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# Evaluation of zirconium dioxide-based sorbents to decrease the matrix effect in avocado and almond multiresidue pesticide analysis followed by gas chromatography tandem mass spectrometry

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ABSTRACT

Two sorbents containing $ZrO_2$ (Z-Sep and Z-Sep+) were tested as a d-SPE clean-up in combination with the QuEChERS and ethyl acetate multiresidue method in the pesticide residues extraction in avocado. All extracts were analysed using gas chromatography coupled with a triple quadrupole mass spectrometer working in multi-reaction monitoring mode. GC QToF was used to compare the amount of matrix compounds present in the final extracts, prepared according to different protocols. The highest number of pesticides with acceptable recoveries and the lowest amount of coextracted matrix compounds were provided by QuEChERS with Z-Sep. Subsequently, this method was fully validated in avocado and almonds. Validation studies were carried out according to DG Sanco guidelines including: the evaluation of recoveries at two levels (10 and 50 μg/kg), limit of quantitation, linearity, matrix effects, as well as interday and intraday precision. In avocado, 166 pesticides were fully validated compared to 119 in almonds. The method was operated satisfactorily in routine analysis and was applied to real samples.


## 1. Introduction

Avocado and almonds are examples of high oil commodities. Avocado contains up to 30% fat whereas the fat content in almonds is around 50%. Both avocado and almond fat contain mainly fatty acids (oleic, palmitic and linoleic) and triglycerides [1,2]. The main problem with these kinds of matrices is in assuring high pesticide recoveries and low levels of co-extracted fat [3]. An additional difficulty in almonds analysis is the low water content of this commodity.

The removal of lipids from the extract before GC analysis is necessary for several reasons. Even small amounts of lipids can damage the column, source and detector [4,5]. Fatty acids interfere with the analysis [6]; they can produce broad peaks which overlap analyte peaks and can also increase matrix effects [7]. The fat content in the extract can be limited by choosing an extraction solvent in which lipid solubility is limited e.g. acetonitrile or methanol [3,8]. The disadvantage of these solvents is the low lipophilic pesticide extraction, where the pesticides remain in the undissolved fat [9]. Ethyl acetate, n-hexane and diethyl ether are better lipid solvents and assure higher recoveries of non-polar pesticides; however, the downside is the high fat content in the extract [3]. Whichever solvent is used, some kind of clean-up is usually necessary. To remove fat from the extract, d-SPE, column SPE, GPC and low temperature fat precipitation can be applied [9].

In the literature, there are numerous examples of pesticide analyses in fatty matrices with GC equipped with detectors such as the nitrogen–phosphorus detector [10–12] or the electron capture detector [10,13–15]; nonetheless, these detectors have limited specificity and DG Sanco guidelines recommend the use of mass detectors [16]. The GC–MS or GC–MS/MS techniques were used in the analysis of different high oil matrices: animal fat [4], milk, bacon [7], avocado [8,17], flaxseeds and peanuts [5]. Lehotay et al. were developing a method for 16 pesticides in avocado. In their studies, matrix solid-phase dispersion was compared with acetate buffered QuEChERS. The authors found the QuEChERS method with C18 and PSA in d-SPE to be the most suitable: this method was the most rapid and ensured the best recoveries [8]. In the other studies, QuEChERS was applied to commodities containing more fat than is present in avocado i.e. flaxseeds and peanuts. Samples were analysed with GC-ToF. During the experiments, the effectiveness of different fat elimination methods were evaluated. The authors tested d-SPE (with C18 and PSA), low temperature fat precipitation (so called freezing-out) and gel permeation

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chromatography (GPC). The best results were obtained from d-SPE [5]. Fernandez Moreno et al. obtained good results in the extraction of 65 GC-amenable pesticides from avocado with an ethyl acetate–cyclohexane mixture; samples were homogenised with polytron. The method was compared to pressurised liquid extraction. Results from both methods were similar so the authors recommended the first as it was faster, cheaper and simpler [17]. To the best of our knowledge, the available literature on pesticide determination methods in almonds is very limited, particularly methods using GC–MS/MS.

The aim of this work was the evaluation of Z-Sep and Z-Sep+ sorbents as the clean-up material for pesticide analysis in high oil matrices. Z-Sep+ is a silica carrier coated with zirconium dioxide and ocadecylosilan groups. Z-Sep is, in fact, a mixture of two sorbents – C18 and silica coated with zirconium dioxide – with a $ZrO_2$/C18 proportion of 2/5.

Zirconium dioxide has hard Lewis acid sites on its surface. These sites are present because zirconium (IV) has vacant 3d orbitals. Lewis acid sites can interact strongly with Lewis bases such as $R–SO_3^-$; $R–PO_3^-$ and $R–OO^-$ creating coordination bonds [18,19]. $ZrO_2$ was found to be a great adsorbent for phospholipids from crude oil. Its adsorption capacity was much higher than other metal oxides – ZnO and $TiO_2$ [20,21]. Zirconia is an amphoteric oxide and at different pHs, its surface can behave as a Brønsted acid or as a Brønsted base. At low pH, the surface is charged positively and behaves like an acid whereas at high pH, the surface charge is negative and zirconia has a basic character [22]. As with phospholipids, $ZrO_2$ is also a good adsorbent for carboxylic acids. Investigation into the adsorption of citric acid suggests the great importance of electrostatic interaction within the adsorption mechanism [23]. Thistlethwaite et al. investigated the adsorption of oleic acid. They concluded that adsorption at low pH occurs thanks to electrostatic interaction between oleate anions and the positively-charged zirconium dioxide surface. However, at pH 9, coordination bonds are responsible for adsorption [18]. In the adsorption of carboxylic acid, the main role is played by the carboxylic group yet the presence of a second $COO^-$ group makes the adsorption stronger. Adsorption is also stronger when a molecule contains a double bond or a hydroxyl group in the α position [24,25]. Apart from carboxylic acids, $COO^-$ groups are also present in proteins and zirconia surface bonds, the molecules of which are very strong [26].

## 2. Experimental

### 2.1. Reagents and materials

All high purity pesticide standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and Riedel-de Haën (Selze, Germany) and they were stored at −30 °C. Individual pesticide stock solutions (1000–2000 mg/L) were prepared in acetonitrile and ethyl acetate and were stored in amber screw-capped glass vials in the dark at −20 °C. Individual standard solutions for optimisation and two standard-mix solutions for calibration were prepared from the stock standards.

Ultra gradient HPLC grade acetonitrile was obtained from Sigma-Aldrich (Steinheim, Germany). HPLC grade methanol was supplied by Panreac (Barcelona, Spain). HPLC-grade ethyl acetate and Trisodium citrate dihydrate were purchased from Fluka (Steinheim, Germany). Primary–secondary amine (PSA) Bond-Elut was obtained from Supelco (Bellefonte, PA, USA). Sodium chloride was purchased from J.T.Baker (Deventer, The Netherlands). Disodium hydrogencitrate sesquihydrate was obtained from Sigma-Aldrich (Steinheim, Germany). Anhydrous magnesium sulphate was supplied by Panreac (Barcelona, Spain). C18 was purchased from Agilent Technologies (Santa Clara, CA). PSA, Z-Sep® and Z-Sep+® were supplied by Supelco (Bellefonte, PA). A Milli-Q-Plus ultra-pure water system from Milli-pore (Milford, MA, USA) was used throughout to hydrate the almonds.

### 2.2. Spiking procedure

For recovery studies, the samples were spiked with the studied pesticides before the corresponding extraction procedure. Samples obtained from the local market were analysed in order to ensure they did not contain any of the studied compounds. Blank samples were spiked with the standard solution in methanol. For avocado, 70 g of minced sample were weighed and transferred to a glass beaker and the sample was fortified with 700 μL of the appropriate working standard solution. Then, the sample was blended for 30 min. Almonds are dehydrated samples, so the spiking procedure was slightly different. 40 g of previously comminuted almonds were placed in a crystallizer. 20 mL of the working standard solution in methanol was added and the mixture was gently blended under a nitrogen stream until dryness. The samples were then allowed to stand at room temperature prior to analysis. The final spiking concentration levels in the samples for recovery studies were 10 and 50 μg/kg.

### 2.3. Extraction methods

Two well known methods were applied to evaluate pesticide extraction from fatty matrices – QuEChERS (using different clean-up sorbents: PSA-C18, Z-Sep and Z-Sep+) and the ethyl acetate method, with and without (Z-Sep and Z-Sep+) clean-up.

#### 2.3.1. QuEChERS method

The QuEChERS sample preparation procedure was applied to the samples. After homogenisation, a 10 g portion of avocado or 5 g of almonds, was weighed in a 50 mL PTFE centrifuge tube (5 mL of water was added to the almond samples. These samples were shaken and left for 30 min). After that, 10 mL of acetonitrile and 50 μL of a mix of surrogate standards at 10 mg/L – triphenyl phosphate (TPP), malathion-$d_{10}$ and dichlorvos-$d_6$ – were added and the samples were shaken in an automatic axial extractor (AGYTAX®, Cirta Lab. S.L., Spain) for 4 min. Afterwards, 4 g of magnesium sulphate, 1 g of sodium chloride, 1 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogencitrate sesquihydrate were added and the samples were again shaken in the automatic axial extractor for 4 min. Then the extract was centrifuged (3700 rpm) for 5 min. 5 mL of the supernatant were transferred to a 15 mL PTFE centrifuge tube containing 750 mg of magnesium sulphate and: (a) 125 mg of PSA and 125 mg of C18, (b) 175 mg of Z-Sep or (c) 175 mg of Z-Sep+. The extract was shaken in a vortex for 30 s and centrifuged again (3700 rpm) for a further 5 min. Subsequently, 100 μL of the extract was evaporated under a gentle nitrogen stream and then it was reconstituted with 100 μL of ethyl acetate (in the case of avocado) or with 50 μL of ethyl acetate (in the case of almond). The vials were vortexed to ensure complete reconstitution. 2 μL (almond samples) or 4 μL (avocado samples) of lindane-$d_6$ 1.25 μg/L were added to each vial as the injection control standard. With this treatment, 1 mL of sample extract represented 1 g of sample.

#### 2.3.2. Ethyl acetate method

For the ethyl acetate method, 10 g of previously homogenised avocado were weighed in a 50 mL PTFE centrifuge tube. Then, 10 mL of ethyl acetate and 50 μL of a mix of surrogate standards at 10 mg/L – triphenyl phosphate (TPP), malathion-$d_{10}$ and dichlorvos-$d_6$ – were added and the mixture was shaken by hand

