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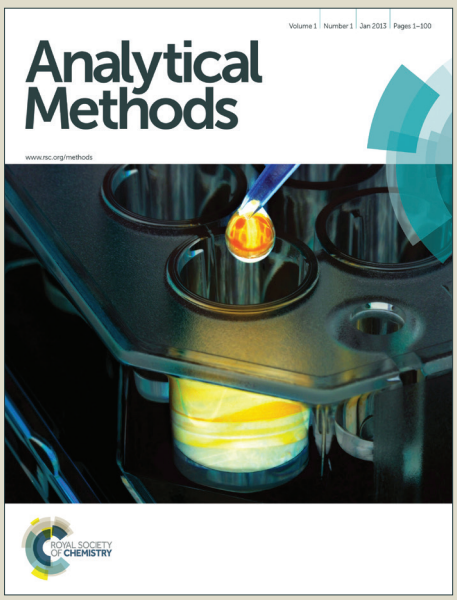

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MULTI-CLASS PESTICIDE DETERMINATION IN ROYAL JELLY BY GAS CHROMATOGRAPHY COUPLED TO TRIPLE QUADRUPOLE TANDEM MASS SPECTROMETRY

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Fast and reliable determination of pesticides in royal jelly using SPE and GC-QqQ-MS/MS. The developed method allows the determination of the target compounds below MRLs established by EU.

Abstract

A solid phase extraction (SPE) procedure using C_{18} cartridges has been developed and validated to extract 127 pesticides from royal jelly. Ethyl acetate and *n*-hexane were used for pesticide elution. Pesticide determination and quantification were performed with gas chromatography coupled to triple quadrupole tandem mass spectrometry (GC-QqQ-MS/MS) using selective reaction monitoring (SRM). Total running time was 23 min. Because of the presence of matrix effect, pesticides were quantified using matrix-matched calibration. Recoveries ranged from 70 to 120% and relative standard deviation (RSD) was lower than 20% (intraday) and 25% (interday) at 10, 50 and 100 μg kg^{-1} for most of the target compounds. Limits of quantification (LOQs) were lower than 10 μg kg^{-1}. The validated method was applied to 6 royal jelly commercial products (liquid and capsule presentations) and no pesticides were detected above the limits of detection.

1. Introduction

Royal jelly is one of the most important products from beehive because of its nutritional and pharmaceutical properties.[1] Different studies have indicated antioxidant, anti-inflammatory, antiviral, anti-ulcerous and antibacterial properties of this product.[2,3] Royal jelly can be sold in fresh state, unprocessed except for being frozen or cooled, mixed with other products, or freeze-dried for further use in other preparations. When it is presented as unprocessed form, it can also be included directly in many food and dietary supplements as well as in medicine-like products or cosmetics.[4]

Dietary supplements are gaining importance because people start searching for optimal nutrition diets that help them to promote health, improve general well-being and reduce the risk of developing certain illnesses.[2] This is accomplished by consuming these products, which can contain a concentrated form of a bioactive agent from a food used to enhance health in dosages that exceed those that could be obtained from the normal food.[5] Bearing in mind this description, royal jelly can be considered a dietary supplement because it can be found as a concentrated form and it has specific nutritional properties, as well as improvements to human health, as mentioned before.

Beehive products, such as royal jelly, could be contaminated by substances, such as pesticides, which can be used in the beehive itself or in the plants where bees collect nectar or pollen.[6] Bogdanov[7] explained that the most common pesticides found in bee products are organochlorines, organophosphorus and carbamates. In Europe, the Regulation 396/2005[8] defines pesticides maximum residue limits (MRLs) for every food and feed, including honey and their derivates at concentrations between 10 and 50 $\mu g\ kg^{-1}$. There are other organizations worldwide that defines pesticides MRLs for royal jelly, such as the Japan Food Chemical Research Foundation (FFCR)[9] with concentrations between 0.3 and 100 $\mu g\ kg^{-1}$, or the Environmental Protection Agency (EPA)[10] in the US that manage concentrations around 30 $\mu g\ kg^{-1}$. This indicates the possible health problem that could be presented because the presence of pesticides in royal jelly. Therefore, analytical methods that could offer reliable determination and quantification of pesticides in royal jelly should be considered.

Although there are several extraction procedures, such as QuEChERS,[11-17] liquid-liquid extraction (LLE)[18-21] and solid phase extraction (SPE),[22,23] which have been used during pesticides determination in honey, up to now, there was only one study concerning pesticides determination in royal jelly. Thus, Karazafiris et al[1] extracted 9

pesticides (organochlorines and organophosphorus) using a SPE procedure with C_{18} as sorbent.

For the determination of pesticides, chromatographic analysis, gas chromatography (GC)[17-22] and liquid chromatography (LC)[11-16,23] were used coupled to different detectors, including ECD,[1,20,22] photodiode array,[23] ion trap (IT),[12,21] single quadrupole mass spectrometry (Q-MS),[18,19] triple quadrupole mass spectrometry (QqQ-MS/MS),[11,14-16] time of flight (TOF)[17] and Orbitrap.[13] The use of MS analyzers allows an increase in the number of pesticides studied, reaching up to 350 compounds when the Orbitrap[13] is used, whereas when ECD is utilized, only 24 compounds can be detected in honey.[19]

Bearing in mind the trend in using more precise analytical methods to determine and quantify pesticides in honey, such as QqQ-MS/MS or QqTOF, much effort should be performed in the analysis of pesticide residues in royal jelly. Therefore, the aim of this work is the development of an analytical methodology to determine and quantify pesticides in royal jelly using GC-QqQ-MS/MS. Bearing in mind the lack of information concerning sample treatment for royal jelly, Karazafaris et al[1] original procedure will be tested against QuEChERS approach that, as discussed before, have provided good results for a similar matrix, such as honey. Also, the use of an advance detector (QqQ-MS/MS) instead of a classical detector (ECD) for pesticide determination and quantification will increase the method precision and the amount of compounds studied.

