137	109	0,01	10	2,62 (13)	2,8 (8)	3,14 (13)
			246	137	0,01	5			
9,7		Propyzamide	173	145	0,01	10	1,96 (14)	1,65 (16)	1,66 (3)
			173	109	0,01	20			
9,9		Pyrimethanil	198	118	0,01	30	1,80 (10)	1,74 (4)	2,47 (6)
			198	156	0,01	20			
9,9		Diazinon	304	179	0,01	10	5,56 (11)	5,74 (16)	5,72 (10)
			276	179	0,01	10			

[a] Average value calculated from matrix-matched standard calibration (four concentration levels) and RSD in parenthesis.
For every compound, the first transition corresponds to quantification (Q) and the second transition to confirmation (q)

Table 1 (cont.). Experimental conditions of the optimized GC-MS/MS method

t_R (min)	Window (min)	Compounds	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell Time (s)	Collision energy (eV)	Orange Q/q ratio [a]	Nectarine Q/q ratio [a]	Spinach Q/q ratio [a]
10,0	9,5-11,5	Disulfoton	274	88	0,01	20	4,02 (6)	2,88 (10)	2,92 (13)
			186	115	0,01	5			
10,2		Tefluthrin	177	137	0,01	10	7,22 (9)	5,04 (4)	2,09 (10)
			197	141	0,01	20			
10,2		δ–HCH	217	181	0,01	10	1,04 (3)	1,04 (8)	1,05 (8)
			219	183	0,01	10			
10,4		Chlorothalonil	264	133	0,01	20	1,66 (3)	1,63 (5)	1,72 (9)
			266	168	0,01	30			
10,4		Etrimfos	181	153	0,01	10	2,32 (2)	1,98 (10)	2,31 (14)
			277	125	0,01	10			
10,7		Endosulfan ether	272	237	0,01	10	1,09 (5)	1,07 (1)	1,11 (3)
			239	204	0,01	10			
10,7		Pirimicarb	238	166	0,01	10	2,24 (5)	1,15(4)	1,52 (9)
			166	96	0,01	10			
11,1		Phosphamidon	127	109	0,01	10	2,86 (5)	3,19 (7)	3,94 (6)
			264	127	0,01	10			
11,2		Metribuzin	198	82	0,01	20	5,41 (12)	5,29 (16)	7,15 (18)
			198	111	0,01	10			
11,3	10,8-11,8	Chlorpyriphos methyl	288	93	0,01	10	3,53 (14)	3,94 (14)	4,48 (6)
			197	169	0,01	30			
11,3		Vinclozolin	285	212	0,01	10	1,04 (13)	1,2 (12)	1,01 (8)
			212	172	0,01	10			
11,4		Parathion methyl	263	109	0,01	10	8,71 (16)	7,82 (6)	9,49 (13)
			233	124	0,01	10			
11,5		Tolclofos methyl	265	250	0,01	10	3,17 (15)	3,13 (14)	2,86 (8)
			265	93	0,01	20			
11,5		Heptachlor	272	237	0,01	10	1,77 (10)	1,60 (1)	1,70 (2)
			274	239	0,01	10			

[a] Average value calculated from matrix-matched standard calibration (four concentration levels) and RSD in parenthesis.
For every compound, the first transition corresponds to quantification (Q) and the second transition to confirmation (q)

Table 1 (cont.). Experimental conditions of the optimized GC-MS/MS method

t_R (min)	Window (min)	Compounds	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell Time (s)	Collision energy (eV)	Orange Q/q ratio [a]	Nectarine Q/q ratio [a]	Spinach Q/q ratio [a]
11,6		Alachlor	188	160	0,01	10	2,20 (16)	1,93 (16)	1,87 (6)
			188	131	0,01	20			
11,5		Carbaryl	144	115	0,01	10	6,73 (10)	8,75 (6)	2,48 (9)
			115	89	0,01	20			
11,8		Metalaxyl	206	132	0,01	20	1,79 (15)	1,85 (9)	1,86 (13)
			206	117	0,01	30			
11,8		Fenchlorphos	285	240	0,01	20	2,98 (12)	3,25 (9)	3,06 (11)
			285	164	0,01	30			
12,2	11,7-13,5	Fenitrothion	260	125	0,01	20	5,62 (10)	6,10 (17)	3,57 (7)
			260	79	0,01	10			
12,3		Methiocarb	168	91	0,01	30	1,27 (12)	1,18 (9)	1,68 (8)
			168	109	0,01	30			
12,3		Pirimiphos methyl	290	233	0,01	10	1,67 (6)	1,5 (8)	1,64 (6)
			290	151	0,01	10			
12,5		Dichlofluanid	224	123	0,01	10	1,86 (10)	2,08 (3)	2,09 (5)
			167	124	0,01	10			
12,6		Aldrin	261	191	0,01	30	1,61 (5)	1,47 (5)	1,43 (12)
			263	193	0,01	20			
12,6		Malathion	127	99	0,01	10	12,6 (5)	15,3 (4)	11,4 (12)
			173	99	0,01	5			
12,7		Metholachlor	238	162	0,01	20	2,91 (14)	2,34 (7)	1,98 (10)
			162	132	0,01	10			
12,9		Fenthion	278	245	0,01	20	6,35 (17)	6,77 (17)	6,50 (2)
			278	108	0,01	10			
12,9		Chlorpyriphos ethyl	199	171	0,01	10	1,01 (6)	1,55 (2)	2,68 (6)
			316	260	0,01	10			
12,9		Parathion ethyl	291	109	0,01	10	70,2 (15)	77,2 (19)	37,0 (19)
			155	124	0,01	10			