**Table 1**
Acquisition and chromatographic parameters for the selected compounds analysed by GC–MS/MS.

| No. | Compound | $t_R$ (min) | SRM1 | CE1 (V) | SRM2 | CE2 (V) | Time segment |
|---|---|---|---|---|---|---|---|
| 1 | 2,4-DDE | 12,2 | 235 > 165 | 20 | 235 > 199 | 15 | 23 |
| 2 | 2,4-DDT | 11,3 | 246 > 176 | 30 | 246 > 211 | 20 | 25 |
| 3 | 4,4-DDD | 12,9 | 235 > 165 | 20 | 235 > 199 | 15 | 27 |
| 4 | 4,4-DDE | 12,1 | 246 > 176 | 30 | 246 > 211 | 20 | 26 |
| 5 | 4,4-DDT | 12,9 | 235 > 165 | 20 | 235 > 199 | 20 | 29 |
| 6 | Acrinathrin | 15,4 | 208 > 181 | 5 | 209 > 141 | 20 | 35 |
| 7 | Alachlor | 8,6 | 188 > 160 | 10 | 188 > 130 | 40 | 11 |
| 8 | Ametryn | 8,7 | 227 > 185 | 5 | 227 > 212 | 8 | 13 |
| 9 | Azoxystrobin | 18,5 | 344 > 329 | 10 | 344 > 156 | 40 | 39 |
| 10 | Benalaxyl | 13,5 | 148 > 105 | 20 | 204 > 176 | 2 | 28 |
| 11 | Bifenox | 14,7 | 311 > 279 | 14 | 311 > 216 | 25 | 31 |
| 12 | Bifenthrin | 14,4 | 181 > 166 | 10 | 181 > 115 | 50 | 32 |
| 13 | Bixafen | 16,9 | 159 > 139 | 15 | 413 > 159 | 12 | 37 |
| 14 | Boscalid | 16,5 | 140 > 112 | 10 | 140 > 76 | 25 | 37 |
| 15 | Bromopropylate | 14,4 | 341 > 185 | 20 | 341 > 155 | 20 | 32 |
| 16 | Bupirimate | 12,6 | 273 > 193 | 5 | 273 > 108 | 15 | 27 |
| 17 | Buprofezin | 12,3 | 305 > 172 | 5 | 305 > 140 | 10 | 26 |
| 18 | Butralin | 10,2 | 266 > 174 | 20 | 266 > 190 | 12 | 15 |
| 19 | Butylate | 3,8 | 156 > 57 | 5 | 174 > 146 | 3 | 1 |
| 20 | Cadusafos | 5,9 | 159 > 97 | 10 | 213 > 73 | 10 | 6 |
| 21 | Carbophenothion | 13,4 | 199 > 143 | 10 | 342 > 157 | 10 | 28 |
| 22 | Carbosulfan | 3,2 | 164 > 149 | 12 | 164 > 122 | 12 | 1 |
| 23 | Chinomethionat | 11,2 | 234 > 206 | 10 | 206 > 148 | 15 | 23 |
| 24 | Chlorbromuron | 3,7 | 233 > 124 | 25 | 233 > 205 | 12 | 1 |
| 25 | Chlorfenapyr | 12,7 | 247 > 227 | 15 | 247 > 200 | 25 | 27 |
| 26 | Chlorfenvinphos | 10,9 | 267 > 159 | 20 | 267 > 81 | 40 | 22 |
| 27 | Chlorobenzilate | 12,8 | 139 > 111 | 15 | 139 > 75 | 30 | 27 |
| 28 | Chlorothalonil | 8,4 | 266 > 231 | 20 | 266 > 133 | 40 | 8 |
| 29 | Chlorpropham | 5,6 | 213 > 171 | 5 | 213 > 127 | 5 | 4 |
| 30 | Chlorpyrifos | 9,6 | 313 > 258 | 15 | 313 > 286 | 5 | 15 |
| 31 | Chlorpyrifos-methyl | 8,3 | 288 > 93 | 26 | 286 > 271 | 16 | 10 |
| 32 | Chlorthal-dimethyl | 9,7 | 330 > 299 | 12 | 330 > 221 | 35 | 14 |
| 33 | Chlozolinate | 10,7 | 259 > 188 | 10 | 331 > 216 | 5 | 18 |
| 34 | Cyfluthrin | 16,3 | 163 > 127 | 5 | 226 > 206 | 10 | 36 |
| 35 | Cypermethrin | 16.5–16.6 | 163 > 127 | 5 | 209 > 141 | 20 | 37 |
| 36 | Cyproconazole | 12,6 | 139 > 111 | 14 | 222 > 125 | 18 | 27 |
| 37 | Cyprodinil | 10,4 | 224 > 208 | 20 | 224 > 197 | 21 | 18 |
| 38 | Deltamethrin | 18,1 | 253 > 93 | 20 | 253 > 172 | 5 | 39 |
| 39 | Diazinon | 7,2 | 304 > 179 | 15 | 304 > 137 | 30 | 8 |
| 40 | Dichlorvos | 3,0 | 185 > 93 | 15 | 185 > 109 | 15 | 1 |
| 41 | Dichlorvos-$d_6$ | 2,9 | 191 > 99 | 15 | 191 > 115 | 20 | 1 |
| 42 | Diclobutrazol | 12,3 | 270 > 159 | 15 | 270 > 201 | 8 | 27 |
| 43 | Dicloran | 6,5 | 206 > 176 | 5 | 206 > 148 | 20 | 7 |
| 44 | Dimethenamid | 8,0 | 230 > 154 | 10 | 154 > 111 | 10 | 8 |
| 45 | Diphenylamine | 5,3 | 169 > 77 | 35 | 168 > 140 | 40 | 4 |
| 46 | Endosulfan alpha | 11,3 | 239 > 204 | 15 | 241 > 206 | 25 | 24 |
| 47 | Endosulfan beta | 12,7 | 241 > 206 | 14 | 239 > 204 | 15 | 27 |
| 48 | Endosulfan sulphate | 13,4 | 387 > 289 | 5 | 387 > 206 | 40 | 29 |
| 49 | EPN | 14,4 | 157 > 77 | 25 | 157 > 110 | 15 | 32 |
| 50 | Epoxiconazole | 13.4 - 14.1 | 192 > 138 | 10 | 192 > 111 | 35 | 29 |
| 51 | Ethion | 13,1 | 231 > 129 | 25 | 231 > 175 | 5 | 27 |
| 52 | Ethofenprox | 16,6 | 163 > 107 | 15 | 163 > 135 | 5 | 37 |
| 53 | Ethofumesate | 9,2 | 207 > 161 | 5 | 207 > 137 | 10 | 13 |
| 54 | Ethoprophos | 5,4 | 158 > 97 | 15 | 158 > 114 | 5 | 4 |
| 55 | Etrimfos | 7,6 | 292 > 181 | 5 | 292 > 153 | 20 | 8 |
| 56 | Fenamidone | 14,6 | 268 > 180 | 20 | 238 > 103 | 20 | 32 |
| 57 | Fenarimol | 15,3 | 139 > 111 | 15 | 219 > 107 | 10 | 34 |
| 58 | Fenazaquin | 14,6 | 160 > 145 | 5 | 160 > 117 | 20 | 31 |
| 59 | Fenbuconazole | 16,2 | 198 > 129 | 5 | 129 > 102 | 15 | 36 |
| 60 | Fenchlorphos | 8,7 | 285 > 270 | 18 | 285 > 240 | 30 | 13 |
| 61 | Fenhexamid | 13,6 | 177 > 78 | 20 | 177 > 113 | 10 | 29 |
| 62 | Fenitrothion | 9,1 | 277 > 260 | 5 | 277 > 109 | 20 | 13 |
| 63 | Fenpropathrin | 14,5 | 181 > 152 | 26 | 265 > 210 | 10 | 32 |
| 64 | Fenpropimorph | 9,6 | 128 > 70 | 12 | 128 > 110 | 10 | 14 |
| 65 | Fenthion | 9,6 | 278 > 109 | 20 | 278 > 169 | 20 | 15 |
| 66 | Fenvalerate/Esfenvalerate RR/SS | 17.5 | 167 > 125 | 12 | 125 > 89 | 22 | 38 |
| 67 | Fenvalerate/Esfenvalerate RS/SR | 17,3 | 167 > 125 | 12 | 125 > 89 | 22 | 38 |
| 68 | Flamprop-isopropyl | 12,9 | 276 > 105 | 5 | 304 > 105 | 12 | 27 |
| 69 | Flamprop-methyl | 12,4 | 276 > 105 | 8 | 230 > 170 | 15 | 25 |
| 70 | Flonicamid | 5.52 | 174 > 146 | 15 | 174 > 126 | 25 | 4 |
| 71 | Fluacrypyrim | 13,4 | 145 > 102 | 30 | 145 > 115 | 15 | 28 |
| 72 | Fluazifop-P-butyl | 12,7 | 282 > 91 | 15 | 282 > 238 | 20 | 26 |
| 73 | Flucythrinate | 16.6–16.8 | 199 > 157 | 5 | 157 > 107 | 15 | 37 |
| 74 | Fludioxonil | 12,4 | 248 > 127 | 30 | 248 > 154 | 25 | 26 |
| 75 | Fluquinconazole | 15,9 | 340 > 298 | 20 | 340 > 286 | 30 | 35 |

**Table 1** (*continued*)

| No. | Compound | $t_R$ (min) | SRM1 | CE1 (V) | SRM2 | CE2 (V) | Time segment |
|---|---|---|---|---|---|---|---|
| 76 | Flusilazole | 12,5 | 233 > 165 | 20 | 233 > 152 | 20 | 27 |
| 77 | Flutolanil | 12,1 | 323 > 173 | 13 | 323 > 281 | 4 | 25 |
| 78 | Flutriafol | 11,9 | 219 > 123 | 12 | 219 > 95 | 20 | 25 |
| 79 | Fluvalinate-tau | 17,5 | 250 > 55 | 18 | 250 > 200 | 22 | 38 |
| 80 | Fonofos | 6,9 | 137 > 109 | 5 | 246 > 137 | 5 | 8 |
| 81 | Formothion | 7,9 | 170 > 93 | 5 | 224 > 125 | 20 | 7 |
| 82 | Fosthiazate | 10,2 | 195 > 103 | 5 | 195 > 139 | 5 | 18 |
| 83 | Heptachlor | 8,4 | 272 > 237 | 10 | 272 > 143 | 40 | 10 |
| 84 | Heptenophos | 4,9 | 124 > 89 | 15 | 215 > 200 | 10 | 3 |
| 85 | Hexaconazole | 11,8 | 214 > 159 | 22 | 214 > 172 | 22 | 25 |
| 86 | Indoxacarb | 18,1 | 203 > 134 | 10 | 264 > 148 | 28 | 39 |
| 87 | Iprodione | 14,4 | 314 > 245 | 10 | 314 > 56 | 20 | 32 |
| 88 | Iprovalicarb | 12,4 | 158 > 116 | 5 | 158 > 98 | 10 | 27 |
| 89 | Isazofos | 7,5 | 161 > 119 | 5 | 257 > 162 | 5 | 8 |
| 90 | Isocarbophos | 9,9 | 136 > 108 | 14 | 230 > 212 | 8 | 16 |
| 91 | Isofenphos-ethyl | 10,9 | 213 > 121 | 15 | 213 > 185 | 3 | 18 |
| 92 | Isofenphos-methyl | 10,4 | 199 > 121 | 10 | 199 > 167 | 10 | 18 |
| 93 | Kresoxim-methyl | 12,5 | 206 > 116 | 5 | 206 > 131 | 10 | 27 |
| 94 | Lambda-cyhalothrin | 15,2 | 197 > 141 | 10 | 197 > 161 | 5 | 34 |
| 95 | Lindane | 6,8 | 219 > 183 | 5 | 181 > 145 | 12 | 7 |
| 96 | Lindane-$d_6$ | 6,7 | 224 > 187 | 5 | 224 > 150 | 20 | 7 |
| 97 | Malathion | 9,4 | 173 > 99 | 15 | 158 > 125 | 8 | 14 |
| 98 | Malathion-$d_{10}$ | 9,3 | 183 > 132 | 5 | 183 > 151 | 3 | 13 |
| 99 | Mecarbam | 10,9 | 159 > 131 | 5 | 329 > 160 | 3 | 21 |
| 100 | Merphos | 12,1 | 169 > 57 | 8 | 169 > 113 | 3 | 26 |
| 101 | Metalaxyl | 8,7 | 206 > 132 | 20 | 266 > 162 | 8 | 13 |
| 102 | Metazachlor | 10,5 | 209 > 133 | 10 | 133 > 117 | 25 | 19 |
| 103 | Metconazole | 14,8 | 125 > 89 | 20 | 125 > 99 | 20 | 32 |
| 104 | Methamidophos | 3,1 | 141 > 95 | 6 | 141 > 79 | 18 | 1 |
| 105 | Methidathion | 11,3 | 145 > 85 | 5 | 145 > 58 | 15 | 24 |
| 106 | Methiocarb | 9,2 | 168 > 153 | 10 | 153 > 109 | 10 | 13 |
| 107 | Methoxychlor | 13,9 | 227 > 169 | 25 | 227 > 115 | 40 | 30 |
| 108 | Metolachlor | 9,5 | 238 > 162 | 8 | 162 > 133 | 12 | 15 |
| 109 | Mevinphos | 3,8 | 127 > 109 | 10 | 127 > 95 | 15 | 2 |
| 110 | Myclobutanyl | 12,4 | 179 > 125 | 10 | 179 > 152 | 5 | 27 |
| 111 | Napropamide | 11,8 | 128 > 72 | 3 | 271 > 128 | 3 | 24 |
| 112 | Nuarimol | 13,8 | 203 > 107 | 10 | 235 > 139 | 12 | 28 |
| 113 | Ofurace | 13,4 | 232 > 158 | 20 | 232 > 186 | 5 | 28 |
| 114 | o-Phenylphenol | 4,5 | 170 > 141 | 30 | 170 > 115 | 40 | 3 |
| 115 | Oxadixyl | 13,1 | 163 > 132 | 15 | 163 > 117 | 25 | 27 |
| 116 | Paclobutrazol | 11,4 | 236 > 125 | 10 | 236 > 167 | 20 | 24 |
| 117 | Parathion-ethyl | 9,7 | 291 > 109 | 10 | 291 > 81 | 10 | 16 |
| 118 | Parathion-methyl | 8,4 | 263 > 109 | 10 | 233 > 124 | 10 | 10 |
| 119 | Pebulate | 4,0 | 128 > 57 | 5 | 161 > 128 | 3 | 1 |
| 120 | Penconazole | 10,6 | 248 > 157 | 25 | 248 > 192 | 15 | 19 |
| 121 | Pendimethalin | 10,5 | 252 > 162 | 10 | 252 > 191 | 10 | 20 |
| 122 | Permethrin | 15.7–15.9 | 163 > 127 | 5 | 183 > 153 | 15 | 35 |
| 123 | Phenothrin | 14.7–14.8 | 123 > 81 | 8 | 183 > 153 | 15 | 32 |
| 124 | Phenthoate | 10,9 | 274 > 121 | 10 | 274 > 246 | 5 | 21 |
| 125 | Phorate | 6,0 | 231 > 129 | 20 | 231 > 175 | 20 | 6 |
| 126 | Phosmet | 14,4 | 160 > 77 | 30 | 160 > 133 | 15 | 32 |
| 127 | Picolinafen | 14,8 | 238 > 145 | 25 | 376 > 238 | 25 | 31 |
| 128 | Picoxystrobin | 11,9 | 335 > 173 | 10 | 303 > 157 | 15 | 24 |
| 129 | Pirimicarb | 7,8 | 238 > 166 | 10 | 166 > 96 | 20 | 9 |
| 130 | Pirimiphos-methyl | 9,1 | 290 > 151 | 15 | 305 > 180 | 5 | 12 |
| 131 | Procymidone | 11,0 | 283 > 96 | 8 | 283 > 255 | 8 | 20 |
| 132 | Profenofos | 12,0 | 337 > 267 | 16 | 337 > 309 | 6 | 25 |
| 133 | Prometon | 6,6 | 225 > 183 | 3 | 225 > 168 | 10 | 6 |
| 134 | Prometryn | 8,7 | 241 > 184 | 12 | 241 > 226 | 8 | 12 |
| 135 | Propaphos | 11,4 | 220 > 140 | 12 | 220 > 125 | 25 | 24 |
| 136 | Propazine | 6,8 | 214 > 172 | 8 | 229 > 187 | 3 | 6 |
| 137 | Propiconazole | 13.5–13.7 | 259 > 173 | 10 | 259 > 191 | 8 | 29 |
| 138 | Propyzamide | 7,0 | 173 > 145 | 16 | 173 > 109 | 32 | 8 |
| 139 | Prosulfocarb | 8,7 | 128 > 86 | 3 | 251 > 128 | 5 | 12 |
| 140 | Prothiofos | 11,9 | 309 > 239 | 15 | 309 > 221 | 25 | 25 |
| 141 | Pyrazophos | 15,4 | 221 > 193 | 10 | 221 > 149 | 15 | 35 |
| 142 | Pyridaben | 15,8 | 147 > 117 | 20 | 147 > 132 | 10 | 35 |
| 143 | Pyrimethanil | 7,1 | 198 > 118 | 25 | 198 > 156 | 25 | 8 |
| 144 | Pyriproxyfen | 15,0 | 136 > 78 | 18 | 136 > 96 | 8 | 34 |
| 145 | Quinalphos | 10,9 | 146 > 91 | 30 | 157 > 129 | 15 | 22 |
| 146 | Quinoxyfen | 13,5 | 307 > 272 | 5 | 307 > 237 | 25 | 29 |
| 147 | Quintozene | 6,9 | 295 > 237 | 15 | 295 > 265 | 10 | 7 |
| 148 | Spirodiclofen | 15,7 | 312 > 259 | 10 | 312 > 109 | 20 | 35 |
| 149 | Spiroxamine I | 8,2 | 100 > 58 | 10 | 100 > 72 | 10 | 9 |
| 150 | Spiroxamine II | 8,9 | 100 > 72 | 10 | 100 > 58 | 10 | 13 |
| 151 | Sulprofos | 13,2 | 156 > 141 | 15 | 322 > 156 | 10 | 28 |