2. Experimental

2.1. Reagents and chemicals

Pesticide reference standards (purity higher than 99%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and Riedel-de-Haën (Seelze-Hannover, Germany). For those pesticides obtained in powder form, stock standard solutions of individual compounds (with concentrations ranging from 200 and 300 mg L^{-1}) were prepared by exact weighing of the powder and dissolved in 50 mL of methanol, acetonitrile or acetone and stored at −18 ºC in the dark. A multicompound working standard solution (1 mg L^{-1} concentration of each compound) was prepared by appropriate dilutions of the stock solutions (prepared previously or commercially available) with acetone and stored under refrigeration at 4 ºC. A caffeine C_{13} solution (20 mg L^{-1}) was also prepared as internal standard (IS) in the same way as the stock standard solutions. Anhydrous

magnesium sulphate and acetic acid were obtained from Panreac (Barcelona, Spain). Sodium chloride, sodium citrate dihydrate and sodium acetate were obtained from J.T. Baker (Deventer, The Netherlands). Primary secondary amine (PSA), graphitized black carbon (GBC), Florisil cartridges (500 mg, 3 mL) and C_{18} cartridges (500 mg, 5 mL) were obtained from Scharlab (Barcelona, Spain). Acetonitrile and methanol were also obtained from Scharlab. Ethyl acetate and disodium hydrogencitrate sesquihydrate were obtained from Sigma-Aldrich (Madrid, Spain). Acetone was obtained from Carlo Erba (Milan, Italy). *n*-Hexane was obtained from VWR international (Radnor, Pennsylvania, USA). All solvents were pesticide residue grade solvents. Highly purified water (Milli-Q, Millipore, Bedford, USA) was used throughout for the preparation of aqueous solutions.

2.2. Instrument and apparatus

Centrifugation was carried out in a high-volume centrifuge equipped with a bucket rotor (4 x 250 mL) from Orto Alresa, Mod. Consul (Madrid, Spain). Sonication was carried out in an ultrasonic bath from J.P. Selecta (Barcelona, Spain). The SPE was assisted with a manifold from Agilent Technologies (Santa Clara, CA, USA).

Chromatographic analyses were carried in a Scion GC system (Bruker corporation, Freemont, CA, USA) equipped with an autosampler from the same company. The column used was a BR-5ms (30 m x 0.25 mm, 0.25 µm particle size) (Bruker) with a constant flow of helium at 1 mL min^{-1}. A fused silica untreated capillary column (2 m x 0.25 mm) from Supelco (Bellefonte, Pennsylvania, USA) was used as a guard column.

Mass spectrometric detection was carried out using a Scion QqQ-MS/MS (Bruker) operating in electron ionization mode (EI,-70 eV).

2.3. Samples

A royal jelly liquid presentation was obtained from a local store, and it was used for blanks, fortified samples for recovery assays and matrix-matched standards for calibration purposes. For the analysis of real samples, 6 royal jelly products (5 liquid and 1 capsule presentations) were obtained from local supermarkets. The samples were storage at 4 ºC prior analysis.

2.4. Sample preparation

2.4.1. Procedure I- QuEChERS methods

The American[24] and European[25] QuEChERS methods were tested following these steps: 2 g of royal jelly were weighted in a 50 mL centrifuge tube; then 8 g of water was added and the solution was vortex for 30 s. After that, 10 mL of a mixture of acetonitrile and acetic acid at 1% (v/v) was added to the solution and vortex for 1 min. After that, 4 g of magnesium sulphate and 1 g of sodium acetate (American version) or 4 g of magnesium sulphate, 1 g of sodium chloride, 1g of sodium citrate dihydrate and 0.5 g of disodium hydrogencitrate sesquihydrate (European version) were added to the mixture and vortex for 1 min. The resultant solution was then centrifuge at 5000 rpm (4126 *g*) for 5 min, and 1 mL was transferred to a tube and evaporated to dryness under a nitrogen stream. Finally, 975 µL of ethyl acetate was added and transferred to a vial with 25 µL of the IS for GC-QqQ-MS/MS analysis.

2.4.2. Procedure II-QuEChERS method + clean-up

Different sorbents were tested for the clean-up process, including PSA, GBC and Florisil. For these methods, only the American QuEChERS version was applied.

PSA: Following Procedure I, after centrifugation, 1.5 mL of the organic phase were transferred to a 2 mL Eppendorf micro tube containing 25 mg of PSA and 200 mg of magnesium sulphate. The tube was then centrifuged at 5000 rpm (4136 *g*) for 5 min and 1 mL was transferred to a tube and evaporated to dryness under a nitrogen stream. Finally, 975 µL of ethyl acetate was added and transferred to a vial with 25 µL of the IS for GC-QqQ-MS/MS analysis.

PSA+GBC: Following Procedure I, after centrifugation, 1.5 mL of the organic phase were transferred to a 2 mL Eppendorf micro tube containing 25 mg of PSA, 100 mg of GBC and 200 mg of magnesium sulphate. The tube was then centrifuged at 5000 rpm (4136 *g*) for 5 min and 1 mL was transferred to a tube and evaporated to dryness under a nitrogen stream. Finally, 975 µL of ethyl acetate was added and transferred to a vial with 25 µL of the IS for GC-QqQ-MS/MS analysis.