[a] Average value calculated from matrix-matched standard calibration (four concentration levels) and RSD in parenthesis.
For every compound, the first transition corresponds to quantification (Q) and the second transition to confirmation (q)

Table 1 (cont.). Experimental conditions of the optimized GC-MS/MS method

t_R (min)	Window (min)	Compounds	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell Time (s)	Collision energy (eV)	Orange Q/q ratio [a]	Nectarine Q/q ratio [a]	Spinach Q/q ratio [a]
13,0		4,4'-Dichlorobenzophenone	250	139	0,01	35	1,32 (16)	1,55 (14)	1,25 (12)
			250	111	0,01	20			
13,5	13,0-14,2	Isodrin	193	157	0,02	20	1,62 (6)	1,66 (4)	1,53 (3)
			195	123	0,02	30			
13,7		Pirimiphos ethyl	304	168	0,02	10	1,53 (3)	1,26 (3)	1,31 (5)
			318	166	0,02	10			
13,8		Cyprodinil	224	207	0,02	10	7,12 (11)	3,81 (12)	3,89 (7)
			225	208	0,02	10			
13,9	13,3-14,3	Heptachlor epoxide	353	263	0,01	10	1,85 (16)	1,44 (16)	2,10(18)
			353	282	0,01	20			
14,0		Oxychlordane	185	121	0,01	20	1,98 (12)	1,94 (18)	1,66 (13)
			235	141	0,01	20			
14,0		Pendimethalin	252	161	0,01	10	0,94 (6)	1,09 (10)	1,16 (5)
			252	191	0,01	10			
14,1		Penconazole	248	157	0,01	20	1,53 (6)	1,71 (7)	1,64 (13)
			248	192	0,01	10			
14,2		Tolyfluanid	137	91	0,01	20	1,04 (8)	1,88 (16)	2,50 (5)
			238	137	0,01	10			
14,2		Chlozolinate	259	188	0,01	10	2,69 (11)	2,94 (16)	1,82 (19)
			188	153	0,01	10			
14,3	13,8-15,1	Chlorfenvinphos	267	159	0,01	10	2,78 (13)	3,15 (16)	2,75 (7)
			323	267	0,01	20			
14,3		Isofenphos	213	121	0,01	20	2,22 (6)	2,27 (7)	2,33 (5)
			255	121	0,01	20			
14,4		Quinalphos	157	129	0,01	10	1,12 (11)	1,07 (7)	1,05 (8)
			157	102	0,01	20			
14,5		Folpet	260	130	0,01	20	1,24 (6)	1,66 (17)	1,18 (14)
			262	130	0,01	10			

[a] Average value calculated from matrix-matched standard calibration (four concentration levels) and RSD in parenthesis.
For every compound, the first transition corresponds to quantification (Q) and the second transition to confirmation (q)

Table 1 (cont.). Experimental conditions of the optimized GC-MS/MS method

t_R (min)	Window (min)	Compounds	Precursor ion (m/z)	Product ion (m/z)	Dwell Time (s)	Collision energy (eV)	Orange Q/q ratio [a]	Nectarine Q/q ratio [a]	Spinach Q/q ratio [a]
14,5		Captan	149	79	0,01	10	1,22 (18)	2,02 (18)	2,22 (9)
			149	105	0,01	10			
14,6		Procymidone	283	96	0,01	10	4,22 (8)	4,58 (11)	5,15 (11)
			283	255	0,01	10			
14,7		*trans*-Chlordane	373	266	0,01	20	1,85 (12)	2,24 (3)	1,82 (11)
			373	264	0,01	20			
14,8		Triflumizole	206	179	0,01	20	6,88 (13)	14,0 (20)	No response
			206	144	0,01	30			
14,9		Methidathion	145	85	0,01	5	14,5 (4)	14,8 (13)	12,1 (12)
			125	79	0,01	5			
15,1	14,4-16,0	Endosulfan I	239	204	0,02	20	1,07 (9)	1,38 (14)	1,18 (6)
			272	237	0,02	10			
15,2		Tetrachlorvinphos	329	109	0,02	20	1,27 (7)	1,14 (16)	8,67 (1)
			331	127	0,02	20			
15,6		Chlorfenson	111	75	0,02	10	1,28 (7)	1,30 (4)	1,47 (5)
			175	111	0,02	10			
16,0	15,2-17,6	Profenofos	339	269	0,01	10	8,02 (20)	9,16 (16)	6,40 (14)
			208	99	0,01	20			
16,0		*p,p'-DDE D_8*	*324*	*254*	*0,01*	*20*	-	-	-
16,0		Dieldrin	263	193	0,01	30	1,30 (8)	1,23 (17)	1,31 (7)
			261	191	0,01	20			
16,1		*p,p'*-DDE	316	246	0,01	20	1,83 (4)	1,85 (12)	1,64 (4)
			318	246	0,01	20			
16,4		Myclobutanil	179	125	0,01	10	4,29 (18)	4,26 (2)	3,57 (11)
			179	90	0,01	20			
16,5		Buprofezin	105	77	0,01	20	18,8 (11)	18,9 (7)	23,0 (13)
			172	115	0,01	10			
16,7		Bupirimate	208	165	0,01	20	5,15 (13)	4,64 (14)	7,11 (10)
			273	193	0,01	10			