**Table 1** (*continued*)

| No. | Compound | $t_R$ (min) | SRM1 | CE1 (V) | SRM2 | CE2 (V) | Time segment |
|---|---|---|---|---|---|---|---|
| 152 | Tebuconazole | 13,8 | 250 > 125 | 20 | 250 > 153 | 12 | 29 |
| 153 | Tebufenpyrad | 14,6 | 333 > 171 | 20 | 333 > 276 | 5 | 31 |
| 154 | Tecnazene | 5,2 | 215 > 179 | 12 | 203 > 143 | 20 | 3 |
| 155 | Tefluthrin | 7,5 | 177 > 127 | 15 | 177 > 137 | 15 | 9 |
| 156 | Terbufos | 6,9 | 231 > 129 | 25 | 231 > 175 | 10 | 8 |
| 157 | Terbumeton | 6,8 | 169 > 154 | 5 | 225 > 169 | 3 | 6 |
| 158 | Terbutryn | 9,1 | 241 > 185 | 3 | 241 > 170 | 10 | 13 |
| 159 | Tetrachlorvinphos | 11,5 | 329 > 109 | 25 | 329 > 79 | 35 | 24 |
| 160 | Tetraconazole | 10,1 | 336 > 204 | 30 | 336 > 218 | 30 | 18 |
| 161 | Tetradifon | 14,8 | 356 > 159 | 10 | 356 > 229 | 10 | 33 |
| 162 | Tetramethrin | 14,4 | 164 > 77 | 30 | 164 > 107 | 15 | 32 |
| 163 | Tolclofos-methyl | 8,5 | 265 > 250 | 15 | 265 > 220 | 25 | 11 |
| 164 | Tolylfluanid | 10,7 | 137 > 91 | 20 | 238 > 137 | 10 | 20 |
| 165 | TPP | 13,9 | 326 > 233 | 10 | 326 > 169 | 35 | 30 |
| 166 | Triadimefon | 9,7 | 208 > 181 | 5 | 208 > 127 | 15 | 15 |
| 167 | Triazophos | 13,3 | 161 > 134 | 5 | 161 > 106 | 10 | 28 |
| 168 | Trifloxystrobin | 13,7 | 222 > 190 | 3 | 222 > 130 | 15 | 29 |
| 169 | Trifluralin | 5,8 | 306 > 264 | 10 | 264 > 160 | 15 | 4 |
| 170 | Vinclozolin | 8,3 | 212 > 172 | 15 | 212 > 109 | 40 | 10 |

for 3 s. After this, 1.5 g of sodium chloride and 8 g of magnesium sulphate were added and the samples were shaken in the automatic axial extractor for 15 min. Following this, the tubes were centrifuged (5 min at 3700 rpm). Afterwards, samples were handled in three different ways: (a) a portion of the extract was transferred into the vials to be directly injected; (b) 5 mL of the extract were placed in a 15 mL PTFE centrifuge tube containing 175 mg of Z-Sep, the extract was shaken in a vortex for 30 s and centrifuged (3700 rpm) for 5 min.; finally a fraction of the extract was directly injected; or (c) the same steps as in (b) but using Z-Sep+ as the sorbent for the clean-up. At the end, 2 µL of lindane-$d_6$ 1.25 µg/L were added to each vial as the injection control standard. With this treatment, 1 mL of sample extract represented 1 g of sample.

## 2.4. Analysis

### 2.4.1. Gas chromatography–triple quadrupole-mass spectrometry analysis

All analyses were done on an Agilent 7890 GC equipped with an Agilent 7693B autosampler and an Agilent 7000 series GC–MS/MS triple quadrupole system (Agilent Technologies, Palo Alto, CA, USA). An Agilent Ultra Inert GC column, HP-5MS UI 15 m × 0.25 mm × 0.25 µm, was used to provide analyte separation. Backflushing was used to shorten the analysis time and reduce system maintenance. Retention Time Locking (RTL) was used to eliminate the need for adjusting the time segment windows of MRM groups, using trifluralin as the locking compound at a retention time of 5.81 min. Sample injections were performed in a 7890A GC multimode inlet, operated using the splitless injection mode through an ultra inert inlet liner, with a glass wool frit from Agilent. The injector operating conditions were as follows: injection volume, 2 µL; the injector temperature was held at 80 °C during the solvent evaporation stage (0.1 min) and then ramped up to 300 °C at 600 °C/min. This temperature was held for 20 min. Helium, with a purity of 99.999%, was used as the carrier gas (working at a constant pressure of 13.172 psi) and the quenching gas; and nitrogen, with a purity of 99.999%, as the collision gas. The oven temperature was as follows: 70 °C for 1 min, programmed to 150 °C at 50 °C/min, then to 200 °C at 6 °C/min and finally to 280 °C at 16 °C/min (4.07 min). The total run time was 20 min with 3 additional minutes for backflushing at 280 °C. The triple quadrupole mass spectrometer was operated in electron impact ionisation (EI) and in the SRM mode. The temperatures of the transfer line, ion source and quadrupole 1 and 2 were 280 °C, 280 °C and 150 °C, respectively. The analysis was performed with a solvent delay of 2 min in order to prevent instrument damage. Mass peak widths were set at wide in the first and third quadrupole. For control and data analysis, Agilent MassHunter B.05.00 software was used.

### 2.4.2. Optimisation of GC–MS/MS parameters

The MS/MS detection method was optimised firstly with individual injections in full-scan mode of each analyte at 1 mg/L – to obtain their retention times and to select the optimal precursor ions. The most intense ion with the highest m/z relationship was selected in most cases. Then, product ion scan methods were automatically created by the MassHunter software with different collision energies, ranging from 5 to 40 V, in order to select the best product ions. After running all of them, the two most intense transitions and their optimal collision energies were selected. The most intense product was selected as the quantifier ion and the second most intense as the qualifier ion. The collision gas flow was 1.5 mL/min and the quenching gas flow was 2.25 mL/min, the optimal values recommended by the manufacturer. A 39-time-segment SRM method was created to obtain adequate sensitivity and signal-to-noise ratio, and the cycle time for each segment was set between 200 and 250 ms. Table 1 shows the instrument parameters and MRM setting for all the compounds studied.

In this method, one precursor ion and two product ions were monitored (MS/MS transitions) in compliance with the identification requirements of the European guidelines [16].

## 2.5. Validation of the analytical procedure

Method accuracy and precision were evaluated by recovery studies using blank matrices at two concentration levels, 10 and 50 µg/kg. All experiments were performed with five replicates for each matrix, in accordance with EU guidelines [16]. Quantification of the compounds in the spiked samples was carried out comparing the peak areas of the samples with those of matrix-matched standard solutions. The level of quantitation (LOQ) was set as the minimum concentration that can be quantified with acceptable accuracy and precision, as described in Document no. SANCO/12495/2011 [16]. Linearity was evaluated both in solvent and matrix, using matrix-matched calibration curves prepared by spiking seven aliquots of the blank extract at seven concentration levels – from 1 to 500 µg/L (corresponding to 1–500 µg/kg in the

sample). The matrix effects were studied by comparing the slopes of the calibration curves in solvent and in matrix. The repeatability of the instrumental method was estimated by determining the inter- and intra-day relative standard deviation (RSD, %) by the repeated analysis ($n=5$) of a spiked matrix extract at the 10 and 50 μg/L levels, from run-to-run over 1 and 5 days, respectively.

### 2.6. Real samples

In order to prove the effectiveness of the validated method and its suitability for routine analysis, it was applied to 25 avocado samples and 18 almond samples purchased at different local markets in Almería (south-eastern Spain), over a three-month time period.

