Application of gas chromatography triple quadrupole mass spectrometry in the quantification-confirmation of pesticides and polychlorinated biphenyls in eggs at trace levels

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Abstract

A new multiresidue method has been developed and validated for the simultaneous analysis of 57 compounds, including organochlorine and organophosphorus pesticide residues (OCPs and OPPs) and polychlorinated biphenyls (PCBs), in eggs at trace levels by gas chromatography coupled to triple quadrupole mass spectrometry (GC-QqQ-MS/MS). Egg samples were extracted by a simple and fast matrix solid-phase dispersion (MSPD) procedure using C18 as sorbent, and ethyl acetate and acetonitrile (85:15 v/v) as elution solvent with a simultaneous clean-up with Florisil on-line. The QqQ analyzer acquired data in selected reaction monitoring (SRM) mode, permitting both quantification and confirmation in a single injection with a running time reduced up to 17.70 min. Recovery was in the range 70–110 % and 70-106 % at 15 and 50 µg/kg, respectively. Precision values expressed as relative standard deviation (R.S.D.) were lower than 20%. Linearity in the range 10-150 µg/kg provided determination coefficients (R^2) higher than 0.98 for all compounds. Limits of detection (LODs) for pesticides were ≤ 2.25 µg/kg and limits of quantification (LOQs) ranged from 0.02 to 7.78 µg/kg. LODs for PCBs were ≤0.41 µg/kg and LOQ were ≤0.71 µg/kg. The method was applied to real samples. Endosulfan sulphate and *p,p'*-DDE were found in two samples at concentrations below the first calibration level.

Keywords: pesticides; polychlorinated biphenyls; mass spectrometry; triple quadrupole; matrix solid phase dispersion; egg; food analysis

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32 **1. Introduction**

33

34 Organochlorine pesticides (OCPs) and organophosphorus pesticides (OPPs) are two groups of
35 compounds which have been extensively applied. Their high effectiveness and lowprice in the control
36 of pests have contributed to the development of the modern agricultural and farming production.
37 Polychlorinated biphenyls (PCBs) have been used in industry as heat exchange fluids, in electric
38 transformers and capacitors as well as additives in pesticides, paint, carbonless copy paper, sealants or
39 plastics [1]. OCPs and OPPs are known of inducing or aggra- vating certain health problems in humans
40 such as cancer or the disruption of hormonal functions [2,3].On the other hand, PCBs adverse effects
41 such as cancer, immunotoxicity, neurotoxicity and endocrine disruption have been also reported [4,5].
42 OCPs and PCBs are included in the group of the so-called persistent organic pollutants (POPs) [6]
43 since they show a high lipophilic character and resistance to degradation. Because of that, they are easy
44 to bioaccumulate along the food chain, especially in fatty tissues such as oils, fats or eggs. Despite the
45 fact that OPPs are less persistent than OCPs and PCBs, they can also bioaccumulate in fatty matrices
46 showing a high acute toxicity. The physicochemical characteristics of these compounds together with
47 their indiscriminate use in the past has led to their occurrence in the environment, biota [7,8] and
48 foodstuffs [9,10],as well as in human tissues [4,11]. Due to the mounting concerns about food safety,
49 the European Union (EU) has established maximum residue levels (MRLs) for OCPs and OPPs in eggs
50 [12,13]. In relation to PCBs, the EU has also set a group of 12 congeners to be monitored in foodstuffs
51 [14–16] since they exhibit toxicological properties similar to dioxins; they are therefore often termed as
52 dioxin- like PCBs. In consequence, improved and powerful analytical methodologies need to be
53 available in order to enforce the international regulations. From other point of view, the study of the
54 levels of these compounds in eggs is of interest since it is currently applied in monitoring for
55 environmental contamination [4,17,18]. The analysis of pesticide residues and PCBs in foodstuffs or
56 environmental samples usually involves the extraction of the analytes from the matrix, the subsequent
57 clean up of the extracts and the final chromatographic analysis. Soxhlet [1,4,9], ultrasonic [19],
58 pressurized solvent (PLE) [20,21], microwave assisted (MAE) [22,23] and solid–liquid [9,20,24]
59 extraction have been applied as extraction techniques in trace analysis. In fatty matrices such as eggs,
60 the clean up is a critical stage due to the high content in lipids and non-volatile compounds of the raw
61 extracts. Gel permeation chromatography (GPC) [5,7,9,20,25] and solid phase extraction (SPE)
62 [1,5,7,26] are commonly used for this purpose. However, the high solvent consumption in GPC and the
63 low recoveries for some compounds in SPE make these options less desirable [24]. In this sense, matrix