Florisil: Following Procedure I, after centrifugation, 2 mL of the organic phase were slowly transferred through a Florisil cartridge. From this resultant solution, 1 mL was transferred to a tube and evaporated to dryness under a nitrogen stream. Finally, 975 µL of ethyl acetated was added and transferred to a vial with 25 µL of the IS for GC-QqQ-MS/MS analysis.

2.4.3. Procedure III-SPE

Karazafaris et al[1] original procedure was tested following these steps: Briefly, 0.5 g of royal jelly was weight in a 50 mL centrifuge tube and 10 mL of a mixture of acetonitrile-water, 1:1 v/v, was added. After that, the tube was sonicated during 15 min at 40 ^{o}C. Then, centrifugation was applied at 3700 rpm (2265 *g*) for 10 min. The supernatant was took from this solution and slowly transferred into a C_{18} pre-treated cartridge with 5 mL of a mixture of ethyl acetate:*n*-hexane (1:1, v/v), 3 mL of acetonitrile and 3 mL of water. Next, the C_{18} cartridges were dried under vacuum for 1 h and 2 mL of ethyl acetate and 2 mL of *n*-hexane were slowly transferred into these cartridges. The final solution was evaporated to dryness under a nitrogen stream and finally 975 µL of ethyl acetate was added and transferred to a vial with 25 µL of the IS for GC-QqQ-MS/MS analysis.

2.5. GC-QqQ-MS/MS analysis

A volume of 3 µL from the final extract was injected into the chromatographic system at a flow rate of 5 µL s^{-1} in the syringe injection. The injector temperature program started at 70 °C and hold for 5 min. Then it was increased with a rate of 200 °C min^{-1} until 300 °C and hold for 20 min. An initial split ratio of 20:1 was set in the injector. Splitless mode was activated at 0.5 min. The column temperature was set at 70 °C at the beginning of the injection and hold for 3.5 min; then the temperature was increased until 180 °C at a 25°C min^{-1} rate, and finally until 325 °C at a rate of 15 °C min^{-1} where it was hold for 5 min. CO_2 was applied as cryogenic cooling when the injector temperature was at 250 °C in order to reach the initial injector temperature as fast as possible before continuing with the next injection. The total running time was 23 min.

The QqQ mass spectrometer was operated in the selected reaction monitoring (SRM) mode. The temperatures of the transfer line, manifold and ionization source were set at 300, 40, and 280 °C, respectively. A filament-multiplier delay of 4.5 min was used for the analysis in order to prevent instrument damage. The electron multiplier voltage was set at 1600 V, which corresponds to+200 V offset above the value obtained in the auto-tuning process. Mass peak widths of 1.5 and 2.0 *m/z* were set in the first and third quadrupole, respectively.

2.6. Validation procedure

The method was properly validated before its application in real samples. Linearity was evaluated using matrix matched standard calibration by analyzing extracted blank

samples of royal jelly spiked with the multi-pesticides standard solution at four concentration levels (5, 10, 50 and 100 μg kg^{-1}). Each matrix-matched standard also contained caffeine C_{13} as IS at a concentration of 500 μg kg^{-1}. Trueness was evaluated in terms of recovery spiking blank samples before the extraction procedure with the corresponding volume of the multi-compound working standard solution. Recovery was evaluated at three different levels, being 10, 50 and 100 μg kg^{-1} respectively, by spiking five blank samples at each level.

Intraday precision (repeatability) and interday precision (intermediate precision) were studied, expressed as relative standard deviation (RSD). Five spiked samples at 10, 50 and 100 μg kg^{-1} were used for the intraday precision. Interday precision was studied at the same concentration levels but processing the samples at five different days.

Finally, limits of detection (LODs) and limits of quantification (LOQs) were obtained by injecting fortified samples at lower concentration levels, being 0.1, 0.5, 1, 2, 5 and 10 μg kg^{-1}. The signal-to-noise ratio (S/N) criteria was used to determine these limits, defining the LOD as the lowest concentration of the analyte yielding a S/N of 3 and the LOQ as the lowest concentration of the analyte yielding a S/N of 10.

3. Results and Discussion

The optimization of the GC-QqQ-MS/MS was carried out previously[26], showing the characteristic GC-QqQ-MS/MS parameters, retention time windows (RTW), precursor ions, product ions and collision energies in Table 1.

3.1. Extraction and clean-up procedure

The procedures mentioned in Section 2.4 were tested with the multipesticide standard solution (177 pesticides) using three replicates at a concentration level of 50 μg kg^{-1}.

First, the American and European QuEChERS methodologies were evaluated following Procedure I (2.4.1). Figure 1a shows the number of pesticides extracted using both methods. It can be seen that American and European QuEChERS obtained similar results, extracting 116 and 110 compounds respectively with recoveries between 70 and 120%. The overall RSD values obtained by these two methods were 11 and 19% respectively. This indicates that American QuEChERS is more suitable to extract a large quantity of pesticides from royal jelly, and it was tested in further experiments.

Next, clean-up steps were evaluated for the American QuEChERS method in order to obtain better recoveries and minimize matrix effect. Figure 1b shows the number of

pesticides extracted using the different sorbents tested as clean-up steps following Procedure II. As it can be seen, when PSA is applied, a large number of pesticides is extracted (150), with recoveries between 70 and 120 %, comparing to the number extracted with Florisil (15) or the PSA+GBC mixture (65). If these results are compared to the ones obtained without clean-up (Figure 1a), it can be seen that the addition of a clean-up step using PSA improves the number of pesticides extracted. This can be explained by the specific compounds removed by the used sorbents. PSA removes fatty acids, other organic acids and sugars, while GBC removes pigments and sterols, and Florisil removes steroids, esters, lactones, glycerides, alkaloids and some carbohydrates.[27] Therefore, it is expected that PSA provides better results with a matrix like royal jelly, which contain high amount of sugars.