## 3. Results and discussion

### 3.1. Method selection

To select the optimal extraction protocol, Z-Sep and Z-Sep+ sorbents were tested as the clean-up in QuEChERS and the ethyl acetate method. To simplify the procedure, only one spiking level (50 μg/kg) in one matrix (avocado) was tested. Fig. 1 presents the percentage of the total number of pesticides with recoveries in the 70–120% range and RSD ≤ 20% ($n=5$). At the outset, the ethyl acetate method was preferred by authors given that the solvent is very suitable for GC analysis [27] and the extract can be injected directly. Acetonitrile has certain drawbacks such as the large expansion volume during vaporisation and the negative influence on some pesticides' stability [28]. For this reason, acetonitrile extracts need to be evaporated and reconstituted in a more suitable solvent and these steps would prolong the sample preparation procedure. However, extracts prepared with ethyl acetate contained a large amount of fat, which was visible after a few hours at −20 °C. A visual comparison did not reveal any particular differences in the fat content of ethyl acetate extracts without clean-up compared to those extracts cleaned with Z-Sep or Z-Sep+. Fat removal from ethyl acetate extracts would be possible with freezing-out or gel permeation chromatography. Nonetheless, both of these methods are time consuming. Moreover, GPC requires optimisation and increases the total amount of solvents used during sample preparation. In the QuEChERS extracts, however, fat was barely visible. In addition to this, the number of pesticides with good recoveries showed that QuEChERS is the better method. Out of 166 pesticides, QuEChERS with Z-Sep ensured recoveries in the 70–120% range and RSD < 20% for 156 pesticides whereas extraction using ethyl acetate resulted in only 143. In all extracts prepared with EtAc, certain pesticides (phorate, propaphos, sulprofos and terbufos) were overestimated i.e. had recoveries higher than 120%. But it is worth noting that ethyl acetate ensured better results for pesticides with low water solubility such as 2,4-DDT, 4,4-DDE, 4,4-DDD, 4,4-DDT, 2,4-DDE and quintozene.

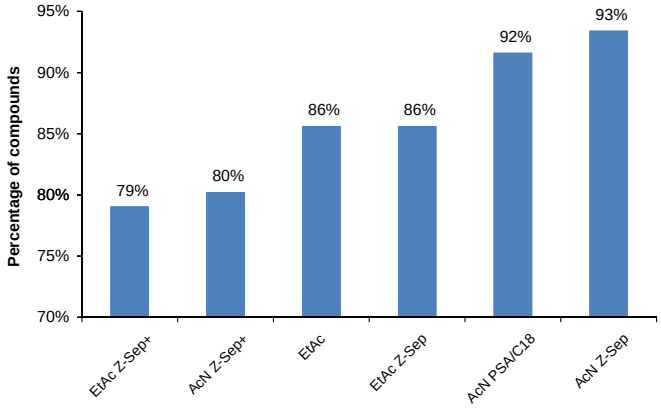


**Fig. 1.** Percentage of total number of evaluated pesticides with recoveries from the range 70–120%, in avocado.

Sapozhnikova and Lehotay compared Z-Sep and Z-Sep+ in the extraction of pesticides and other contaminants from fish tissue. Their findings are in agreement with results presented in this paper – Z-Sep sorbent provides cleaner extracts and better recoveries than Z-Sep+ or PSA+C18 [29].

Based on the recovery results and a comparison of GC-QToF full-scan chromatograms (described in Section 3.6), the authors chose QuEChERS with Z-Sep clean-up for further validation in avocado and almonds.

### 3.2. Recovery studies

In both matrices investigated, pesticide recoveries were characterised by low relative standard deviation (RSD) values. At both the 50 μg/kg and 10 μg/kg spiking levels, the majority of analytes had RSD equal to, or lower than, 10%. Only three pesticides exceeded the RSD value of 20%, which is recommended by DG Sanco guidelines. Those pesticides were chlorthalonil at the 50 μg/kg spiking level and butralin with deltamethrin at 10 μg/kg in the almond matrix. The recognised reason of low recoveries and consistent results (low RSD values) permits recoveries lower than 70% to be accepted [16]. Accordingly, pesticide results in the 60–120% range may be accepted. Recovery values and RSDs are shown in Table 2.

In the avocado samples, 13 pesticides were not detected at 10 μg/kg. The rest of the analytes were extracted with recoveries equal to or higher than 60%.

In almonds, as with avocado, two spiking levels (10 and 50 μg/kg) were selected for recovery studies. However, this matrix turned out to be more problematic than avocado. In general it can be said that, in almonds, the vast majority of pesticides had lower recoveries than in avocado. Because of the higher fat content, pesticide extraction was more difficult. This statement refers especially to the most lipophilic pesticides such as merphos, fluvalinate-tau, DDD, DDE, DDT and ethofenprox. At the 50 μg/kg level, 119 pesticides had recoveries in the 60–120% range; 46 had recoveries below 60% and the recovery of 1 analyte (spiroxamine) was above 120%. At the 10 μg/kg level, 107 pesticides had acceptable recoveries, 58 were not detected or were extracted with low recoveries and, likewise at this level, spiroxamine had a recovery higher than 120%.

To check if the lower pesticide recoveries were indeed a result of the fat content and not a consequence of adsorption onto Z-Sep, the following experiment was carried out: 5 mL of a 100 μg/L acetonitrile solution of all the investigated pesticides were placed in contact with 175 mg of Z-Sep. Then 50 μL was evaporated and subsequently redissolved in ethyl acetate and analysed by GC QqQ. To eliminate the influence of the evaporation step, standard solutions used for quantitation were also prepared in acetonitrile, evaporated and redissolved in ethyl acetate. Consequently, no correlation was noticed between low recoveries in this experiment and low recoveries from spiked samples. For example, merphos ($pK_{ow}$ 7.67) had recoveries as follows: from solvent 92%, from avocado 71%, from almonds 0%. This pattern (high recoveries from solvent, lower from avocado and the lowest from almonds) repeated in numerous pesticides with high $pK_{ow}$ and therefore

**Table 2**
Recoveries and relative standard deviation at 10 and 50 μg/kg ($n=5$) in the two matrices.

| No. | Compound | Avocado | | | | Almond | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 10 μg/Kg | | 50 μg/Kg | | 10 μg/Kg | | 50 μg/Kg | |
| | | Rec, % | RSD, % | Rec, % | RSD, % | Rec, % | RSD, % | Rec, % | RSD, % |
| 1 | 2,4-DDE | 66 | 6 | 67 | 4 | 34 | 2 | 32 | 6 |
| 2 | 2,4-DDT | 62 | 3 | 61 | 7 | 34 | 7 | 31 | 9 |
| 3 | 4,4-DDD | 71 | 5 | 67 | 3 | 42 | 2 | 40 | 2 |
| 4 | 4,4-DDE | 65 | 3 | 60 | 4 | – | – | 28 | 5 |
| 5 | 4,4-DDT | 71 | 5 | 67 | 3 | 42 | 2 | 40 | 2 |
| 6 | Acrinathrin | 93 | 8 | 91 | 3 | 63 | 14 | 62 | 6 |
| 7 | Alachlor | 86 | 2 | 84 | 2 | 77 | 10 | 75 | 2 |
| 8 | Ametryn | 93 | 3 | 91 | 1 | 72 | 5 | 75 | 2 |
| 9 | Azoxystrobin | 89 | 7 | 93 | 2 | – | – | 88 | 4 |
| 10 | Benalaxyl | 90 | 4 | 83 | 7 | – | – | 86 | 2 |
| 11 | Bifenox | 92 | 8 | 83 | 6 | 78 | 5 | 72 | 3 |
| 12 | Bifenthrin | 72 | 5 | 72 | 3 | 46 | 2 | 41 | 6 |
| 13 | Bixafen | 92 | 7 | 90 | 2 | 99 | 4 | 86 | 3 |
| 14 | Boscalid | 89 | 4 | 93 | 3 | 92 | 3 | 79 | 3 |
| 15 | Bromopropylate | 73 | 9 | 76 | 4 | 59 | 3 | 54 | 3 |
| 16 | Bupirimate | 91 | 10 | 84 | 3 | 81 | 9 | 79 | 6 |
| 17 | Buprofezin | 83 | 18 | 80 | 12 | – | – | 57 | 4 |
| 18 | Butralin | 81 | 16 | 78 | 6 | 53 | 26 | 45 | 7 |
| 19 | Butylate | 75 | 8 | 72 | 3 | 60 | 4 | 42 | 20 |
| 20 | Cadusafos | 87 | 3 | 84 | 3 | 72 | 4 | 67 | 3 |
| 21 | Carbophenothion | 83 | 15 | 86 | 4 | 55 | 8 | 51 | 2 |
| 22 | Carbosulfan | – | – | 107 | 6 | – | – | 71 | 6 |
| 23 | Chinomethionat | 70 | 5 | 96 | 3 | 30 | 2 | 37 | 7 |
| 24 | Chlorbromuron | 112 | 5 | 107 | 3 | 84 | 18 | 79 | 2 |
| 25 | Chlorfenapyr | – | – | 81 | 10 | – | – | 55 | 18 |
| 26 | Chlorfenvinphos | 90 | 7 | 88 | 1 | 73 | 4 | 74 | 4 |
| 27 | Chlorobenzilate | 85 | 5 | 82 | 6 | 72 | 1 | 70 | 2 |
| 28 | Chlorpropham | 86 | 6 | 82 | 4 | 67 | 3 | 79 | 8 |
| 29 | Chlorpyrifos | 79 | 6 | 77 | 6 | 54 | 4 | 60 | 5 |
| 30 | Chlorpyrifos-methyl | 95 | 6 | 77 | 3 | 69 | 4 | 60 | 2 |
| 31 | Chlorothalonil | 85 | 17 | 85 | 5 | – | – | 25 | 27 |
| 32 | Chlozolinate | 82 | 9 | 86 | 4 | – | – | 74 | 3 |
| 33 | Cyfluthrin | 90 | 3 | 92 | 4 | 64 | 3 | 62 | 4 |
| 34 | Cypermethrin | – | – | 81 | 4 | 62 | 3 | 50 | 2 |
| 35 | Chlorthal-dimethyl | 79 | 3 | 83 | 7 | 71 | 10 | 65 | 5 |
| 36 | Cyproconazole | 85 | 10 | 87 | 4 | 73 | 2 | 70 | 4 |
| 37 | Cyprodinil | 87 | 1 | 76 | 2 | – | – | 52 | 3 |
| 38 | Deltamethrin | 82 | 10 | 82 | 2 | 75 | 22 | 55 | 4 |
| 39 | Diazinon | 82 | 6 | 84 | 4 | – | – | 67 | 1 |
| 40 | Dichlorvos | 81 | 13 | 83 | 3 | 73 | 7 | 77 | 4 |
| 41 | Diclobutrazol | 88 | 8 | 87 | 2 | 80 | 1 | 77 | 6 |
| 42 | Dicloran | 87 | 10 | 81 | 6 | – | – | 76 | 8 |
| 43 | Dimethenamid | 93 | 6 | 86 | 4 | 79 | 3 | 78 | 2 |
| 44 | Diphenylamine | 97 | 7 | 76 | 5 | 56 | 2 | 57 | 3 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 45 | Endosulfan alpha | – | – | 65 | 8 | – | – | 39 | 3 |
| 46 | Endosulfan beta | – | – | 83 | 4 | – | – | 55 | 3 |
| 47 | Endosulfan sulphate | – | – | 90 | 6 | – | – | 75 | 5 |
| 48 | EPN | 84 | 6 | 90 | 3 | 64 | 8 | 70 | 4 |
| 49 | Epoxiconazole | 89 | 5 | 91 | 3 | 84 | 1 | 82 | 6 |
| 50 | Ethion | 83 | 4 | 83 | 6 | 67 | 4 | 62 | 5 |
| 51 | Ethofenprox | 85 | 4 | 74 | 3 | 50 | 4 | 44 | 6 |
| 52 | Ethofumesate | 91 | 9 | 97 | 4 | 86 | 3 | 82 | 6 |
| 53 | Ethoprophos | 88 | 6 | 85 | 2 | 75 | 2 | 76 | 3 |
| 54 | Etrimfos | 93 | 5 | 85 | 4 | 66 | 3 | 67 | 5 |
| 55 | Fenvalerate/Esfenvalerate RR/SS | 77 | 5 | 81 | 4 | 71 | 5 | 52 | 7 |
| 56 | Fenvalerate/Esfenvalerate RS/SR | 76 | 6 | 83 | 5 | 66 | 6 | 53 | 6 |
| 57 | Fenamidone | 86 | 11 | 84 | 5 | 96 | 5 | 84 | 3 |
| 58 | Fenarimol | 94 | 6 | 101 | 2 | 83 | 5 | 74 | 1 |
| 59 | Fenazaquin | 70 | 8 | 72 | 3 | 44 | 5 | 44 | 3 |
| 60 | Fenbuconazole | 91 | 2 | 91 | 3 | 90 | 5 | 86 | 2 |
| 61 | Fenchlorphos | 77 | 7 | 74 | 4 | 54 | 6 | 48 | 2 |
| 62 | Fenhexamid | 91 | 5 | 88 | 2 | 70 | 6 | 79 | 2 |
| 63 | Fenitrothion | 86 | 14 | 85 | 3 | 81 | 12 | 66 | 1 |
| 64 | Fenpropathrin | 101 | 18 | 84 | 5 | 61 | 11 | 61 | 6 |
| 65 | Fenpropimorph | 109 | 3 | 104 | 5 | 64 | 12 | 61 | 5 |
| 66 | Fenthion | 78 | 7 | 82 | 5 | 73 | 4 | 79 | 5 |
| 67 | Flamprop-isopropyl | 90 | 1 | 90 | 3 | 89 | 7 | 83 | 2 |
| 68 | Flamprop-methyl | 94 | 5 | 92 | 6 | 84 | 6 | 79 | 4 |
| 69 | Flonicamid | 86 | 2 | 95 | 2 | 84 | 4 | 86 | 11 |
| 70 | Fluacrypyrim | – | – | 89 | 6 | 95 | 5 | 81 | 2 |
| 71 | Fluazifop-P-butyl | 83 | 8 | 90 | 4 | 91 | 7 | 72 | 6 |
| 72 | Flucythrinate | 79 | 8 | 92 | 5 | 86 | 4 | 69 | 6 |
| 73 | Fludioxonil | 86 | 9 | 91 | 7 | – | – | 83 | 6 |
| 74 | Fluquinconazole | 90 | 4 | 85 | 1 | 78 | 3 | 76 | 2 |
| 75 | Flusilazole | 88 | 7 | 87 | 3 | 78 | 5 | 83 | 3 |
| 76 | Flutolanil | 96 | 5 | 90 | 3 | 83 | 1 | 80 | 1 |
| 77 | Flutriafol | – | – | 94 | 4 | – | – | 91 | 5 |
| 78 | Fluvalinate-tau | 86 | 2 | 82 | 3 | 59 | 11 | 54 | 3 |
| 79 | Fonofos | 88 | 6 | 85 | 1 | 70 | 8 | 62 | 7 |
| 80 | Formothion | 82 | 13 | 86 | 6 | – | – | 51 | 12 |
| 81 | Fosthiazate | 91 | 4 | 86 | 2 | 83 | 15 | 88 | 9 |
| 82 | Heptachlor | 60 | 2 | 61 | 5 | – | – | 36 | 7 |
| 83 | Heptenophos | 89 | 7 | 87 | 1 | 83 | 12 | 87 | 4 |
| 84 | Hexaconazole | 83 | 4 | 86 | 3 | – | – | 88 | 10 |
| 85 | Indoxacarb | 89 | 2 | 91 | 3 | 80 | 9 | 85 | 4 |
| 86 | Iprodione | 93 | 2 | 88 | 6 | 76 | 5 | 80 | 5 |
| 87 | Iprovalicarb | 89 | 12 | 80 | 7 | 77 | 15 | 94 | 1 |
| 88 | Isazofos | 85 | 3 | 87 | 5 | 99 | 3 | 80 | 4 |
| 89 | Isocarbophos | 102 | 8 | 109 | 2 | 83 | 11 | 84 | 5 |
| 90 | Isofenphos-ethyl | 86 | 5 | 98 | 4 | 74 | 2 | 78 | 3 |
| 91 | Isofenphos-methyl | 93 | 6 | 94 | 3 | 79 | 2 | 75 | 3 |
| 92 | Kresoxim-methyl | 94 | 9 | 91 | 3 | 79 | 5 | 85 | 4 |
| 93 | Lambda-cyhalothrin | 84 | 12 | 87 | 2 | 62 | 4 | 61 | 8 |
| 94 | Lindane | 76 | 11 | 77 | 5 | 60 | 6 | 53 | 4 |
| 95 | Malathion | 83 | 7 | 86 | 4 | 89 | 8 | 88 | 3 |
| 96 | Mecarbam | – | – | 86 | 5 | – | – | 119 | 3 |
| 97 | Merphos | 73 | 5 | 71 | 1 | – | – | – | – |
| 98 | Metalaxyl | 84 | 13 | 87 | 2 | 91 | 9 | 95 | 5 |
| 99 | Methamidophos | 78 | 7 | 77 | 5 | – | – | 74 | 9 |
| 100 | Metazachlor | 83 | 12 | 87 | 3 | 95 | 9 | 82 | 3 |
| 101 | Metconazole | 93 | 9 | 89 | 3 | 81 | 8 | 74 | 5 |
| 102 | Methidathion | 85 | 7 | 85 | 2 | 75 | 7 | 82 | 6 |
| 103 | Methiocarb | 111 | 17 | 115 | 5 | 78 | 13 | 74 | 9 |
| 104 | Metolachlor | 97 | 2 | 91 | 3 | 77 | 3 | 75 | 1 |