64 solid phase dispersion (MSPD) is an alternative to the traditional techniques since it permits to perform
65 both extraction and clean up in a single step with a minimal amount of solvent. This technique shows a
66 high flexibility and selectivity due to the variety of possible combinations of both sorbents and elution
67 solvents [7,27–30]. These characteristics, together with its simplicity and high throughput, have
68 increased the use of MSPD to extract pesticides, PCBs and other organic environmental pollutants from
69 food [10,25,31], biological [32], and environmental samples [33,34]. In this work, a new method for
70 the simultaneous analysis of OCPs, OPPs and PCBs with MSPD extraction has been developed. Gas
71 chromatography (GC) coupled to electron capture detection (ECD) is widely used [5,10,18,19,26,33] in
72 pesticide residue and PCB analysis. However, mass spectrometry (MS) is currently one of the most
73 powerful tools in simultaneous quantification-confirmation of organic compounds, because of its high
74 selectivity and sensitivity according to the analyzer. Single quadrupole (Q) [1,4,8,17,31] and ion trap
75 (IT) [20,22,34] analyzers have been applied in the analysis of the target com- pounds in fatty matrices.
76 The Q analyzer only permits data acquisition in single ion monitoring (SIM) with the subsequent lack
77 of confirmation capability. The IT methods allow confirming the positive results but the running time is
78 relatively high when multiresidue methods (MRM) have to be developed, due to its lower scan speed
79 compared to the triple quadrupole analyzer (QqQ) speed. The QqQ analyzer is able to solve those two
80 negative aspects since it provides higher scan speed and confirmation is ensured by operating in
81 selected reaction monitoring (SRM) mode. The high selectivity and sensitivity of the QqQ analyzer
82 also allows the simplification of the sample pre- treatment by reducing or even removing the clean up
83 stage. In addition, the large volume injection technique (LVI) together with an injector operating in
84 programmed temperature vaporization (PTV) is currently applied in trace analysis [35,36] since it
85 permits or avoids the need for pre-concentration steps and increases sensitivity. Nevertheless, the use of
86 QqQ analyzers focused on the analysis of OCPs, OPPs and PCBs is still reduced and it is extremely
87 reduced in fatty matrix applications [9,11,25,35,37]. To our knowledge, this is the first approach in the
88 simultaneous quantification-confirmation of more than 50 pesticide residues and PCBs in egg with a
89 QqQ analyzer. The developed method- ology is adequate to determine OCPs, OPPs and PCBs in such
90 samples due to the effectiveness of the extraction procedure and the fast chromatographic analysis (less
91 than 18 min), providing adequate performance characteristics.
92
93 **2. Experimental**
94 ***2.1. Materials and reagents***
95 Chlorfenvinphos, chlorpyriphos ethyl, chlorpyriphos methyl, dichlorvos, endosulfan sulphate, ethion,
96 famphur, fenamiphos, heptachlor, heptachlor epoxide endo, heptachlor epoxide exo,