Although good results were obtained at a concentration of 50 μg kg^{-1} using Procedure II, when this procedure was tested at 10 μg kg^{-1}, only 68 pesticides obtained recoveries between 70 and 120%. Also, a pre-concentration procedure involving evaporation of 2 and 5 mL of the solvent and reconstitution in 1 mL was tested following the same conditions, but results were not improved. Keeping in mind that most of the MRL cited in Regulation 396/2005[8] for royal jelly are equal to 10 μg kg^{-1}, it is important that the developed method could quantify pesticides at the MRLs set by EU. Therefore, and bearing in mind that during the application of the QuEChERS procedure, a dilution of the target compounds were performed, another method was evaluated. Procedure III, based on SPE, was tested at 10 μg kg^{-1}, extracting 101 pesticides with recoveries between 70 and 120%. Figure 1c compared both procedures, concluding that, at this concentration level, SPE provided better results than the QuEChERS methodologies. Therefore, SPE was selected for method validation.

Finally, in Figure 2 the total ion chromatograms (TIC) for a solvent (Figure 2a), matrix using the QuEChERS approach (Figure 2b), and matrix using the SPE procedure (Figure 2c) are shown. All the samples are fortified to 10 μg kg^{-1} with the multipesticide standard solution. It can be seen that the SPE procedure improves pesticide signals and, therefore, quantification became more suitable.

3.2. Method validation

The proposed methodology was validated in order to ensure the reliability of the method for its application in routine analysis. In this case, only the pesticides that provided good

recoveries applying the optimized procedure were analyzed, including those that had recoveries between 60 and 70% (127 pesticides).

First, matrix effect was evaluated for all the pesticides studied, calculated as the ratio between the slope from the matrix calibration curve and the slope from the solvent calibration curve. If this effect is not presented, the values obtained should be between 0.8 and 1.2. Figure 3 shows the results, and it can be seen that for 58% of the pesticides studied there is not matrix effect. For 30% of them, the ratio was higher than 1.2, indicating matrix enhancement, whereas matrix suppression was only observed for 12% of the studied pesticides (ratio lower than 0.8).

In order to avoid these effects, quantification was performed using matrix-matched calibration standards with concentration levels from 5 to 100 μg kg^{-1} (5, 10, 50 and 100 μg kg^{-1}). Linearity was first evaluated in the whole range by least-squares regression of relative peak area (analyte/IS) versus concentration. Overall, determination coefficient (R^2) was higher than 0.98 for all the cases. In addition, the deviation of each individual level from the calibration curve was $\leq$ 20%. In this case, most of the compounds obtained good linearity at the proposed levels (5, 10, 50 and 100 μg kg^{-1}) except for those compounds with LOQs higher than 5 μg kg^{-1}. In this case, linearity was evaluated from 10 to 100 μg kg^{-1}.

Recoveries were studied in order to evaluate trueness. These results can be seen in Table 2, finding recoveries between 70 and 120% for most of the pesticides studied at three concentration levels (10, 50 and 100 μg kg^{-1}), except for azinphos-methyl, azoxystrobin, benfluralin, boscalid, fenthion, fonofos, hexaconazole, parathion methyl, phosmet, pyridafenthion and quintozene that have recoveries between 60 and 64% at 50 μg kg^{-1}. Also, chlorbenside and phosmet presented recoveries of 60 and 61% respectively at 10 μg kg^{-1}, and captan and fenthion got low recoveries (61 and 60% respectively) at 100 μg kg^{-1}. These low recoveries can be explained because the different nature from the pesticides studied and the stronger interactions with the SPE compounds.

Repeatability was studied, expressed as RSD, obtaining values below 20% for all the pesticides studied at the same concentration levels aforementioned (Table 2). The interday precision was also studied, expressed as RSD, obtaining values below 25% for most of the pesticides studied at the same concentration levels (Table 2), except for bruprofezin, captan, cyanofenphos, cypermethrin, furathiocarb and quintozene that provided RSD values between 30 and 37% at 10 μg kg^{-1}.

Finally, LODs and LOQs were also estimated, obtaining LODs between 1 and 5 μg kg^{-1} and LOQs between 2 and 10 μg kg^{-1} for all the pesticides studied. This indicates that the method is suitable to determine pesticides in royal jelly at low concentrations because the LOQs obtained are below the MRLs cited in Regulation 396/2005[8].

3.3. Application to real samples

Six royal jelly products (five liquid and one capsule presentation) were tested for pesticide residues using the validated method. An internal quality control was performed in order to ensure quality results. This implies a matrix-matched calibration, a reagent blank and a spiked sample at 10 μg kg^{-1}. No pesticides were found on the analyzed samples, indicating that the selected products fulfill European legislation[8] and can be considered healthy for consumers.

4. Conclusions

A reliable method to determine pesticides in royal jelly has been developed. A SPE procedure provided suitable results for the analysis of target compounds at low concentrations. QuEChERS like methods were also proposed but recoveries were not suitable at low concentration levels. This is important bearing in mind that the lowest MRLs of European Legislation are equal to 10 μg kg^{-1} and the SPE method used provided LOQs lower than this value. GC-QqQ-MS/MS was used to pesticides quantification. The method was validated obtaining good trueness and precision values. When the method was applied to real samples, no positive samples were detected. Nevertheless, the latent danger of pesticides presence in nutraceutical products, like royal jelly, has to be considered and future investigations should be focused on the development of robust and precise methodologies in order to warranty food safety in these products.