**Table 2** (*continued*)

| No. | Compound | Avocado | | | | Almond | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 10 μg/Kg | | 50 μg/Kg | | 10 μg/Kg | | 50 μg/Kg | |
| | | Rec, % | RSD, % | Rec, % | RSD, % | Rec, % | RSD, % | Rec, % | RSD, % |
| 105 | Methoxychlor | 82 | 10 | 81 | 7 | 73 | 6 | 61 | 1 |
| 106 | Mevinphos | 74 | 14 | 77 | 2 | 57 | 12 | 67 | 12 |
| 107 | Myclobutanyl | 100 | 4 | 90 | 3 | 81 | 8 | 81 | 2 |
| 108 | Napropamide | 79 | 7 | 96 | 4 | 86 | 1 | 84 | 6 |
| 109 | Nuarimol | 84 | 4 | 84 | 3 | 84 | 9 | 81 | 1 |
| 110 | o-Phenylphenol | 79 | 7 | 83 | 4 | 82 | 2 | 77 | 4 |
| 111 | Ofurace | 91 | 9 | 96 | 3 | 88 | 4 | 80 | 6 |
| 112 | Oxadixyl | 102 | 9 | 91 | 5 | 88 | 8 | 90 | 3 |
| 113 | Paclobutrazol | 91 | 6 | 92 | 2 | – | – | 83 | 8 |
| 114 | Parathion-ethyl | 88 | 5 | 87 | 3 | – | – | 72 | 1 |
| 115 | Parathion-methyl | 97 | 6 | 90 | 4 | – | – | 77 | 9 |
| 116 | Pebulate | 71 | 5 | 76 | 3 | 67 | 5 | 48 | 13 |
| 117 | Penconazole | 85 | 10 | 82 | 3 | 73 | 2 | 73 | 4 |
| 118 | Pendimethalin | 84 | 12 | 85 | 4 | – | – | 48 | 2 |
| 119 | Permethrin | 80 | 5 | 73 | 3 | 60 | 7 | 47 | 4 |
| 120 | Phenothrin | 78 | 8 | 73 | 5 | – | – | 53 | 9 |
| 121 | Phenthoate | 87 | 2 | 85 | 2 | 73 | 6 | 75 | 2 |
| 122 | Phorate | 73 | 7 | 85 | 4 | – | – | 72 | 8 |
| 123 | Phosmet | 82 | 10 | 86 | 5 | 78 | 8 | 82 | 3 |
| 124 | Picolinafen | 82 | 4 | 83 | 1 | 71 | 4 | 65 | 6 |
| 125 | Picoxystrobin | – | – | 91 | 5 | 103 | 8 | 78 | 10 |
| 126 | Pyrimethanil | 71 | 14 | 75 | 5 | – | – | 61 | 3 |
| 127 | Pirimicarb | 83 | 7 | 81 | 2 | 78 | 3 | 78 | 5 |
| 128 | Pirimiphos-methyl | 83 | 9 | 85 | 3 | 70 | 9 | 66 | 2 |
| 129 | Procymidone | 86 | 8 | 79 | 4 | 77 | 2 | 77 | 1 |
| 130 | Profenofos | 84 | 5 | 78 | 7 | – | – | 55 | 10 |
| 131 | Prometon | 89 | 5 | 85 | 3 | 91 | 3 | 77 | 2 |
| 132 | Prometryn | 90 | 6 | 93 | 2 | – | – | 68 | 2 |
| 133 | Propaphos | 105 | 9 | 108 | 2 | 117 | 3 | 97 | 3 |
| 134 | Propazine | 79 | 3 | 90 | 5 | 80 | 6 | 76 | 4 |
| 135 | Propiconazole | 79 | 6 | 85 | 2 | 77 | 6 | 76 | 5 |
| 136 | Propyzamide | 94 | 7 | 88 | 1 | 71 | 1 | 82 | 5 |
| 137 | Prosulfocarb | 72 | 8 | 77 | 9 | 69 | 8 | 62 | 4 |
| 138 | Prothiofos | 75 | 4 | 72 | 3 | 38 | 14 | 37 | 6 |
| 139 | Pyrazophos | 92 | 7 | 87 | 4 | 81 | 10 | 76 | 2 |
| 140 | Pyridaben | 73 | 10 | 77 | 4 | 52 | 2 | 46 | 5 |
| 141 | Pyriproxyfen | 90 | 6 | 85 | 6 | 58 | 5 | 53 | 1 |
| 142 | Quinalphos | – | – | 87 | 2 | – | – | 63 | 2 |
| 143 | Quinoxyfen | 66 | 9 | 66 | 4 | 45 | 3 | 40 | 5 |
| 144 | Quintozene | 66 | 18 | 63 | 5 | – | – | 33 | 2 |
| 145 | Spirodiclofen | 78 | 11 | 75 | 6 | 64 | 8 | 54 | 2 |
| 146 | Spiroxamine I | – | – | 105 | 5 | 154 | 12 | 159 | 5 |
| 147 | Spiroxamine II | – | – | 78 | 3 | 89 | 9 | 116 | 3 |
| 148 | Sulprofos | 82 | 2 | 84 | 3 | 75 | 6 | 63 | 4 |
| 149 | Tebuconazole | 95 | 4 | 88 | 4 | 76 | 5 | 85 | 1 |
| 150 | Tebufenpyrad | 79 | 9 | 78 | 3 | 66 | 6 | 62 | 5 |
| 151 | Tecnazene | 61 | 5 | 71 | 7 | 49 | 6 | 41 | 6 |
| 152 | Tefluthrin | 78 | 7 | 76 | 4 | 57 | 5 | 51 | 1 |
| 153 | Terbufos | 93 | 8 | 89 | 5 | 72 | 8 | 71 | 7 |
| 154 | Terbumeton | 84 | 13 | 87 | 7 | 91 | 3 | 75 | 2 |
| 155 | Terbutryn | 94 | 7 | 89 | 3 | 77 | 10 | 67 | 2 |
| 156 | Tetrachlorvinphos | 86 | 2 | 84 | 7 | 75 | 16 | 81 | 6 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 157 | Tetraconazole | 82 | 1 | 98 | 1 | 78 | 16 | 80 | 4 | | | | | | |
| 158 | Tetradifon | 73 | 9 | 76 | 2 | 43 | 7 | 45 | 4 | | | | | | |
| 159 | Tetramethrin | 74 | 11 | 80 | 3 | 82 | 3 | 70 | 3 | | | | | | |
| 160 | Tolclofos-methyl | 92 | 1 | 90 | 4 | 67 | 5 | 62 | 4 | | | | | | |
| 161 | Tolylfluanid | 84 | 17 | 100 | 7 | – | – | 16 | 6 | | | | | | |
| 162 | Triadimefon | 91 | 10 | 86 | 6 | 86 | 1 | 79 | 6 | | | | | | |
| 163 | Triazophos | 85 | 4 | 88 | 6 | 75 | 1 | 87 | 2 | | | | | | |
| 164 | Trifloxystrobin | 86 | 1 | 91 | 3 | 81 | 10 | 80 | 2 | | | | | | |
| 165 | Trifluralin | 80 | 6 | 77 | 1 | 57 | 6 | 56 | 5 | | | | | | |
| 166 | Vinclozolin | 85 | 11 | 87 | 5 | 65 | 5 | 69 | 5 | | | | | | |

confirms that recoveries of lipophilic pesticides depend on the amount of fat in the matrix. Some of the more polar pesticides (e.g. dichlorvos, methamidophos, mevinphos, spiroxamine) had very low recoveries from pure solvent, but recoveries from the matrices were satisfactory.