[a] Average value calculated from matrix-matched standard calibration (four concentration levels) and RSD in parenthesis.
For every compound, the first transition corresponds to quantification (Q) and the second transition to confirmation (q)

Table 1 (cont.). Experimental conditions of the optimized GC-MS/MS method

t_R (min)	Window (min)	Compounds	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell Time (s)	Collision energy (eV)	Orange Q/q ratio [a]	Nectarine Q/q ratio [a]	Spinach Q/q ratio [a]
16,7		Endrin	263	193	0,01	30	1,10 (13)	1,02 (17)	1,22 (11)
			261	191	0,01	20			
17,1		Endosulfan II	193	123	0,01	30	1,21 (13)	1,07 (14)	2,26 (16)
			241	170	0,01	20			
17,5	17,2-19,8	*p,p'*-DDD	235	165	0,05	20	1,68 (10)	1,68 (8)	1,74 (2)
			237	165	0,05	20			
17,6		*p,p'*-DDT	235	165	0,05	30	1,77 (4)	1,46 (8)	1,93 (3)
			237	165	0,05	10			
17,7		Oxadixyl	163	132	0,05	10	3,24 (16)	2,76 (9)	2,40 (5)
			163	117	0,05	20			
17,8		Ethion	231	129	0,05	20	7,40 (4)	7,71 (5)	7,61 (13)
			231	175	0,05	20			
18,3	17,8-19,8	Triazophos	161	134	0,05	5	1,24 (8)	1,21 (15)	1,40 (7)
			257	162	0,05	10			
18,6		Endosulfan sulfate	274	239	0,05	20	1,04 (3)	1,14 (5)	1,07 (3)
			272	237	0,05	10			
18,7		Propiconazole I	173	145	0,05	10	1,04 (2)	1,04 (7)	1,02 (4)
			173	109	0,05	20			
18,9		Propiconazole II	173	145	0,05	10	1,11 (1)	1,08 (4)	1,06 (3)
			173	109	0,05	20			
19,3		Tebuconazole	125	89	0,05	10	2,62 (8)	3,02 (5)	1,73 (4)
			250	125	0,05	10			
20,4	19,8-21,3	Iprodione	314	245	0,01	20	2,08 (12)	1,74 (3)	1,22 (9)
			187	124	0,01	10			
20,5		Phosmet	160	77	0,01	20	1,48 (2)	1,33 (9)	1,63 (2)
			160	133	0,01	10			
20,6		Bromopropylate	183	155	0,01	10	1,16 (4)	2,08 (5)	3,47 (12)
			343	185	0,01	10			

[a] Average value calculated from matrix-matched standard calibration (four concentration levels) and RSD in parenthesis.
For every compound, the first transition corresponds to quantification (Q) and the second transition to confirmation (q)

Table 1 (cont.). Experimental conditions of the optimized GC-MS/MS method

t_R (min)	Window (min)	Compounds	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell Time (s)	Collision energy (eV)	Orange Q/q ratio [a]	Nectarine Q/q ratio [a]	Spinach Q/q ratio [a]
20,8		Bifenthrin	181	166	0,01	10	1,16 (6)	1,04 (3)	1,01 (4)
			181	165	0,01	20			
20,9		Dicofol	251	139	0,01	10	1,44 (13)	1,60 (11)	No response
			251	111	0,01	30			
20,9		Methoxychlor	227	169	0,01	30	1,87 (5)	1,24 (5)	1,01 (2)
			227	141	0,01	20			
21,5	20,7-22,6	Tetradifon	356	229	0,02	20	1,22 (9)	1,22(3)	1,26 (16)
			356	159	0,02	10			
21,9		Phosalone	182	111	0,02	10	5,25 (8)	5,01 (9)	4,43 (6)
			367	182	0,02	10			
21,9		Azinphos methyl	160	77	0,02	20	5,96 (13)	7,61 (6)	8,88 (4)
			160	132	0,02	10			
22,0		Mirex	272	237	0,02	10	1,85 (5)	1,81(5)	1,82 (6)
			274	239	0,02	10			
22,1		Pyriproxyfen	136	96	0,02	20	3,80 (14)	3,84 (9)	3,94 (10)
			136	78	0,02	30			
22,4	22,0-23,6	*lambda*-Cyhalothrin I	181	152	0,05	20	1,09 (8)	2,49 (11)	2,20 (6)
			208	181	0,05	10			
22,8		*lambda*-Cyhalothrin II	181	152	0,05	20	1,06 (9)	2,53 (16)	2,05 (7)
			208	181	0,05	10			
22,8		Fenarimol	251	139	0,05	10	1,45 (5)	1,42 (6)	1,42 (6)
			219	107	0,05	10			
23,2		Pyrazophos	221	193	0,05	10	2,60 (11)	2,47 (5)	2,67 (3)
			221	149	0,05	10			
23,3		Acrinathrin	181	152	0,05	10	0,97 (10)	2,01 (13)	1,87 (2)
			208	181	0,05	20			
24,1	23,6-24,8	Permethrin I	183	153	0,05	10	1,76 (6)	2,32 (6)	1,95 (1)
			183	165	0,05	10			

[a] Average value calculated from matrix-matched standard calibration (four concentration levels) and RSD in parenthesis.
For every compound, the first transition corresponds to quantification (Q) and the second transition to confirmation (q)