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Table 1. Retention time windows (RTWs) and MS/MS parameters of the selected pesticides

Compound	RTW (min)	Precursor ion	Product ions (collision energy, eV)[a]
2,4-DDD	13.95-13.96	235	**165 (25);** 199 (15)
2,4-DDT	13.92-13.98	235	**165 (25);** 199 (15)
4,4-DDD	14.39-14.42	235	**165 (25);** 199 (15)
4,4-DDE	13.82-13.89	318	176 (50); **246 (20)**
4,4-DDT	14.83-14.89	235	**165 (25);** 199 (15)
4,4'-Dichlorobenzophenone	12.76-12.86	250	**139 (15);** 215 (10)
Aclonifen	14.40-14.52	264	182 (25); **194 (15)**
Acrinathrin	15.99-16.06	181	127 (30)
		289	**93 (10)**
Alachlor	12.02-12.08	269	160 (20); **188 (10)**
Aldrin	12.67-12.73	263	**193 (35);** 228 (20)
α-HCH	10.87-10.93	219	109 (35); **183 (10)**
Azinphos-ethyl	16.20-16.31	160	105 (10); **132 (5)**
Azinphos-methyl	15.37-15.42	160	**105 (10);** 132 (5)
Azoxystrobin	18.38-18.44	344	**156 (40);** 172 (45)
Benalaxyl	14.63-14.67	266	**148 (15)**
		325	148 (25)
Benfluralin	10.47-10.54	292	160 (25); **264 (10)**
β-HCH	10.88-10.93	219	109 (35); **183 (10)**
Bifenox	15.61-15.67	341	**189 (20);** 281 (15)
Bifenthrin	15.15-15.23	181	115 (50); **165 (25)**
Boscalid	17.25-17.29	204	169 (15)
		342	**140 (15)**
Bromophos ethyl	13.34-13.37	359	**303 (12);** 331 (10)
Bromophos methyl	12.83-12.89	331	286 (30); **316 (20)**
Bromopropylate	15.43-15.48	341	157 (40); **183 (20)**
Buprofezin	13.88-13.93	249	106 (25); **193 (10)**
Bupirimate	13.82-13.89	273	150 (10); **193 (10)**
Butralin	12.55-12.73	266	**190 (15);** 220 (15)
Cadusafos	10.67-10.72	213	73 (10); **89 (15)**
Captan	13.18-13.27	117	82 (30)
		149	**70 (20)**
Carbophenothion	14.67-14.72	157	**45 (10)**
		342	157 (15)
Chlorbenside	13.41-13.50	268	89 (40); **125 (15)**
Chlordane	13.51-13.59	373	**266 (22);** 301 (10)
Chlorfenapyr	13.96-14.02	247	200 (30); **227 (15)**
Chlorfenson	13.71-13.77	175	75 (30); **111 (10)**
Chlorfenvinphos	13.07-13.09	267	**159 (15)**

		323	267 (15)
Chloropropylate	14.19-14.26	251	111 (30); **139 (10)**
Chlorothalonil	11.52-11.58	266	**168 (28);** 231 (20)
Chlorpyrifos ethyl	12.50-12.57	314	**258 (15);** 286 (10)
Chlorpyrifos methyl	11.94-12.01	286	136 (25); **241 (30)**
Chlorthal-dimethyl	12.60-12.66	301	**223 (25);** 273 (15)
Chlozolinate	12.99-13.06	331	186 (15); **259 (10)**
Clodinafop propargyl	14.71-14.81	349	238 (15); **266 (10)**
Cyanofenphos	14.68-14.74	185	**157 (10)**
		157	110 (15)
Cycloate	10.40-10.45	154	72 (10); **83 (5)**
Cyfluthrin	17.00-17.06	163	**127 (10)**
		226	206 (20)
Cynidon ethyl	19.11-19.19	358	302 (30); **330 (10)**
Cypermethrin	17.22-17.26	163	**127 (10)**
		181	127 (30)
δ-HCH	11.30-11.35	219	109 (35); **183 (10)**
Deltamethrin	18.27-18.32	172	**93 (10)**
		253	93 (20)
Diazinon	11.02-11.36	304	137 (35); **179 (15)**
Dichlofenthion	11.84-11.91	279	**222 (15);** 251 (5)
Dicofol o,p	14.98-15.05	251	111 (35); **139 (20)**
Dicofol p,p	15.61-15.65	251	111 (35); **139 (20)**
Dieldrin	13.65-13.69	263	**193 (35);** 228 (20)
Difenoconazole	18.13-18.17	323	202 (35); **265 (15)**
Diflufenican	14.94-14.98	394	238 (40); **266 (15)**
Endosulfan α	13.52-13.63	195	125 (25)
		241	**170 (25)**
Endosulfan β	14.40-14.43	195	**125 (25)**
		241	170 (25)
Endosulfan sulfate	14.32-14.37	270	**235 (18)**
		387	289 (10)
Endrin	14.27-14.31	263	**193 (35)**; 228 (20)
Ethion	14.28-14.35	231	**175 (15);** 203 (10)
Etrimfos	11.50-11.54	292	152 (20); **181 (10)**
Famoxadone	18.56-18.68	330	196 (25); **224 (10)**
Fenitrothion	12.33-12.40	260	109 (15); **125 (15)**
Fenoxicarb	15.41-15.53	255	157 (25); **186 (10)**
Fenpropathrin	15.42-15.52	265	181 (30); **210 (10)**
Fenthion	12.58-12.63	278	125 (40); **245(10)**
Fentoate	13.11-13.19	274	121 (10); **125 (18)**
Fenvalerate+Esfenvalerate	17.73-17.78	225	**119 (20);** 147 (10)
Fipronil	12.98-13.02	367	**213 (30);** 255 (22)
Flucythrinate	17.20-17.26	225	**119 (20);** 147 (10)
Fonofos	11.33-11.42	246	**109 (18);** 137 (10)