### 3.3. Limits of quantitation

DG Sanco guidelines define the limit of quantitation as the lowest validated spike level meeting the method performance acceptability criteria [16]. Only two spiking levels were investigated (10 and 50 μg/kg); thus only those two LOQ values were possible. In avocado, 153 pesticides had LOQs of 10 μg/kg and 13 had 50 μg/kg. As was mentioned above, the extraction of pesticides from almonds was more difficult. Ninety eight pesticides had LOQs of 10 μg/kg and 21 pesticides had LOQs of 50 μg/kg. The remaining 47 pesticides were not detected or their recoveries were below 60%. Detailed limits of quantitation values are shown in Table 3.

### 3.4. Linearity

Linearity was checked in the range from 1 μg/L up to 500 μg/L. The linear ranges of all compounds are presented in Table 3. Detector response was considered linear when the coefficient of determination ($r^2$) was equal to or higher than 0.99. The lowest calibrated level always had a qualifying transition with $S/N \geq 6$. For the majority of pesticides in avocado extracts, the detector response was linear from at least 10 μg/L (in some cases even from 1 or 2 μg/L) up to 500 μg/L. Only a few pesticides showed lower sensitivity. The final almond extract had a 0.5 g/mL concentration and to achieve satisfactory sensitivity, samples had to be concentrated up to 1 g/mL. Even after this, for 16 analytes, the linear range began at 50 μg/L. Matrix effects were probably responsible for the difference in sensitivity between these two matrices (see Section 3.6).

### 3.5. Inter- and intra-day precision

In Supporting information (Table S1), precision values are presented for the chromatographic method expressed as intra-day ($n=5$) and inter-day (over 5 days) precision. The criterion of $RSD \leq 20\%$ recommended by DG-Sanco guidelines [16] was fulfilled by almost all analytes; only spiroxamine in almonds exceeded 20%.

### 3.6. Matrix effects

Signal suppression or enhancement phenomena of the analyte injected in matrix, compared to when this analyte is injected in pure solvent, are known as matrix effects. Enhancement appears because matrix components block active sites (silanols, metal ions etc.) present in the column or inlet. When active sites are blocked, more analyte molecules can reach the detector. Signal suppression, on the other hand, can be observed when non-volatile compounds accumulated in the GC system create new active sites. A matrix effect higher than 20% must be eliminated or compensated for e.g. by application of analyte protectants or by using matrix-matched calibration. Matrix effects vary according to the commodity and the analyte combination and they change over time [30–32]. In this work, matrix effects were calculated from calibration curve slopes in solvent and in matrix according to the equation

$$ME(\%) = \left( \left( \frac{Slope\ of\ calibration\ curve\ in\ matrix}{Slope\ of\ calibration\ curve\ in\ solvent} \right) - 1 \right) \times 100$$

**Table 3**
Limits of quantification, concentration range and matrix effects for the selected matrices studied.

| No. | Compound | LOQ (μg/kg) | | $R^2$ | | Instrumental concentration range (μg/L) | | ME(%)=((slope matrix/slope solvent)−1)×100 | |
|---|---|---|---|---|---|---|---|---|---|
| | | Avocado | Almond | Avocado | Almond | Avocado | Almond | Avocado | Almond1 |
| 1 | 2,4-DDE | 10 | – | 0.997 | 1.000 | 10–500 | 10–500 | 25 | 3 |
| 2 | 2,4-DDT | 10 | – | 1.000 | 0.999 | 10–500 | 10–500 | 76 | 27 |
| 3 | 4,4-DDD | 10 | – | 0.996 | 0.999 | 1–500 | 10–500 | 58 | 36 |
| 4 | 4,4-DDE | 10 | – | 0.996 | 1.000 | 10–500 | 10–500 | 36 | 8 |
| 5 | 4,4-DDT | 10 | – | 0.996 | 0.999 | 1–500 | 10–500 | 57 | 36 |
| 6 | Acrinathrin | 10 | 10 | 0.994 | 0.992 | 10–500 | 10–500 | 300< | 139 |
| 7 | Alachlor | 10 | 10 | 0.996 | 0.996 | 10–500 | 10–500 | 49 | 31 |
| 8 | Ametryn | 10 | 10 | 0.996 | 0.998 | 10–500 | 10–500 | 76 | 51 |
| 9 | Azoxystrobin | 10 | 50 | 0.994 | 0.998 | 10–500 | 10–500 | 300< | 169 |
| 10 | Benalaxyl | 10 | 50 | 0.995 | 0.999 | 20–500 | 10–500 | 61 | 38 |
| 11 | Bifenox | 10 | 10 | 0.990 | 0.991 | 10–500 | 10–500 | 35 | 37 |
| 12 | Bifenthrin | 10 | - | 0.996 | 0.998 | 10–500 | 10–500 | 64 | 35 |
| 13 | Bixafen | 10 | 10 | 0.996 | 0.999 | 1–500 | 10–500 | 300< | 99 |
| 14 | Boscalid | 10 | 10 | 0.996 | 0.998 | 1–500 | 10–500 | 227 | 69 |
| 15 | Bromopropylate | 10 | – | 0.997 | 0.999 | 2–500 | 10–500 | 164 | 118 |
| 16 | Bupirimate | 10 | 10 | 0.998 | 0.997 | 2–500 | 10–500 | 46 | 44 |
| 17 | Buprofezin | 10 | – | 0.991 | 1.000 | 10–500 | 50–500 | 41 | 28 |
| 18 | Butralin | 10 | – | 0.995 | 0.992 | 10–500 | 10–500 | 121 | 59 |
| 19 | Butylate | 10 | – | 0.997 | 0.999 | 1–500 | 10–500 | 39 | 25 |
| 20 | Cadusafos | 10 | 10 | 0.995 | 0.999 | 10–500 | 10–500 | 91 | 58 |
| 21 | Carbophenothion | 10 | – | 0.994 | 0.999 | 10–500 | 10–500 | 172 | 67 |
| 22 | Carbosulfan | 50 | 50 | 0.999 | 0.990 | 50–500 | 50–500 | −65 | −57 |
| 23 | Chinomethionat | 10 | – | 0.994 | 0.999 | 2–500 | 10–500 | 71 | 42 |
| 24 | Chlorbromuron | 10 | 10 | 0.997 | 0.999 | 2–500 | 10–500 | 19 | −13 |
| 25 | Chlorfenapyr | 50 | – | 0.996 | 0.996 | 50–500 | 50–500 | 61 | 25 |
| 26 | Chlorfenvinphos | 10 | 10 | 0.997 | 0.998 | 10–500 | 10–500 | 156 | 42 |
| 27 | Chlorobenzilate | 10 | 10 | 0.997 | 0.999 | 10–500 | 10–500 | 69 | 48 |
| 28 | Chlorpropham | 10 | 10 | 0.996 | 0.999 | 1–500 | 10–500 | 154 | 41 |
| 29 | Chlorpyrifos | 10 | 50 | 0.996 | 0.999 | 2–500 | 10–500 | 42 | 29 |
| 30 | Chlorpyrifos-methyl | 10 | 10 | 0.996 | 0.996 | 2–500 | 10–500 | 104 | 55 |
| 31 | Chlorothalonil | 10 | – | 0.993 | 0.996 | 2–500 | 10–500 | 223 | 77 |
| 32 | Chlozolinate | 10 | 50 | 0.997 | 0.998 | 10–500 | 10–500 | 41 | 25 |
| 33 | Cyfluthrin | 10 | 10 | 0.995 | 0.996 | 10–500 | 10–500 | 297 | 76 |
| 34 | Cypermethrin | 50 | – | 0.997 | 0.997 | 20–500 | 10–500 | 205 | 63 |
| 35 | Chlorthal-dimethyl | 10 | 10 | 0.996 | 0.999 | 10–500 | 10–500 | 14 | 34 |
| 36 | Cyproconazole | 10 | 10 | 0.996 | 0.993 | 10–500 | 10–500 | 63 | 84 |
| 37 | Cyprodinil | 10 | – | 0.996 | 0.999 | 1–500 | 10–500 | 60 | 22 |
| 38 | Deltamethrin | 10 | – | 0.998 | 0.998 | 2–500 | 10–500 | 300< | 123 |
| 39 | Diazinon | 10 | 50 | 0.996 | 0.999 | 10–500 | 50–500 | – | – |
| 40 | Dichlorvos | 10 | 10 | 0.997 | 0.999 | 1–500 | 10–500 | 62 | 65 |
| 41 | Diclobutrazol | 10 | 10 | 0.996 | 0.999 | 10–500 | 10–500 | 198 | 148 |
| 42 | Dicloran | 10 | 50 | 0.992 | 0.993 | 2–500 | 10–500 | 117 | 30 |
| 43 | Dimethenamid | 10 | 10 | 0.996 | 0.998 | 2–500 | 10–500 | 73 | 28 |
| 44 | Diphenylamine | 10 | – | 0.996 | 0.999 | 10–500 | 10–500 | 39 | 14 |
| 45 | Endosulfan alpha | 50 | – | 0.994 | 0.999 | 20–500 | 50–500 | 23 | 11 |
| 46 | Endosulfan beta | 50 | – | 0.997 | 0.998 | 20–500 | 50–500 | 24 | 4 |
| 47 | Endosulfan sulphate | 50 | 50 | 0.995 | 1.000 | 20–500 | 50–500 | – | – |
| 48 | EPN | 10 | 10 | 0.993 | 0.992 | 10–500 | 10–500 | 253 | 70 |
| 49 | Epoxiconazole | 10 | 10 | 0.997 | 0.997 | 2–500 | 10–500 | 176 | 79 |
| 50 | Ethion | 10 | 10 | 0.995 | 0.998 | 2–500 | 10–500 | 132 | 62 |
| 51 | Ethofenprox | 10 | – | 0.996 | 0.999 | 10–500 | 10–500 | 151 | 44 |
| 52 | Ethofumesate | 10 | 10 | 0.998 | 0.998 | 10–500 | 10–500 | 40 | 50 |
| 53 | Ethoprophos | 10 | 10 | 0.995 | 0.998 | 1–500 | 10–500 | 166 | 70 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 54 | Etrimfos | 10 | 10 | 0.995 | 0.998 | 10–500 | 10–500 | 79 | 52 |
| 55 | Fenvalerate/Esfenvalerate RR/SS | 10 | – | 0.993 | 0.994 | 1–500 | 10–500 | 300 < | 84 |
| 56 | Fenvalerate/Esfenvalerate RS/SR | 10 | – | 0.994 | 0.994 | 1–500 | 10–500 | 300 < | 93 |
| 57 | Fenamidone | 10 | 10 | 0.997 | 0.999 | 2–500 | 10–500 | 74 | 26 |
| 58 | Fenarimol | 10 | 10 | 0.997 | 0.999 | 10–500 | 10–500 | 98 | 54 |
| 59 | Fenazaquin | 10 | – | 0.997 | 0.999 | 10–500 | 10–500 | 80 | 37 |
| 60 | Fenbuconazole | 10 | 10 | 0.995 | 0.999 | 10–500 | 10–500 | 262 | 58 |
| 61 | Fenchlorphos | 10 | – | 0.995 | 0.999 | 2–500 | 10–500 | 77 | 61 |
| 62 | Fenhexamid | 10 | 10 | 0.996 | 1.000 | 10–500 | 10–500 | 300 < | 248 |
| 63 | Fenitrothion | 10 | 10 | 0.992 | 0.988 | 10–500 | 10–500 | 243 | 131 |
| 64 | Fenpropathrin | 10 | 10 | 0.996 | 0.999 | 10–500 | 10–500 | 63 | 46 |
| 65 | Fenpropimorph | 10 | 10 | 0.996 | 0.998 | 2–500 | 10–500 | 56 | 53 |
| 66 | Fenthion | 10 | 10 | 0.995 | 0.998 | 10–500 | 10–500 | 203 | 300 < |
| 67 | Flamprop-isopropyl | 10 | 10 | 0.997 | 0.999 | 10–500 | 10–500 | 55 | 43 |
| 68 | Flamprop-methyl | 10 | 10 | 0.997 | 1.000 | 10–500 | 10–500 | 33 | 24 |
| 69 | Flonicamid | 10 | 10 | 0.997 | 1.000 | 2–500 | 10–500 | 185 | 3 |
| 70 | Fluacrypyrim | 50 | 10 | 0.996 | 0.999 | 20–500 | 10–500 | 58 | 35 |
| 71 | Fluazifop-P-butyl | 10 | 10 | 0.996 | 0.999 | 10–500 | 10–500 | 97 | 52 |
| 72 | Flucythrinate | 10 | 10 | 0.994 | 0.992 | 10–500 | 10–500 | 300 < | 145 |
| 73 | Fludioxonil | 10 | 50 | 0.997 | 0.999 | 10–500 | 50–500 | 100 | 53 |
| 74 | Fluquinconazole | 10 | 10 | 0.996 | 0.998 | 1–500 | 10–500 | 108 | 54 |
| 75 | Flusilazole | 10 | 10 | 0.995 | 0.999 | 10–500 | 10–500 | 48 | 37 |
| 76 | Flutolanil | 10 | 10 | 0.993 | 0.999 | 10–500 | 10–500 | 98 | 61 |
| 77 | Flutriafol | 50 | 50 | 0.995 | 0.999 | 20–500 | 10–500 | 106 | 79 |
| 78 | Fluvalinate-tau | 10 | – | 0.990 | 0.990 | 2–500 | 10–500 | 300 < | 149 |
| 79 | Fonofos | 10 | 10 | 0.996 | 0.998 | 10–500 | 10–500 | 77 | 77 |
| 80 | Formothion | 10 | – | 0.994 | 0.994 | 10–500 | 50–500 | – | – |
| 81 | Fosthiazate | 10 | 10 | 0.994 | 0.994 | 10–500 | 10–500 | – | – |
| 82 | Heptachlor | 10 | – | 0.993 | 0.997 | 10–500 | 50–500 | 62 | 23 |
| 83 | Heptenophos | 10 | 10 | 0.996 | 1.000 | 10–500 | 10–500 | 300 < | 97 |
| 84 | Hexaconazole | 10 | 50 | 0.997 | 0.998 | 10–500 | 10–500 | 74 | 55 |
| 85 | Indoxacarb | 10 | 10 | 0.997 | 1.000 | 2–500 | 10–500 | 115 | 43 |
| 86 | Iprodione | 10 | 10 | 0.996 | 0.998 | 2–500 | 10–500 | 300 < | 135 |
| 87 | Iprovalicarb | 10 | 10 | 0.997 | 0.999 | 10–500 | 10–500 | 242 | 115 |
| 88 | Isazofos | 10 | 10 | 0.995 | 0.997 | 10–500 | 10–500 | 65 | 45 |
| 89 | Isocarbophos | 10 | 10 | 0.997 | 0.995 | 10–500 | 10–500 | 178 | 106 |
| 90 | Isofenphos-ethyl | 10 | 10 | 0.997 | 0.997 | 1–500 | 10–500 | 68 | 44 |
| 91 | Isofenphos-methyl | 10 | 10 | 0.995 | 0.998 | 2–500 | 10–500 | 75 | 43 |
| 92 | Kresoxim-methyl | 10 | 10 | 0.996 | 1.000 | 10–500 | 10–500 | 49 | 35 |
| 93 | Lambda-cyhalothrin | 10 | 10 | 0.996 | 0.996 | 10–500 | 10–500 | 220 | 71 |
| 94 | Lindane | 10 | – | 0.996 | 0.999 | 2–500 | 10–500 | 37 | 28 |
| 95 | Malathion | 10 | 10 | 0.994 | 0.998 | 2–500 | 10–500 | 131 | 66 |
| 96 | Mecarbam | 50 | 50 | 0.997 | 0.999 | 20–500 | 50–500 | 59 | 31 |
| 97 | Merphos | 10 | – | 0.997 | 0.999 | 10–500 | 10–500 | 65 | 45 |
| 98 | Metalaxyl | 10 | 10 | 0.996 | 0.999 | 10–500 | 10–500 | 37 | 30 |
| 99 | Methamidophos | 10 | 50 | 0.997 | 0.998 | 10–500 | 50–500 | 300 < | 44 |
| 100 | Metazachlor | 10 | 10 | 0.995 | 0.996 | 10–500 | 10–500 | 94 | 49 |
| 101 | Metconazole | 10 | 10 | 0.996 | 0.999 | 10–500 | 10–500 | 83 | 50 |
| 102 | Methidathion | 10 | 10 | 0.994 | 0.995 | 2–500 | 10–500 | 300 < | 66 |
| 103 | Methiocarb | 10 | 10 | 0.996 | 0.993 | 10–500 | 10–500 | 300 < | 159 |
| 104 | Metolachlor | 10 | 10 | 0.996 | 0.999 | 10–500 | 10–500 | 62 | 38 |
| 105 | Methoxychlor | 10 | 10 | 0.999 | 0.997 | 10–500 | 10–500 | 181 | 5 |
| 106 | Mevinphos | 10 | 50 | 0.995 | 0.999 | 1–500 | 10–500 | 300 < | 173 |
| 107 | Myclobutanyl | 10 | 10 | 0.997 | 0.998 | 2–500 | 10–500 | 58 | 30 |
| 108 | Napropamide | 10 | 10 | 0.996 | 1.000 | 10–500 | 10–500 | 76 | 67 |
| 109 | Nuarimol | 10 | 10 | 0.997 | 0.999 | 10–500 | 10–500 | 79 | 42 |
| 110 | o-Phenylphenol | 10 | 10 | 0.997 | 1.000 | 1–500 | 10–500 | 166 | 61 |
| 111 | Ofurace | 10 | 10 | 0.995 | 1.000 | 2–500 | 10–500 | 133 | 53 |
| 112 | Oxadixyl | 10 | 10 | 0.997 | 1.000 | 10–500 | 10–500 | 71 | 29 |
| 113 | Paclobutrazol | 10 | 50 | 0.996 | 0.998 | 10–500 | 50–500 | – | – |