Table 1 (cont). Experimental conditions of the optimized GC-MS/MS method

t_R (min)	Window (min)	Compounds	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell Time (s)	Collision energy (eV)	Orange Q/q ratio [a]	Nectarine Q/q ratio [a]	Spinach Q/q ratio [a]
24,3		Pyridaben	147	117	0,05	20	1,95 (12)	2,12 (6)	2,28 (7)
			147	132	0,05	10			
24,3		Permethrin II	183	153	0,05	10	1,69 (4)	2,23 (4)	1,98 (2)
			183	165	0,05	10			
24,6		Coumaphos	362	226	0,05	20	1,24 (5)	1,07 (3)	1,29 (5)
			226	163	0,05	10			
25,3	24,1-27,1	Cyfluthrin I	163	91	0,05	10	1,14 (5)	1,32 (10)	4,82 (7)
			163	127	0,05	20			
25,5		Cyfluthrin II	163	91	0,05	10	1,26 (5)	1,38 (4)	5,10 (6)
			163	127	0,05	20			
25,6		β-Cyfluthrin	163	91	0,05	10	1,13 (2)	1,31 (7)	5,10 (6)
			163	127	0,05	20			
25,7		Cyfluthrin III	163	91	0,05	10	1,19 (3)	1,34 (4)	5,71 (4)
			163	127	0,05	20			
25,9		Cypermethrin I	163	91	0,05	10	1,47 (7)	1,52 (11)	5,05 (6)
			163	127	0,05	10			
26,1		Cypermethrin II	163	91	0,05	10	1,12 (6)	1,51 (8)	5,19 (10)
			163	127	0,05	10			
26,2		Cypermethrin III	163	91	0,05	10	1,16 (9)	1,29 (6)	4,99 (3)
			163	127	0,05	10			
26,3		Cypermethrin IV	163	91	0,05	10	1,21 (6)	1,42 (6)	4,94 (5)
			163	127	0,05	10			
26,4		Etofenprox	163	106	0,05	10	5,37 (14)	9,33 (1)	4,60 (13)
			163	134	0,05	10			
27,6	27,0-35,0	Fenvalerate	181	152	0,05	10	1,61 (9)	1,91 (5)	1,76 (4)
			225	119	0,05	20			
28,0		Esfenvalerate	181	152	0,05	10	1,71 (8)	1,90 (3)	2,54 (8)
			225	91	0,05	20			

[a] Average value calculated from matrix-matched standard calibration (four concentration levels) and RSD in parenthesis.
For every compound, the first transition corresponds to quantification (Q) and the second transition to confirmation (q)

Table 1 (cont.). Experimental conditions of the optimized GC-MS/MS method

t_R (min)	Window (min)	Compounds	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell Time (s)	Collision energy (eV)	Orange Q/q ratio [a]	Nectarine Q/q ratio [a]	Spinach Q/q ratio [a]
28,0		*tau*-Fluvalinate I	252	200	0,05	20	6,20 (7)	5,29 (14)	2,60 (9)
			250	200	0,05	20			
28,2		*tau*-Fluvalinate II	252	200	0,05	20	5,64 (13)	5,37 (13)	2,55 (8)
			250	200	0,05	20			
29,0		Deltamethrin	181	152	0,05	20	2,92 (2)	2,39 (14)	2,64 (4)
			253	93	0,05	10			
29,6		Azoxystrobin	344	329	0,05	10	79,1 (12)	77,4 (15)	44,5 (14)
			344	156	0,05	30			

[a] Average value calculated from matrix-matched standard calibration (four concentration levels) and RSD in parenthesis.
For every compound, the first transition corresponds to quantification (Q) and the second transition to confirmation (q)

Table 2. Average recovery (%) and RSD (in parenthesis) after the application of the GC-MS/MS procedure to orange, nectarine and spinach samples (n=5) at two concentration levels. Limits of detection (LOD).