Fosalone	15.86-15.90	367	111 (30); **182 (10)**
Furathiocarb	15.62-15.70	194	**161 (10);** 179 (10)
Heptachlor	12.21-12.27	237	143 (25)
		272	**237 (15)**
Heptachlor epoxide cis	13.21-13.27	289	219 (28); **253 (10)**
Heptachlor epoxide trans	13.15-13.23	353	**263 (15);** 282 (15)
Hexaconazole	13.74-13.76	214	124 (30); **159 (25)**
Isocarbophos	12.85-12.91	230	155 (25); **198 (10)**
Isodrin	12.68-12.73	263	**193 (35);** 228 (20)
Isofenphos	13.01-13.07	213	**121 (15);** 185 (5)
Isofenphos methyl	12.86-12.90	241	**121 (20);** 199 (10)
Kresoxim methyl	13.85-13.87	206	116 (10); **132 (10)**
Lambda cyhalothrin	15.94-16.02	181	127 (30)
		197	**161 (10)**
Lindane	11.31-11.34	219	109 (35); **183 (10)**
Malathion	12.38-12.43	173	**99 (15);** 127 (5)
Methoxychlor	15.48-15.53	227	**169 (30);** 184 (20)
Mirex	16.25-16.32	272	**237 (15)**
		332	262 (35)
Oxadiazon	13.73-13.77	175	**112 (15)**
		258	112 (25)
Oxyfluorfen	13.81-13.86	300	**223 (20)**
		361	300 (15)
Parathion ethyl	12.63-12.67	291	91 (22); **109 (15)**
Parathion methyl	12.04-12.10	263	79 (28); **109 (15)**
Penconazole	13.05-13.09	248	**157 (25);** 192 (15)
Pendimethalin	12.95-13.01	252	**161 (15);** 191 (10)
Pentachloroaniline	11.89-11.93	265	**194 (25);** 230 (15)
Permethrin	16.61-16.66	163	**127 (10)**
		183	128 (25)
Phosmet	15.43-15.49	160	**77 (25);** 133 (12)
Phtalimide	9.36-9.62	147	**103 (10);** 104 (10)
Pirimiphos ethyl	12.71-12.78	333	163 (10); **168 (25)**
Pirimiphos methyl	12.26-12.27	290	**125 (25);** 151 (20)
Prochloraz	16.55-16.81	180	**138 (15)**
		308	70 (15)
Procymidone	13.23-13.27	283	67 (30); **96 (10)**
Propanil	11.97-12.03	161	**99 (25);** 126 (20)
Propargite	14.83-14.90	173	**135 (15)**
		350	201 (10)
Prophenophos	13.77-13.79	339	251 (30); **269 (15)**
Propiconazole	14.79-14.83	259	**173 (18);** 191 (10)
Prothiofos	13.71-13.75	309	221 (30); **239 (15)**
Pyrazophos	16.13-16.17	265	138 (30); **210 (10)**
Pyridaben	16.69-16.76	309	132 (35); **147 (15)**
Pyridafenthion	15.27-15.33	340	**199 (10);** 203 (25)

Pyrifenox	13.42-13.46	262	192 (18); **200 (18)**
Pyriproxyfen	15.90-15.96	136	41 (10); **96 (12)**
Quinalphos	13.16-13.21	298	**156 (12);** 190 (10)
Quinometionate	13.42-13.52	234	148 (25); **206 (10)**
Quintozene	11.26-11.28	295	**237 (18);** 265 (10)
S-421	12.29-12.31	132	95 (20); **97 (20)**
Silafluorfen	17.41-17.44	286	207 (10); **258 (15)**
Sulfotep	10.53-10.59	322	**146 (28);** 266 (10)
Tau fluvalinate	17.79-17.83	250	**55 (12);** 200 (20)
Tebuconazole	15.01-15.05	250	**125 (20);** 153 (10)
Tefluthrin	11.40-11.45	177	87 (30); **127 (15)**
Terbutryn	12.32-12.34	241	170 (15); **185 (10)**
Tetrachlorvinphos	13.42-13.46	329	**109 (20)**
		331	316 (20)
Tetradifon	15.80-15.84	229	166 (20); **201 (15)**
Tolclophos methyl	12.05-12.09	265	220 (25); **250 (15)**
Trichloronate	12.82-13.19	297	223 (22); **269 (15)**
Trifluralin	10.45-10.49	306	159 (25); **264 (10)**
Vinclozolin	11.97-12.03	212	145 (25); **172 (15)**