**Table 3** (*continued*)

| No. | Compound | LOQ (μg/kg) | | $R^2$ | | Instrumental concentration range (μg/L) | | ME(%)=((slope matrix/slope solvent)−1)×100 | |
|---|---|---|---|---|---|---|---|---|---|
| | | Avocado | Almond | Avocado | Almond | Avocado | Almond | Avocado | Almond1 |
| 114 | Parathion-ethyl | 10 | 50 | 0.990 | 0.992 | 10–500 | 50–500 | – | – |
| 115 | Parathion-methyl | 10 | 50 | 0.998 | 0.990 | 10–500 | 10–500 | 300 < | 135 |
| 116 | Pebulate | 10 | – | 0.997 | 1.000 | 2–500 | 10–500 | 50 | 28 |
| 117 | Penconazole | 10 | 10 | 0.996 | 0.996 | 10–500 | 10–500 | 60 | 42 |
| 118 | Pendimethalin | 10 | – | 0.989 | 0.997 | 2–500 | 10–500 | 102 | 44 |
| 119 | Permethrin | 10 | – | 0.996 | 0.999 | 2–500 | 10–500 | 138 | 52 |
| 120 | Phenothrin | 10 | – | 0.995 | 0.999 | 10–500 | 10–500 | 149 | 43 |
| 121 | Phenthoate | 10 | 10 | 0.994 | 0.993 | 10–500 | 10–500 | 108 | 48 |
| 122 | Phorate | 10 | 50 | 0.994 | 0.997 | 10–500 | 50–500 | 300 < | 300 < |
| 123 | Phosmet | 10 | 10 | 0.993 | 0.996 | 2–500 | 10–500 | 300 < | 201 |
| 124 | Picolinafen | 10 | 10 | 0.997 | 1.000 | 2–500 | 10–500 | 94 | 43 |
| 125 | Picoxystrobin | 50 | 10 | 0.996 | 0.999 | 10–500 | 10–500 | 55 | 52 |
| 126 | Pyrimethanil | 10 | 50 | 0.996 | 0.997 | 10–500 | 10–500 | 83 | 50 |
| 127 | Pirimicarb | 10 | 10 | 0.995 | 0.997 | 2–500 | 10–500 | 57 | 34 |
| 128 | Pirimiphos-methyl | 10 | 10 | 0.997 | 0.998 | 10–500 | 10–500 | 51 | 41 |
| 129 | Procymidone | 10 | 10 | 0.997 | 0.999 | 10–500 | 10–500 | 29 | 22 |
| 130 | Profenofos | 10 | – | 0.996 | 1.000 | 10–500 | 50–500 | 164 | 85 |
| 131 | Prometon | 10 | 10 | 0.996 | 0.999 | 10–500 | 10–500 | 74 | 35 |
| 132 | Prometryn | 10 | 50 | 0.995 | 0.998 | 10–500 | 10–500 | 44 | 36 |
| 133 | Propaphos | 10 | 10 | 0.994 | 0.997 | 2–500 | 10–500 | 300 < | 300 < |
| 134 | Propazine | 10 | 10 | 0.997 | 0.998 | 2–500 | 10–500 | 66 | 51 |
| 135 | Propiconazole | 10 | 10 | 0.997 | 1.000 | 2–500 | 10–500 | 80 | 51 |
| 136 | Propyzamide | 10 | 10 | 0.995 | 0.999 | 2–500 | 10–500 | 72 | 39 |
| 137 | Prosulfocarb | 10 | 10 | 0.996 | 0.999 | 10–500 | 10–500 | 49 | 47 |
| 138 | Prothiofos | 10 | – | 0.997 | 0.999 | 10–500 | 10–500 | 61 | 50 |
| 139 | Pyrazophos | 10 | 10 | 0.995 | 0.996 | 10–500 | 10–500 | 300 < | 117 |
| 140 | Pyridaben | 10 | – | 0.996 | 0.997 | 10–500 | 10–500 | 170 | 61 |
| 141 | Pyriproxyfen | 10 | – | 0.997 | 0.999 | 10–500 | 10–500 | 128 | 44 |
| 142 | Quinalphos | 50 | 50 | 0.996 | 0.998 | 20–500 | 10–500 | 70 | 23 |
| 143 | Quinoxyfen | 10 | – | 0.997 | 0.999 | 1–500 | 10–500 | 52 | 38 |
| 144 | Quintozene | 10 | – | 0.993 | 0.993 | 10–500 | 20–500 | 87 | 49 |
| 145 | Spirodiclofen | 10 | – | 0.997 | 0.998 | 10–500 | 10–500 | 114 | 89 |
| 146 | Spiroxamine I | 50 | – | 0.996 | 0.998 | 10–500 | 10–500 | 120 | 68 |
| 147 | Spiroxamine II | 50 | 10 | 0.996 | 0.998 | 20–500 | 10–500 | 65 | 45 |
| 148 | Sulprofos | 10 | 10 | 0.996 | 0.998 | 10–500 | 10–500 | 258 | 300 < |
| 149 | Tebuconazole | 10 | 10 | 0.997 | 0.997 | 10–500 | 10–500 | 144 | 86 |
| 150 | Tebufenpyrad | 10 | 10 | 0.997 | 0.999 | 1–500 | 10–500 | 79 | 36 |
| 151 | Tecnazene | 10 | – | 0.995 | 0.998 | 2–500 | 10–500 | 121 | 53 |
| 152 | Tefluthrin | 10 | – | 0.996 | 0.997 | 1–500 | 10–500 | 35 | 21 |
| 153 | Terbufos | 10 | 10 | 0.995 | 0.997 | 2–500 | 10–500 | 300 < | 300 < |
| 154 | Terbumeton | 10 | 10 | 0.997 | 0.998 | 2–500 | 10–500 | 63 | 44 |
| 155 | Terbutryn | 10 | 10 | 0.997 | 0.999 | 10–500 | 10–500 | 69 | 43 |
| 156 | Tetrachlorvinphos | 10 | 10 | 0.995 | 0.994 | 10–500 | 10–500 | – | – |
| 157 | Tetraconazole | 10 | 10 | 0.994 | 0.999 | 10–500 | 10–500 | 48 | 13 |
| 158 | Tetradifon | 10 | – | 0.996 | 0.999 | 2–500 | 10–500 | 48 | 30 |
| 159 | Tetramethrin | 10 | 10 | 0.996 | 0.999 | 10–500 | 10–500 | 151 | 60 |
| 160 | Tolclofos-methyl | 10 | 10 | 0.996 | 0.998 | 10–500 | 10–500 | 65 | 33 |
| 161 | Tolylfluanid | 10 | – | 0.990 | 0.995 | 10–500 | 10–500 | 150 | 40 |
| 162 | Triadimefon | 10 | 10 | 0.997 | 0.999 | 2–500 | 10–500 | 57 | 43 |
| 163 | Triazophos | 10 | 10 | 0.996 | 0.998 | 2–500 | 10–500 | 300 < | 96 |
| 164 | Trifloxystrobin | 10 | 10 | 0.997 | 0.999 | 10–500 | 10–500 | 122 | 52 |
| 165 | Trifluralin | 10 | – | 0.990 | 0.990 | 2–500 | 10–500 | 101 | 41 |
| 166 | Vinclozolin | 10 | 10 | 0.994 | 0.998 | 10–500 | 10–500 | 40 | 39 |