Compounds	Orange			Nectarine			Spinach		
	Fortification levels (mg/Kg)		LOD (mg/Kg)	Fortification levels (mg/Kg)		LOD (mg/Kg)	Fortification levels (mg/Kg)		LOD (mg/Kg)
	0,01	0,05		0,01	0,05		0,01	0,05	
Acephate[1]	64(20)	70 (8)	0,002	74 (17)	109 (15)	0,004	-	-	-
Acrinathrin[2]	71 (8)	111 (17)	0,001	74 (12)	101 (23)	0,0003	-	-	-
Alachlor[1]	88 (17)	85 (5)	0,002	81 (18)	94 (9)	0,004	104 (10)	98 (15)	0,002
Aldrin[1]	51 (16)	95 (4)	0,005	85 (15)	103 (10)	0,003	73 (12)	114 (9)	0,0004
Atrazine[2]	70 (11)	91 (15)	0,004	72 (15)	78 (11)	0,002	107 (11)	79 (9)	0,004
Azinphos methyl[1]	71 (11)	36 (10)	0,003	108 (18)	22 (17)	0,002	-	107 (13)	0,02
Azoxystrobin[1]	80 (8)	87 (19)	0,002	109 (17)	93 (12)	0,001	104 (13)	85 (12)	0,004
Bifenthrin[2]	75 (4)	94 (18)	0,0001	71 (10)	99 (9)	0,0006	89 (12)	68 (10)	0,001
Bromopropylate[1]	90 (17)	89 (12)	0,0002	91 (13)	117 (10)	0,002	110 (1)	126 (19)	0,002
Bupirimate[1]	79 (10)	99 (38)	0,005	81 (10)	-	0,003	104 (19)	102 (15)	0,01
Buprofezin[1]	106 (9)	106 (20)	0,006	82 (7)	87 (22)	0,008	105 (19)	95 (6)	0,006
Captan[1]	-	100 (18)	-	-	59 (4)	-	-	-	-
Carbaryl[2]	78 (6)	99 (16)	0,0007	86 (15)	98 (12)	0,002	102 (10)	72 (15)	0,001
trans-Chlordane[1]	80 (8)	94 (13)	0,001	90 (17)	96 (7)	0,0005	100 (11)	85 (8)	0,001
Chlorfenson[1]	90 (8)	96 (8)	0,0001	93 (19)	101 (7)	0,0001	102 (7)	84 (10)	0,0005
Chlorfenvinphos[2]	70 (8)	81 (20)	0,007	70 (7)	105 (21)	0,002	99 (12)	76 (8)	0,01
Chlorothalonil[1]	100 (14)	86 (5)	0,001	117 (9)	94 (15)	0,003	75 (17)	90 (11)	0,005
Chlorpropham[1]	109 (12)	85 (4)	0,002	79 (7)	118 (12)	0,001	107 (13)	118 (12)	0,004
Chlorpyriphos ethyl[2]	90 (12)	100 (13)	0,001	89 (6)	117 (17)	0,002	110 (12)	72 (9)	0,0002
Chlorpyriphos methyl[2]	70 (15)	81 (14)	0,003	69 (15)	88 (12)	0,002	95 (11)	65 (3)	0,0004
Chlozolinate[1]	103 (20)	96 (16)	0,01	86 (15)	91 (7)	0,01	85 (11)	98 (24)	0,01
Coumaphos[2]	82 (9)	88 (20)	0,004	87 (6)	102 (13)	0,003	104 (22)	61 (8)	0,008
Cyfluthrin I[2]	78 (8)	100 (8)	0,002	84 (13)	110 (21)	0,001	62 (10)	72 (11)	0,004
Cyfluthrin II[2]	89 (8)	93 (10)	0,002	80 (12)	109 (20)	0,001	53 (15)	67 (3)	0,003
Cyfluthrin III[2]	86 (11)	90 (6)	0,002	76 (15)	101 (17)	0,0004	62 (14)	64 (2)	0,003
β-Cyfluthrin[2]	74 (10)	95 (6)	0,002	78 (5)	102 (17)	0,0006	84 (20)	68 (10)	0,003
lambda-Cyhalothrin I[2]	85 (9)	114 (13)	0,002	82 (1)	100 (21)	0,001	-	-	-
lambda-Cyhalothrin II[2]	83 (9)	109 (15)	0,002	102 (8)	104 (19)	0,001	-	-	-

[1,2,3] The number indicates the I.S. used for each analyte: 1, *HCB-*$^{13}C_6$; 2, *Terbutylazine* D_6 ; 3, *p,p'-DDE* D_8.

Table 2 (cont.). Average recovery (%) and RSD (in parenthesis) after the application of the GC-MS/MS procedure to orange, nectarine and spinach samples (n=5) at two concentration levels. Limits of detection (LOD).

Compounds	Orange			Nectarine			Spinach		
	Fortification levels (mg/Kg)		LOD (mg/Kg)	Fortification levels (mg/Kg)		LOD (mg/Kg)	Fortification levels (mg/Kg)		LOD (mg/Kg)
	0,01	0,05		0,01	0,05		0,01	0,05	
Cypermethrin I[2]	106 (14)	92 (12)	0,001	105 (7)	94 (12)	0,001	105 (22)	76 (12)	0,008
Cypermethrin II[2]	98 (13)	93 (7)	0,003	79 (1)	105 (18)	0,002	107 (7)	71 (11)	0,01
Cypermethrin III[2]	73 (15)	92 (10)	0,001	73 (11)	-	0,003	98 (18)	71 (13)	0,01
Cypermethrin IV[2]	80 (15)	102 (11)	0,003	86 (4)	-	0,004	114 (7)	73 (14)	0,01
Cyprodinil[1]	76 (18)	93 (7)	0,005	90 (22)	108 (8)	0,008	92 (20)	108 (17)	0,01
p,p'-DDD[3]	65 (6)	73 (15)	0,0002	65 (5)	70 (3)	0,0001	102 (5)	65 (6)	0,0003
p,p'-DDE[3]	64 (2)	70 (7)	0,001	67 (6)	73 (4)	0,0002	109 (5)	70 (7)	0,0004
p,p'-DDT[3]	65 (2)	89 (8)	0,0003	60 (6)	74 (10)	0,0001	102 (3)	68 (16)	0,01
Deltamethrin[2]	90 (9)	78 (14)	0,002	82 (6)	124(24)	0,002	113 (13)	80 (11)	0,004
Diazinon[2]	63 (5)	89 (10)	0,001	57 (8)	91 (10)	0,0005	90 (9)	73 (6)	0,001
Dichlofluanid[1]	88 (16)	91 (4)	0,002	84 (19)	85 (7)	0,001	47 (31)	70 (4)	0,0004
4,4'-Dichlorbenzophenone[1]	69 (12)	111 (12)	0,01	82 (14)	-	0,009	90 (11)	85 (20)	0,002
Dichlorvos[1]	70 (18)	69 (6)	0,0003	57 (17)	83 (8)	0,0006	98 (17)	64 (9)	0,001
Dicofol[1]	48 (14)	103 (6)	0,01	55 (12)	69 (5)	0,01	-	-	-
Dieldrin[1]	87 (24)	114 (13)	0,005	67 (7)	106 (7)	0,002	92 (9)	124 (11)	0,004
Dimethoate[1]	110 (16)	76 (20)	0,001	105 (17)	81 (5)	0,003	102 (13)	115 (10)	0,01
Diphenylamine[1]	78 (5)	87 (16)	0,001	68 (16)	78 (7)	0,0003	87 (19)	102 (10)	0,001
Disulfoton[2]	87 (5)	111 (14)	0,01	75 (20)	92 (21)	0,009	-	-	-
Endosulfan I[1]	99 (18)	102 (16)	0,01	79 (20)	103 (7)	0,01	97 (16)	100 (15)	0,01
Endosulfan II[1]	85 (20)	86 (16)	0,01	-	89 (15)	0,02	110 (12)	106 (27)	0,006
Endosulfan ether[1]	79 (8)	84 (10)	0,005	79 (19)	87 (7)	0,005	72 (15)	90 (13)	0,004
Endosulfan sulfate[1]	105 (12)	107 (12)	0,002	97 (11)	103 (15)	0,001	112 (9)	96 (10)	0,0005
Endrin[1]	92 (15)	106 (13)	0,005	91 (20)	126 (33)	0,005	108 (11)	115 (17)	0,005
Esfenvalerate[2]	85 (8)	87 (19)	0,001	75 (5)	99 (13)	0,002	121 (15)	68 (9)	0,002
Ethion[2]	74 (9)	101 (20)	0,0004	77 (6)	96 (8)	0,0001	113 (10)	66 (9)	0,003
Ethoprophos[1]	94 (11)	85 (8)	0,001	82 (16)	115 (12)	0,001	89 (12)	118 (11)	0,003
Etofenprox[2]	64 (5)	89 (11)	0,002	79 (16)	105 (18)	0,001	111 (1)	65 (10)	0,005