[a] Quantifier ion in bold

Table 2. Validation results of the developed method

Compound	Recovery(%)[a]			Intermediate precision (%)[b]			LOD	LOQ
	10 µg kg^{-1}	50 µg kg^{-1}	100 µg kg^{-1}	10 µg kg^{-1}	50 µg kg^{-1}	100 µg kg^{-1}	(µg kg^{-1})	(µg kg^{-1})
2,4-DDD	91 (16)	90 (13)	108 (10)	18	12	10	1	2
2,4-DDT	95 (15)	90 (11)	105 (11)	18	10	10	1	2
4,4-DDD	95 (15)	90 (11)	105 (11)	15	10	8	1	2
4,4-DDE	83 (10)	83 (8)	95 (7)	14	11	11	2	5
4,4-DDT	92 (16)	85 (11)	97 (8)	15	11	7	2	5
4,4'-Diclorobenzophenone	82 (13)	74 (11)	92 (7)	19	14	9	2	5
Aclonifen	103 (17)	79 (13)	91 (13)	25	21	13	5	10
Acrinatrin	102 (14)	95 (8)	116 (6)	16	13	13	2	5
Alachlor	70 (15)	70 (15)	76 (16)	24	15	16	2	5
Aldrin	79 (20)	70 (13)	72 (8)	21	14	16	2	5
α-HCH	82 (14)	72 (7)	85 (7)	17	8	7	1	2
Azinphos-ethyl	100 (16)	76 (9)	93 (11)	20	12	15	5	10
Azinphos-methyl	71 (16)	61 (9)	70 (13)	19	19	11	5	10
Azoxystrobin	72 (16)	60 (10)	70 (12)	21	18	14	5	10
Benalaxyl	88 (18)	76 (11)	90 (11)	21	13	15	1	2
Benfluralin	72 (16)	64 (6)	76 (11)	19	14	15	1	2
ß-HCH	80 (19)	83 (10)	100 (13)	17	8	8	2	5
Bifenox	109 (17)	92 (14)	106 (11)	11	19	17	5	10
Bifenthrin	91 (17)	93 (11)	113 (9)	13	10	11	1	2
Boscalid	71 (18)	60 (11)	70 (13)	21	15	17	2	5
Bromophos ethyl	84 (17)	86 (11)	99 (9)	18	16	12	2	5
Bromophos methyl	82 (13)	86 (10)	96 (11)	15	10	11	2	5
Bromopropylate	93 (16)	92 (10)	110 (12)	10	10	12	1	2
Bruprofezin	109 (15)	91 (15)	105 (10)	35	10	5	5	10
Bupirimate	101 (12)	83 (11)	101 (14)	15	12	16	2	5
Butralin	96 (19)	76 (13)	90 (13)	17	8	16	2	5
Cadusafos	72 (17)	71 (12)	77 (15)	19	11	10	2	5
Captan	108 (17)	70 (13)	61 (15)	34	25	11	5	10
Carbophenothion	83 (16)	84 (10)	101 (8)	16	14	12	2	5
Chlorbenside	60 (16)	70 (13)	70 (7)	22	15	12	1	2
Chlordane	81 (11)	89 (10)	107 (6)	24	19	12	5	10
Chlorfenapyr	109 (14)	101 (6)	119 (7)	25	11	7	5	10
Chlorfenson	95 (16)	85 (10)	97 (11)	10	11	9	1	2
Chlorfenvinphos	89 (16)	72 (13)	70 (10)	15	10	10	2	5
Chlorpyrifos ethyl	84 (16)	79 (11)	94 (13)	16	13	11	1	2
Chlorpyrifos methyl	77 (18)	75 (11)	89 (16)	18	13	7	2	5
Chloropropylate	92 (16)	91 (11)	106 (11)	13	9	12	1	2
Chlorothalonil	96 (17)	70 (12)	72 (7)	17	14	17	5	10
Chlorthal-dimethyl	87 (17)	83 (10)	95 (13)	16	7	11	2	5
Chlozolinate	75 (20)	83 (13)	95 (13)	18	12	11	2	5
Clodinafop propargyl	99 (13)	92 (12)	109 (11)	17	10	8	1	2
Cyanofenphos	103 (18)	96 (10)	116 (13)	30	9	17	5	10
Cycloate	99 (19)	94 (9)	111 (8)	18	12	11	2	5
Cyfluthrin	102 (15)	98 (11)	120 (8)	18	9	12	2	5
Cynidon ethyl	94 (16)	92 (8)	113 (10)	11	8	10	2	5
Cypermethrin	100 (19)	88 (13)	120 (9)	34	20	24	1	2
δ-HCH	81 (19)	85 (10)	102 (12)	21	8	5	1	2
Deltamethrin	102 (19)	95 (11)	118 (7)	25	10	7	5	10
Diazinon	83 (12)	72 (10)	85 (14)	21	12	7	2	5
Dichlofention	74 (19)	71 (10)	82 (16)	15	19	13	2	5