97 hexachlorobenzene, malathion, mirex, *o,p'*-DDT, parathion ethyl, parathion-methyl, *p,p'*-DDE and
98 vinclozoline standards, as well as the internal standards (I.S.) used, caffeine and 3'-fluoro-2,4,4'-
99 trichlorobiphenyl (PCB 28F, 100 mg/L in isooctane), were provided by Dr. Ehrenstorfer GmbH
100 (Augsburg, Germany). Bromophos ethyl, bromophos methyl, chloropropylate, endosulfan alpha, EPN,
101 etrimfos, fenthion, isofenphos, methamidophos, metolachlor, pirimiphos ethyl, pirimiphos methyl, p,p'-
102 DDD, prothiophos, quintozene and sulfotep were purchased from Riedel-de Haën (Seelze, Germany).
103 Aldrin, *o,p'*-DDD and tetrachlorvinphos were supplied by Chem Service (West Chester, PA, USA);
104 purity was always ≥ 94.0 %. Individual PCBs standards with IUPAC Nos 18, 28, 31, 44, 52, 77, 81,
105 101, 105, 114, 118, 123, 138, 153, 156, 157, 167, 169 and 180 were purchased from Dr. Ehrenstorfer
106 GmbH, with purities ≥ 97.0 %. Acetone, *n*-hexane, ethyl acetate (EtAc) and acetonitrile (ACN) were
107 supplied by J.T.Baker (Deventer, Holland); cyclohexane and dichloromethane were supplied by Riedel-
108 de Haën (Seelze, Germany) and methanol was supplied by Sigma-Aldrich (Steinheim, Germany),
109 always in residue analysis grade. Individual stock standard solutions were prepared by exact weighing
110 and dissolution in acetone (concentrations in the range from 100 to 500 µg/kg); these solutions were
111 stored under refrigeration (T ≤ 5ºC). A multipesticide working standard solution (2 µg/L concentration
112 of each compound) was prepared by appropriate dilution of the stock solutions with acetone. A multi-
113 PCB working standard solution (1 µg/L concentration of each compound) was prepared in the same
114 way. Both solutions were stored in a fridge (T ≤ 5ºC). Finally, a working standard solution of caffeine
115 (20 mg/L) and PCB 28F (4 mg/L) were prepared by appropriate dilution of the stock solution with
116 acetone and stored under the aforementioned conditions. Reagent-grade anhydrous magnesium
117 sulphate (purity > 98 %) was supplied by Riedel-de Haën. Preparative-grade (100 g, bulk) C18-bonded
118 silica material with 40-µm particle size, 18 % carbon load and end capped as well as 12-mL SPE
119 reservoirs with two frits were provided by Varian (Harbour City, CA, USA). Florisil sorbent of
120 pesticide-residue grade with a 150–250 µm particle size and 60–100 mesh (250 g, bulk) was purchased
121 from Merck (Darmstadt, F.R. Germany). Preparative-grade (50 g, bulk) aminopropil-bonded silica with
122 15-35-µm particle size, 9-nm pore size was obtained from Fluka (Steinheim, Germany). Primary
123 secondary amine (PSA)-bonded silica (100 g, bulk) was supplied by Supelco (Bellefonte, PA, USA).
124 19.8-mm filters of glass fibre were purchased from Dionex Corporation (Sunnyvale, CA, USA).
125
126 ***2.2. Apparatus***
127 GC-QqQ-MS/MS analysis were performed with a GC system Varian 3800 (Varian Instruments,
128 Sunnyvale, CA, USA) equipped with electronic flow control (EFC) and cryogenic cooling with carbon

129 dioxide (CO_2, 99.9 %). A Varian 1200L triple quadrupole mass spectrometer was coupled to the gas
130 chromatograph (mass range from *m/z* 10 to 1500). Samples were injected with a Combi Pal
131 autosampler (CTC Analytics, Zwingen, Switzerland) into a 1079 split/splitless septum-equipped
132 programmable injector (SPI) operating in the LVI technique. The glass liner was equipped with a
133 Carbofrit plug (Resteck, Bellefonte, PA, USA). A fused-silica untreated capillary column 2 m x 0.25
134 mm i.d. from Supelco was used as guard column connected to a Varian FactorFour Capillary Column
135 VF-5ms analytical column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). The instrument data system
136 also held and EI-MS/MS library specially created for the target analytes under our experimental
137 conditions. Other EI-MS/MS libraries were also available. The mass spectrometer scale was weekly
138 calibrated with perfluorotributylamine. Varian Workstation software was used for instrument control
139 and data analysis. SPE extractions were performed with an SPE manifold system supplied by Waters
140 (Milford, MA, USA).
141
142 ***2.3. Egg sample extraction and clean-up***
143 Hen eggs samples were purchased in several supermarkets in Almería. They were stored under
144 refrigeration until analysis (T ≤ 5ºC). Pesticide and PCBs-free samples were used in the validation
145 procedure and matrix-matched standard calibrations. Samples were homogenized with a glass bar in a
146 flask. Samples for recovery studies were spiked with the corresponding volume of both working
147 solutions and left for 1 h before performing the extraction process.
148 0.5-g portion of the homogenized egg sample was weighed in a glass mortar. Next, 2.0 g of C18
149 sorbent (previously washed with two volumes each of *n*-hexane, dichloromethane and methanol) and 1
150 g of anhydrous $MgSO_4$ was added. The sample was blended using a glass pestle with moderated
151 pressure for 2 min, obtaining a slightly yellow mixture. The aforementioned mixture was transferred
152 into a 12-mL SPE reservoir containing 2 g of Florisil (previously activated by heating to 130ºC
153 overnight). A glass fibre filter was placed on top of the transferred material. The mixture was fitted
154 with a glass bar, avoiding the formation of voids or channels. The SPE cartridge was eluted with 1.5
155 mL of ACN saturated in n-hexane (85:15, v/v) and 8.5 mL of EtAc (3+3+2.5 mL), previously used in
156 washing both mortar and pestle. The elution of the cartridges was performed in a SPE vacuum
157 manifold with 10-mL glass test tubes by applying a low vacuum of 250 Torr at a flow rate of 0.5
158 mL/min approximately. The final extract was evaporated to near dryness with a nitrogen stream. The
159 residue was re-dissolved with 950 µL of cyclohexane, and 25 µL of each I.S. working solution
160 (caffeine and PCB 28F).