[1,2,3] The number indicates the I.S. used for each analyte: 1, *HCB-*$^{13}C_6$; 2, *Terbutylazine* D_6 ; 3, *p,p'-DDE* D_8.

Table 2 (cont.). Average recovery (%) and RSD (in parenthesis) after the application of the GC-MS/MS procedure to orange, nectarine and spinach samples (n=5) at two concentration levels. Limits of detection (LOD).

Compounds	Orange			Nectarine			Spinach		
	Fortification levels (mg/Kg)		LOD (mg/Kg)	Fortification levels (mg/Kg)		LOD (mg/Kg)	Fortification levels (mg/Kg)		LOD (mg/Kg)
	0,01	0,05		0,01	0,05		0,01	0,05	
Etrimfos[2]	66 (9)	84 (19)	0,001	63 (5)	90 (10)	0,001	93 (9)	65 (8)	0,002
Fenarimol[1]	102 (8)	102 (15)	0,001	102 (19)	76 (12)	0,0005	110 (17)	88 (9)	0,001
Fenchlorphos[2]	68 (6)	88 (16)	0,0009	63 (1)	87 (10)	0,002	98 (11)	67 (7)	0,0002
Fenitrothion[2]	81 (16)	97 (11)	0,004	87 (16)	94 (9)	0,009	97 (19)	83 (12)	0,001
Fenthion[2]	77 (2)	90 (14)	0,01	71 (10)	72 (12)	0,005	100 (14)	60 (12)	0,0007
Fenvalerate[2]	90 (6)	93 (13)	0,002	76 (4)	102 (13)	0,003	113 (9)	80 (10)	0,006
tau-Fluvalinate I[2]	74 (16)	77 (16)	0,003	72 (12)	123 (20)	0,002	-	-	-
tau-Fluvalinate II[2]	93 (17)	76 (16)	0,002	89 (17)	95 (16)	0,002	-	-	-
Folpet[1]	64 (13)	99 (8)	0,01	74 (17)	67 (11)	0,007	-	-	-
Fonofos[1]	72 (11)	88 (8)	0,001	83 (4)	86 (4)	0,0003	77 (14)	91 (10)	0,0004
HCB[1]	71 (5)	80 (4)	0,002	70 (7)	91 (10)	0,001	107 (3)	90 (9)	0,003
α-HCH[1]	83 (6)	83 (4)	0,001	81 (13)	104 (8)	0,0005	75 (7)	104 (10)	0,002
β-HCH[1]	85 (11)	87 (4)	0,001	78 (15)	108 (17)	0,002	78 (8)	118 (12)	0,001
δ-HCH[1]	95 (11)	83 (4)	0,002	86 (13)	118 (9)	0,004	105 (12)	123 (14)	0,005
γ-HCH[1]	88 (14)	90 (14)	0,002	105 (9)	119 (14)	0,003	90 (12)	127 (13)	0,002
Heptachlor[1]	86 (10)	88 (4)	0,002	79 (15)	100 (12)	0,001	73 (13)	104 (10)	0,0002
Heptachlor epoxide[1]	-	110 (26)	0,05	-	96 (11)	0,04	-	111 (11)	0,02
Heptenophos[1]	94 (6)	90 (5)	0,0002	85 (15)	101 (7)	0,003	109 (7)	96 (10)	0,001
Iprodione[1]	99 (20)	92 (17)	0,0004	-	-	-	101 (17)	78 (11)	0,004
Isodrin[1]	81 (8)	94 (12)	0,003	92 (9)	91 (6)	0,005	94 (12)	88 (6)	0,003
Isofenphos[2]	69 (7)	87 (13)	0,003	71 (11)	94 (8)	0,001	99 (14)	68 (7)	0,005
Malathion[2]	70 (5)	93 (19)	0,002	71 (4)	101 (8)	0,0007	110 (10)	65 (10)	0,001
Metalaxyl[1]	101 (17)	87 (8)	0,002	104 (15)	104 (14)	0,002	108 (12)	97 (15)	0,0001
Methacrifos[1]	70 (12)	81 (7)	0,0008	69 (5)	79 (4)	0,0006	111 (1)	72 (3)	0,001
Methidathion[2]	70 (5)	-	0,001	70 (9)	98 (12)	0,0005	108 (11)	65 (13)	0,001
Methiocarb[2]	71 (12)	101 (20)	0,006	90 (16)	99 (17)	0,009	104 (19)	67 (16)	0,004
Metholachlor[1]	81 (8)	86 (9)	0,0005	84 (16)	98 (9)	0,0008	108 (15)	88 (13)	0,0005