Dicofol o,p	90 (17)	92 (10)	108 (10)	17	10	10	1	2
Dicofol p,p	89 (14)	109 (16)	119 (9)	22	16	12	2	5
Dieldrin	105 (15)	95 (9)	116 (12)	21	6	10	5	10
Difenoconazole	92 (16)	85 (10)	104 (11)	19	10	12	2	5
Diflufenican	98 (17)	94 (11)	113 (9)	16	9	8	1	2
Endosulfan α	114 (14)	82 (10)	96 (14)	22	19	17	5	10
Endosulfan ß	97 (19)	93 (14)	108 (14)	22	17	16	5	10
Endosulfan sulfate	92 (14)	94 (12)	114 (9)	15	12	14	2	5
Endrin	91 (11)	90 (11)	112 (9)	19	16	8	5	10
Ethion	95 (18)	93 (11)	111 (9)	15	9	8	2	5
Etrimfos	76 (18)	71 (15)	80 (8)	24	18	6	1	2
Famoxadone	110 (13)	95 (10)	117 (10)	18	10	9	5	10
Fenitrothion	72 (14)	73 (9)	73 (11)	21	16	12	1	2
Fenoxicarb	104 (16)	72 (12)	93 (7)	24	18	11	5	10
Fenpropathrin	112 (17)	92 (10)	109 (11)	17	9	11	5	10
Fenthion	74 (18)	60 (11)	60 (14)	25	16	18	2	5
Fentoate	86 (17)	86 (10)	100 (13)	19	17	13	2	5
Fenvalerate+Esfenvalerate	100 (15)	95 (11)	114 (9)	12	9	10	2	5
Fipronil	92 (15)	95 (9)	110 (13)	13	12	18	2	5
Flucythrinate	102 (21)	101 (15)	118 (10)	20	16	14	5	10
Fonofos	72 (17)	63 (8)	78 (8)	19	17	17	2	5
Fosalone	84 (20)	95 (8)	114 (11)	15	8	15	5	10
Furathiocarb	104 (23)	78 (13)	119 (9)	33	24	12	5	10
Heptachlor	71 (14)	70 (10)	71 (11)	20	16	13	1	2
Heptachlor epoxide cis	91 (11)	83 (8)	102 (13)	25	13	9	2	5
Heptachlor epoxide trans	96 (19)	77 (10)	92 (14)	19	19	15	2	5
Hexaconazole	74 (12)	62 (10)	71 (8)	15	9	8	5	10
Isocarbophos	92 (19)	86 (11)	98 (12)	23	12	13	2	5
Isodrin	73 (11)	70 (10)	73 (11)	18	15	14	5	10
Isofenphos	86 (16)	85 (10)	97 (10)	12	9	8	1	2
Isofenphos methyl	86 (10)	85 (10)	95 (8)	14	12	11	2	5
Kresoxim methyl	94 (13)	82 (10)	99 (13)	25	9	14	2	5
Lambda cyhalothrin	99 (15)	95 (11)	119 (8)	15	14	8	2	5
Lindane	84 (12)	71 (7)	86 (14)	24	12	8	1	2
Malathion	71 (19)	71 (12)	71 (11)	19	13	14	1	2
Methoxychlor	93 (16)	89 (12)	105 (10)	11	9	12	5	10
Mirex	86 (18)	85 (10)	103 (8)	18	9	11	1	2
Oxadiazon	84 (19)	92 (11)	111 (12)	19	8	12	2	5
Oxyfluorfen	100 (19)	93 (8)	115 (11)	14	16	14	2	5
Parathion ethyl	83 (13)	79 (10)	93 (14)	25	12	14	2	5
Parathion methyl	70 (19)	64 (5)	70 (14)	21	17	13	2	5
Penconazole	71 (18)	72 (8)	74 (12)	20	18	13	1	2
Pendimethalin	88 (19)	79 (9)	93 (14)	22	17	9	2	5
Pentachloroaniline	80 (19)	75 (10)	89 (9)	20	16	11	5	10
Permethrin	92 (17)	93 (11)	116 (8)	18	11	7	2	5
Phosmet	61 (15)	63 (11)	70 (11)	17	15	14	2	5
Phtalimide	106 (18)	72 (10)	81 (14)	16	9	15	2	5
Pirimiphos ethyl	91 (19)	91 (15)	101 (8)	19	17	17	1	2
Pirimiphos methyl	80 (16)	80 (11)	93 (11)	16	11	8	1	2
Prochloraz	113 (17)	84 (14)	108 (12)	23	16	18	5	10
Procymidone	71 (12)	71 (8)	74 (13)	19	19	17	2	5
Propanil	116 (20)	71 (18)	86 (13)	24	15	12	5	10
Propargite	92 (17)	91 (15)	114 (7)	23	16	10	5	10
Prophenophos	95 (10)	91 (10)	109 (12)	13	10	15	2	5
Propiconazole	86 (13)	71 (10)	78 (8)	23	20	19	5	10
Prothiofos	85 (16)	87 (10)	101 (9)	14	7	11	2	5

Pyrazophos	107 (15)	93 (12)	108 (12)	10	5	7	5	10
Pyridaben	89 (14)	91 (12)	108 (9)	17	10	11	5	10
Pyridafenthion	70 (11)	63 (10)	71 (9)	19	9	9	2	5
Pyrifenox	72 (11)	72 (10)	73 (10)	23	16	16	2	5
Pyriproxyfen	95 (14)	90 (11)	107 (11)	17	9	8	2	5
Quinalphos	78 (14)	77 (9)	93 (14)	19	17	17	5	10
Quinometionate	74 (16)	75 (10)	83 (14)	20	13	17	2	5
Quintozene	72 (17)	63 (13)	72 (7)	37	25	19	2	5
S-421	75 (18)	71 (9)	82 (14)	24	15	9	1	2
Silafluorfen	92 (17)	91 (10)	110 (8)	12	9	10	2	5
Sulfotep	71 (10)	70 (8)	72 (10)	20	19	5	1	2
Tau fluvalinate	99 (16)	98 (10)	118 (7)	15	9	11	2	5
Tebuconazole	70 (12)	70 (10)	71 (10)	19	12	14	1	2
Tefluthrin	76 (13)	70 (8)	86 (13)	24	12	19	1	2
Terbutryn	70 (10)	72 (6)	70 (10)	17	17	9	2	5
Tetrachlorvinphos	71 (17)	71 (9)	75 (8)	18	12	15	2	5
Tetradifon	89 (17)	93 (12)	109 (11)	12	11	11	2	5
Tolclophos methyl	80 (15)	76 (11)	89 (12)	16	10	10	2	5
Trichloronate	105 (16)	75 (7)	95 (12)	17	13	11	5	10
Trifluralin	70 (18)	70 (4)	71 (8)	25	14	12	1	2
Vinclozolin	78 (15)	72 (7)	87 (14)	16	9	10	2	5

[a] R.S.D values are given in brackets (n = 5)

[b] n = 5.

Figure captions

Figure 1. Recoveries obtained for all the pesticides studied following Procedure I using American and European QuEChERS (a), Procedure II using PSA, Florisil and PSA+GBC, as clean-up steps (b), and Procedure III comparing a solid phase extraction with QuEChERS (c).

Figure 2. Total ion chromatograms (TIC) corresponding to a standard mixture of pesticides: in solvent (a), matrix using American QuEChERS (b) and matrix using a solid phase extraction (SPE) procedure (c), all fortified at 10 μg kg^{-1}.

Figure 3. Evaluation of matrix effect for all the pesticides studied.

80x40mm (300 x 300 DPI)