161

162 ***2.4. GC-QqQ-MS/MS analysis***

163 Ten microlitres of the final extract were injected into the chromatographic system at 1 μL/s. The
164 initial temperature of the injector was set at 70ºC (hold for 0.5 min), and then it was increased up to
165 300ºC at 100ºC/min (hold for 8.0 min). The split ratio was 30:1 until 0.5 min. The splitless mode was
166 activated from 0.5 to 3.5 min. The split ratio was 100:1 at 3.5 min and 30:1 at 10 min. The initial
167 temperature of the column oven was 70ºC (hold for 3.5 min). This temperature was increased at a rate
168 of 50ºC/min up to 180ºC; next, the temperature was increased up to 300ºC (hold for 8 min) at a rate of
169 30ºC/min. Cryogenic cooling with CO_2 was applied when the injector temperature was 185ºC in order
170 to reach the initial conditions in a short time. Helium (99.9999%) at a constant flow-rate of 1 mL/min
171 was used as carrier gas; argon (99.99 %) at a pressure in the range 1.90-2.10 mTorr was used as
172 collision gas. The running time was of 17.7 min, divided into seven segments.

173 The QqQ mass spectrometer was operated in electron ionization (EI) at 70 eV in the selected
174 reaction monitoring (SRM) mode. The transfer line, manifold and ionization source temperatures were
175 set at 300, 40 and 280ºC, respectively. A filament multiplier delay of 4.5 min was fixed in order to
176 prevent instrument damages. The electron multiplier voltage was set at 1400 V (+200 V offset above
177 the auto-tuning process). The scan time was of 0.25 s which resulted in dwell times (scan time divided
178 by number of transitions) ranging from 0.005 to 0.036 s. Peak widths of *m/z* 2.0 and 1.5 were set in the
179 first (Q1) and third quadrupole (Q3), respectively. The specific MS/MS conditions are shown in Table
180 1.

181

182 **3. Results and discussion**

183 ***3.1. Optimization of the MSPD extraction***

184 The optimization of MSPD procedure was performed with blank egg samples spiked at 50 μg/kg. The
185 use of porcelain mortars was avoided since analyte losses had been previously reported [31]. A
186 preliminary experience was carried out to determine the most appropriate ratio of sample-to-bonded-
187 phase solid support. This ratio depends on the sample nature, although ratios of 1:4 and 1:2 are
188 frequently applied [10,25,30]. Egg matrix is a fatty and highly viscous sample; in this sense, the ratio
189 1:4 was better than the ratio 1:2. Moreover, a great decrease of sensitivity was observed when the ratio
190 1:2 was applied due to the higher matrix content in the final extract. Volumes of 1 and 0.5 mL were
191 tested in the final re-dissolution step; 1-mL volume was chosen since the same decrease of sensitivity
192 was observed with 0.5 mL.