[1,2,3] The number indicates the I.S. used for each analyte: 1, *HCB-*$^{13}C_6$; 2, *Terbutylazine* D_6 ; 3, *p,p'-DDE* D_8.

Table 2 (cont). Average recovery (%) and RSD (in parenthesis) after the application of the GC-MS/MS procedure to orange, nectarine and spinach samples (n=5) at two concentration levels. Limits of detection (LOD).

Compounds	Orange			Nectarine			Spinach		
	Fortification levels (mg/Kg)		LOD (mg/Kg)	Fortification levels (mg/Kg)		LOD (mg/Kg)	Fortification levels (mg/Kg)		LOD (mg/Kg)
	0,01	0,05		0,01	0,05		0,01	0,05	
Methoxychlor[3]	44 (12)	84 (16)	0,0003	48 (15)	75 (11)	0,004	53 (20)	-	0,01
Metribuzin[2]	65 (18)	57 (23)	0,009	74 (20)	49(7)	0,007	115 (19)	72 (18)	0,003
Mevinphos[1]	87 (6)	93 (7)	0,0001	84 (17)	109 (10)	0,0006	106 (6)	94 (9)	0,0004
Mirex[1]	69 (9)	95 (4)	0,0003	72 (14)	100 (8)	0,0001	93 (14)	84 (10)	0,0003
Myclobutanil[1]	95 (14)	94 (9)	0,0004	100 (19)	98 (6)	0,001	105 (10)	86 (10)	0,0006
Omethoate[1]	61 (7)	56 (8)	0,001	74 (13)	-	0,005	-	68 (11)	0,01
Oxadixyl[1]	89 (4)	96 (4)	0,001	90 (16)	90 (7)	0,0003	106 (11)	89 (11)	0,001
Oxychlordane[3]	84 (19)	85 (10)	0,003	69 (2)	67 (12)	0,003	84 (17)	73 (10)	0,006
Parathion ethyl[2]	71 (10)	91 (11)	0,007	70 (10)	98 (13)	0,003	107 (9)	72 (13)	0,0004
Parathion methyl[2]	72 (13)	85 (17)	0,002	72 (15)	107 (20)	0,003	93 (16)	84 (11)	0,0002
Penconazole[1]	95 (11)	91 (9)	0,003	88 (19)	96 (7)	0,001	110 (10)	94 (11)	0,006
Pendimethalin[1]	86 (19)	83 (6)	0,01	95 (23)	110 (3)	0,009	125 (27)	121 (14)	0,0003
Pentachlorobenzene[1]	60 (8)	74 (9)	0,0005	45 (1)	70 (11)	0,003	63 (23)	60 (15)	0,01
Permethrin I[2]	81 (3)	99 (12)	0,002	89 (6)	103 (20)	0,002	111 (9)	61 (5)	0,004
Permethrin II[2]	86 (9)	97 (15)	0,002	80 (4)	100 (18)	0,002	123 (4)	66 (10)	0,003
Phorate[1]	81 (8)	94 (6)	0,0005	76 (18)	81 (5)	0,0004	69 (14)	60 (8)	0,002
Phosalone[2]	71 (8)	89 (17)	0,001	78 (9)	94 (12)	0,001	112 (14)	68 (7)	0,005
Phosmet[2]	76 (4)	89 (20)	0,0001	82 (5)	107 (13)	0,001	124 (27)	67 (10)	0,003
Phosphamidon[2]	75 (8)	83 (14)	0,001	80 (3)	98 (13)	0,001	113 (13)	70 (6)	0,0003
Pirimicarb[2]	73 (6)	102 (18)	0,001	69 (4)	-	0,001	107 (9)	60 (13)	0,0002
Pirimiphos methyl[2]	69 (6)	87 (13)	0,003	58 (11)	90 (9)	0,003	86 (8)	71 (5)	0,0003
Pirimiphos ethyl[2]	67 (6)	90 (9)	0,003	66 (3)	90 (9)	0,001	98 (15)	68 (7)	0,0002
Procymidone[1]	86 (14)	87 (13)	0,008	98 (19)	99 (8)	0,002	90 (8)	95 (11)	0,005
Profenofos[2]	76 (10)	87 (20)	0,008	77 (9)	93 (10)	0,002	110 (10)	63 (8)	0,008
Propiconazole I[1]	95 (14)	95 (5)	0,0004	96 (18)	115 (11)	0,002	102 (8)	125 (13)	0,01
Propiconazole II[1]	102 (12)	98 (7)	0,0003	93 (16)	118 (7)	0,001	112 (8)	128 (15)	0,001
Propyzamide[1]	65 (4)	87 (20)	0,001	67 (8)	99 (10)	0,0003	112 (8)	73 (7)	0,0005