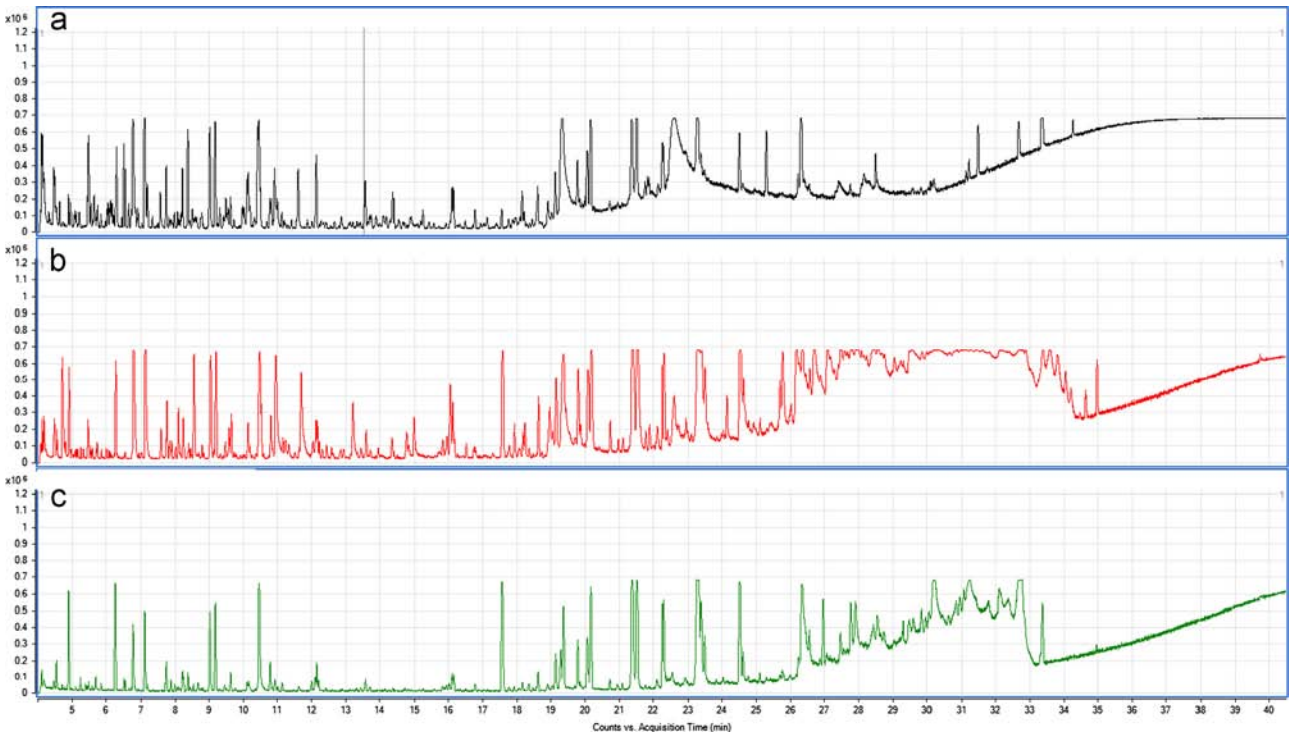


**Fig. 2.** GC-QToF full scan chromatograms of avocado extracts (a) ethyl acetate extraction; (b) QuEChERS with PSA/C18 clean-up; and (c) QuEChERS with Z-Sep.

Matrix effect values are presented in Table 3. In avocado extracts, only two pesticides exhibited matrix effects smaller than 20% (chlorthal-dimethyl and chlorbromuron). Medium matrix effects (20–50%) appeared in 25 analytes. The remaining pesticides showed strong matrix effects (suppression/enhancement > 50%). In the presented results, signal enhancement was more common, only one pesticide (carbosulfan) was suppressed.

Most of the pesticides with very strong matrix effects (e.g. phosmet, fenhexamid, azoxystrobin, fluvalinate-tau) eluted from the column with long retention times. In that part of chromatogram also eluted large amount of matrix compounds. These compounds were responsible for blocking active sites in the column and, in consequence, for pesticide signal enhancement. However not all the compounds were equally vulnerable to enhancement. For example bifenthrin and iprodion eluted with the same retention time but the first exhibited 63% enhancement whereas the latter 444%.

In almond extracts, the matrix effects were smaller. In the case of 9 pesticides, MEs were below 20%, 73 were in the 20–50% range and the signals of the rest were strongly affected (ME > 50%). Again, the majority of pesticides had higher sensitivity in matrix than in solvent. The signals of only two pesticides (carbosulfan and chlorbromuron) were suppressed.

Seven pesticides (diazinon, endosulphan sulphate, formothion, fosthiazate, paclobutrazol, parathion ethyl and tetrachlorvinphos) had low sensitivity in solvent and only two points with the highest concentrations (200 and 500 μg/L) were detected. Thus it was impossible to calculate the matrix effects according to the equation presented above.

To compare the amount of coextracted matrix compounds, blank avocado extracts were injected into GC-QToF working in full-scan mode. The chromatograms obtained are depicted in Fig. 2. These experiments revealed some differences in coextractives removal between the methods. Visual comparison suggests that Z-Sep (Fig. 2c) ensured better clean-up than PSA/C18 (Fig. 2b), especially in the case of compounds eluting from 19 to 35 min. To identify compounds which are adsorbed better by Z-Sep than by PSA/C18, the obtained data were compared with the NIST library. Among compounds more effectively removed by Z-Sep were some typical avocado constituents such as palmitoleic acid, oleic acid, gamma-sitosterol, campesterol, phytol and methyl hexadecanoate. All of these contain an oxygen atom in the molecule and adsorption probably occurs via coordination of electrons from oxygen by zirconium atoms. Other identified compounds, which were removed more efficiently by Z-Sep than by PSA/C18 (1-heptatriacotanol, cis,cis-7,10,-hexadecadienal, (Z)-9-octadecenamide), had a long carbon chain and one or two functional groups containing oxygen. The full-scan chromatogram of ethyl acetate extract (Fig. 2a) shows that this extract contained many more coextracted compounds with short retention times than the extract cleaned with Z-Sep. Moreover, the last part of the chromatogram (after 34 min.) is more intensive than in the case of the two other extracts. However, using the NIST library, out of the typical compounds present in avocado, only gamma-sitosterol and small amounts of palmitoleic acid and methyl hexadecanoate were identified. The general conclusion from a comparison of the full-scan chromatograms is that Z-Sep sorbent ensures the cleanest samples. This is very important because it reduces the need for system maintenance.

### 3.7. Real samples

The fully validated method was employed in real sample analysis. Twenty five avocado and 18 almond samples were bought at local shops in Almería.

Five avocado samples contained one pesticide at a concentration above the limit the quantitation and one sample contained two. The most frequently detected pesticide was permethrin. It was present in four samples at concentrations from 16 to 32 μg/kg. Other quantified pesticides were deltamethrin (11 μg/kg), phenothrin (11 μg/kg), and tetramethrin (56 μg/kg). Taking into account an uncertainty value of 50% [33], only tetramethrin exceeded the European Union Maximum Residue Level (EU MRL) value [34] – for tetramethrin, the EU MRL is 10 μg/kg. Apart from

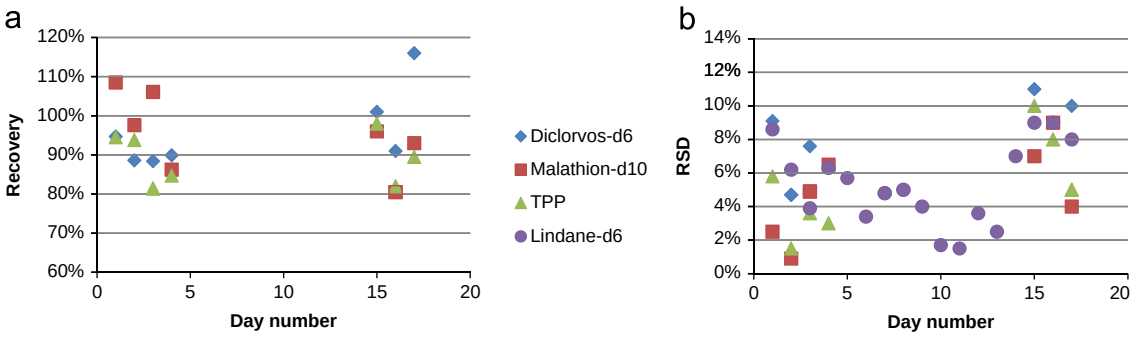


**Fig. 3.** (a) Recoveries of quality control compounds; (b) RSD of quality control compounds. Injections made from 23rd of November until 26th of March.

the quantified pesticides, some analytes were detected but at concentrations below 10 μg/kg. These were the cases with chlopyriphos (3 samples), fludioxonil (1 sample), lambda cyhalotrin (3 samples) and picolinafen (1 sample).

Thirteen almond samples contained at least one pesticide. In total there were 23 positive findings. However, in all cases, pesticide concentrations were below 10 μg/kg. Amongst the detected pesticides were: ethofenprox (9 samples), chlorpyriphos (3 samples), quinoxyfen, iprodione (2 samples), 4,4-DDD, 4,4-DDT, boscalid, chlorpropham, deltamethrin, o-phenylphenol and phosmet (1 sample).

The obtained results were compared with the EURL database [35]. Only residues of permethrin, chlorpyriphos and fludioxonil in avocado and chlorpyriphos in almonds were reported in these commodities over the time period from 2002 to 2012.

### 3.8. *Quality control*

Throughout all of the experiments, the quality of results was controlled in two stages. Extraction was controlled using three surrogate standards – triphenyl phosphate (TPP), dichlorvos-$d_6$ and malathion-$d_{10}$. Extraction was considered to be carried out correctly when surrogate standard recoveries were in the 70–120% range and the RSD was lower than 20%. When these criteria were not met, extractions were repeated. The second stage was injection control. For this purpose, lindane-$d_6$ was used. A set of injections was accepted if the RSD of lindane-$d_6$ peak area was below 20%. When the RSD was higher, injections were repeated. The quality control data are shown in Fig. 3.

Apart from TTP and deuterated standards, fenvalerate/esfenvalerate RS/SR, fenpropimorph, methiocarb, phosmet, propaphos and tetraconazole should also be included in the quality control and monitored regularly because their inter-day variability was equal to 20%.

## 4. Conclusions

The QuEChERS method with Z-Sep sorbent ensured better removal of coextracted matrix compounds than PSA and C18. Extracts were also cleaner than those prepared with the ethyl acetate method. The application of Z-Sep did not have a negative influence on recoveries. Z-Sep was efficient in the removal of fatty acids, esters of fatty acids and sterols. The extraction of pesticides from almonds was more “difficult” than from avocado. High fat matrices such as avocado and almonds represent a challenge due to low fat solubility in acetonitrile. This situation is more pronounced in almonds than in avocado. This point was especially problematic for highly lipophilic analytes. However, the presented method is characterised by very good precision, with RSD values below 10% in most cases.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.09.053.

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