193 In relation to the extraction sorbent, C18-bonded silica was initially selected since the target
194 compounds showed mainly non-polar character. However, the application of aminopropyl-bonded
195 silica was also evaluated since it could provide higher recoveries for more polar OPPs. Considering the
196 egg matrix complexity because of its high content in fat, two clean-up methodologies, using Florisil
197 coupled on-line to the extraction sorbent and a dispersive solid-phase extraction (D-SPE) with PSA,
198 were tested. ACN, ACN saturated in *n*-hexane and EtAc were the elution solvents studied. The use of
199 aminopropil as sorbent provided very poor recoveries as well as the application of D-SPE with PSA;
200 therefore, C18 and Florisil were selected. The extracts eluted with AcN and AcN saturated in *n*-hexane
201 were cleaner than the EtAc extracts, however, recoveries were slightly better with this last solvent (Fig.
202 1). A compromise solution was chosen with an elution solvent mixture EtAc:ACN saturated in *n*-
203 hexane (85:15 v/v), which provided recoveries in the range 70-106 % at 50 μg/kg (Table 2). The
204 elution with this low percentage of ACN saturated in *n*-hexane permitted to obtain higher recoveries for
205 more polar pesticides such as dichlorvos and methamidophos.
206
207 ***3.2. GC-QqQ-MS/MS analysis***
208 The chromatographic separation is not a critical stage in the development of a multiresidue method
209 with QqQ analyzers because of the possibility of monitoring co-eluted compounds in SRM (Fig. 2).
210 The high QqQ acquisition speed permits the application of fast temperature gradients in order to
211 diminish the chromatographic analysis since the analyzer is able to monitor a high number of
212 transitions simultaneously. The only limit is due to the shape peak and the number of scans or points
213 per peak. A minimum of 6-8 scans (including those of the baseline) were set [35,38]. The final
214 temperature program carried out the separation of 57 compounds in less than 18 min.
215 In the optimization of the MS/MS conditions, full scan spectra were obtained to select the precursor
216 ions (Table 1). Selection of the precursor ion was carried out trying to choose the ion with the highest
217 *m/z* ratio (increase in selectivity) and abundance (increase in sensitivity). Then, product ion spectra
218 were acquired by collision-induced dissociation (CID) with argon. Collision energies (CE) from 0 to 50
219 eV were applied. The aforementioned criterion was also applied to choose the more suitable product
220 ions. The final purpose was to develop a SRM method with 2 or 3 reactions or transitions per
221 compound. In PCBs, due to the low sensitivity and/or the confirmation requirements, more than one
222 precursor ion was selected to achieve at least two MS/MS transitions (Fig. 3).
223 The scan time was optimized in order to evaluate its influence on sensitivity and peak shape. Scan
224 time values of 0.15, 0.25, 0.35 and 0.45 s were tested. A scan time of 0.25 s was selected as result of a
225 compromise solution between sensitivity (high scan time and dwell time) and peak shape (low scan

226 time and dwell time). Higher values did not provide enough scans per peak; on the contrary, a scan
227 time of 0.15 s did not provide suitable sensitivity and peak shape obtained was not adequate (Fig. 4).
228 The selected scan time yielded dwell times from 0.008 to 0.036 s.

229 Finally, the LVI technique was applied together with a PTV since it was mandatory to increase the
230 sensitivity due to the non-concentration of the analytes but also dilution of them after performing the
231 extraction procedure.

232

233 ***3.3. Validation of the final method***

234 A validation protocol of the overall analytical procedure was carried out in order to establish the
235 performance characteristics of the method which ensure the correct quantification and confirmation of
236 OCPs, OPPs and PCBs in egg matrix. Accuracy, precision, linearity, limits of detection (LODs), limits
237 of quantification (LOQs) and confirmation criteria were established.

238

239 *3.3.1. Identification and confirmation of the target compounds*

240 Identification of the target compounds was based on the use of retention time windows (RTWs). The
241 RTW was defined as the retention time (RT) average plus or minus 3 standard deviations (SD) of the
242 RT (RT ± 3SD) when 10 blank samples spiked at the second level of calibration were injected.

243 Confirmation was carried out by comparing the sample spectrum with a reference spectrum obtained
244 from a blank egg sample spiked at the second calibration level. Comparison was performed with a
245 forward search which compared the sample spectrum (product ions obtained) with the reference
246 spectrum. The result of this comparison gave a value ranging from 1 to 1000 (arbitrary units, a.u.)
247 which was named FIT by the software. In general, a FIT ≥ 700 (a.u.) confirmed a positive result.