[1,2,3] The number indicates the I.S. used for each analyte: 1, *HCB-$^{13}C_6$* ; 2, *Terbutylazine D_6* ; 3, *p,p'-DDE D_8.*

Table 2 (cont). Average recovery (%) and RSD (in parenthesis) after the application of the GC-MS/MS procedure to orange, nectarine and spinach samples (n=5) at two concentration levels. Limits of detection (LOD).

Compounds	Orange			Nectarine			Spinach		
	Fortification levels (mg/Kg)		LOD (mg/Kg)	Fortification levels (mg/Kg)		LOD (mg/Kg)	Fortification levels (mg/Kg)		LOD (mg/Kg)
	0,01	0,05		0,01	0,05		0,01	0,05	
Pyrazophos[2]	74 (10)	87 (14)	0,0006	73 (10)	91 (11)	0,0004	109 (16)	67 (9)	0,002
Pyridaben[1]	92 (20)	96 (10)	0,0002	80 (17)	103 (12)	0,0004	109 (15)	84 (10)	0,004
Pyrimethanil[1]	100 (11)	98 (15)	0,001	94 (20)	81 (34)	0,005	109 (13)	119 (6)	0,001
Pyriproxyfen[1]	111 (14)	105 (12)	0,003	90 (7)	104 (6)	0,005	108 (8)	91 (18)	0,01
Quinalphos[2]	78 (8)	98 (12)	0,007	73 (9)	94 (9)	0,003	110 (13)	70 (5)	0,003
Quintozene[1]	86 (17)	87 (7)	0,003	93 (16)	115 (16)	0,001	75 (14)	122 (7)	0,003
Simazine[2]	74 (17)	85 (19)	0,009	80 (19)	73 (15)	0,01	111 (14)	84 (5)	0,01
Tebuconazole[1]	107 (9)	96 (10)	0,0003	109 (15)	68 (17)	0,001	110 (2)	88 (11)	0,001
Tecnazene[1]	64 (2)	81 (4)	0,002	63 (12)	76 (5)	0,002	110 (12)	84 (5)	0,009
Tefluthrin[2]	69 (8)	94 (15)	0,002	66 (12)	93 (8)	0,003	-	-	-
Terbufos[1]	77 (10)	87 (4)	0,001	78 (14)	95 (8)	0,0003	84 (9)	88 (11)	0,001
Terbutylazine[2]	72 (1)	87 (22)	0,002	70 (7)	78 (6)	0,003	96 (12)	70 (9)	0,003
Tetrachlorvinphos[2]	65 (5)	111 (14)	0,005	70 (3)	104 (19)	0,002	105 (9)	95 (16)	0,004
Tetradifon[1]	107 (8)	94 (10)	0,001	103 (15)	105 (7)	0,01	104 (7)	100 (17)	0,01
Tolclofos methyl[1]	60 (6)	81 (14)	0,001	62 (8)	89 (11)	0,0007	83 (8)	70 (8)	0,0001
Tolyfluanid[1]	90 (16)	80 (6)	0,002	93 (19)	108 (11)	0,001	71 (10)	110 (15)	0,01
Triazophos[2]	74 (7)	88 (19)	0,0005	74 (8)	103 (12)	0,001	104 (14)	68 (9)	0,001
Triflumizole[1]	73 (17)	-	0,003	86 (18)	-	0,002	-	-	-
Trifluralin[1]	86 (5)	91 (10)	0,0003	83 (11)	90 (7)	0,0002	104 (11)	80 (10)	0,002
Vinclozolin[1]	102 (17)	97 (13)	0,004	95 (19)	97 (6)	0,005	100 (20)	96 (14)	0,0004

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Table 3. Pesticides found in orange, nectarine and spinach samples after application of the overall procedure (concentrations expressed in mg/Kg).

Compounds	Orange 1	Orange 2	Nectarine 1	Nectarine 2	Spinach 1	Spinach 2
Chlorpyriphos ethyl	-	0.016	d	-	-	-
p,p'-DDD	d	-	-	-	d	-
p-p'-DDE	-	d	-	-	-	d
p,p'-DDT	d	-	-	-	-	
Deltamethrin	-	-	0.021	-	-	-
Endosulfan sulfate	d	-	-	-	-	-
Malathion	-	-	d	-	-	-
Mirex	-	d	-	d	-	d
Phosmet	-	-	0.015	-	-	-

d, detected

FIGURE CAPTIONS

Figure 1. (a) Absolute and relative matrix effect for nectarine samples.**(b)** Relative matrix effect for spinach samples in the GC-MS/MS determination of selected pesticides.

Figure 2. GC-MS/MS SRM chromatograms for selected pesticides (within a wide range of retention times) pesticides in orange, nectarine and spinach samples fortified at 0.01 mg/Kg. Only the quantification transition is shown.

Figure 3. GC-MS/MS SRM chromatograms for pesticides detected in a nectarine sample (nectarine 1, Table 3). (Q) quantification transition, (q) confirmative transition.