248 The European Commission Decision 2002/657/EC [39] introduced the concept of identification
249 points (IPs) for the confirmation stage [9,11,25,35]. The number of IPs depends on the spectrometric
250 technique used. In the case of low resolution mass spectrometry (LR-MS^n) such as QqQ-MS, this
251 document set a minimum of 3 IPs for the confirmation of OCPs, OPPs and PCBs. In this work, the
252 analysis of the target compounds involved the monitoring of a minimum of two product ions which
253 resulted in 3 IPs, 1.5 IPs each. Therefore, the MRM method permitted to obtain from 3 to 4.5 IPs
254 according to the aforementioned regulation.

255

256 *3.3.2. Quantification of the target compounds*

257 One of the main problems in trace analysis of complex matrices is the suppression/enhancement
258 matrix effect. In this work, matrix-matched standard calibration was used for quantification purposes in
259 order to avoid matrix effect.
260 Linearity was studied in the range 10-150 μg/kg (10, 50 150 μg/kg) and linear calibration graphs
261 were plotted by least-squares regression of concentration versus relative peak area (analyte/IS) of the
262 calibration standards. PCB 28F was the IS for PCBs, whereas caffeine was the IS for pesticide residues.
263 The selection of caffeine is based on its chromatographic properties that are similar to some pesticides.
264 Its chromatographic behaviour is well known in our laboratories and the peak shape and intensity of
265 this compound is easily interpreted by our analysts in routine analysis. Nevertheless, the use of labelled
266 pesticide as IS is very interesting because they have identical physical–chemical properties to the non-
267 labelled pesticide.
268 Determination coefficient (R^2) values between 0.9807 and 0.9999 were obtained for all the target
269 compounds.
270 Accuracy and precision were evaluated by injecting five replicate blank samples spiked at two levels
271 of concentration, 15 and 50 μg/kg. Recoveries were in the range 70-110 % at 15 μg/kg and 70-106 % at
272 50 μg/kg. Precision was expressed as relative standard deviation (RSD). RDS values obtained were
273 lower than 20 % in both levels (Table 2). These values of RSD were slightly higher in comparison with
274 other QqQ works [11,35] but this increase was mainly due to the pre-treatment sample since MSPD
275 usually provided higher RSD [29].
276 LODs and LOQs were calculated in blank extracts as the lowest analyte concentration that yielded a
277 signal-to-noise (S/N) ratio of 3 and 10, respectively. In the case of pesticides, LODs and LOQs were in
278 the range 0.01-2.25 μg/kg and 0.02-7.78 μg/kg, respectively; whereas for PCBs, LODs ranged from
279 0.03 to 0.41 μg/kg and LOQs from 0.09 to 0.71 μg/kg.
280

281 ***3.4. Application to real samples***

282 Twenty real egg samples were analyzed with the developed method, performing several internal
283 quality controls in order to guarantee that the measurement process was under statistical control. Each
284 batch of samples was processed together with a matrix blank which was obtained with a blank sample
285 plus the corresponding volumes of the IS. The matrix blank eliminated a false positive as result of
286 contamination in the extraction process, instrument or chemicals used as well as to identify the possible
287 matrix interferences. A reagent blank was obtained by performing the whole process without sample.
288 This sample eliminated possible false positives produced by contamination in the instrument or solvent

289 used. A blank extract spiked at the second calibration level permitted to control the extraction
290 efficiency. Calibration curves were prepared daily obtaining determination coefficients ≥ 0.98. The
291 analysis showed the presence of endosulfan sulphate and *p,p'*-DDE (OCPs) in two samples with
292 concentrations below the first calibration point (Fig. 6). PCBs were not found in the analyzed samples.
293
294 **4. Conclusions**
295 In the present work, the potentiality of GC-QqQ-MS/MS in the quantification and confirmation of
296 OCPs, OPPs and PCBs in eggs at trace levels has been demonstrated. The results obtained are proof of
297 the capability of QqQ-MS in the analysis of trace compounds in complex matrices. The instrumental
298 analysis of the target compounds was carried out in a single run of less than 18 min which contributed
299 to reduce the whole analysis time. The simple and fast MSPD procedure optimized is able to perform
300 the simultaneous extraction and clean-up of the samples. MSPD has been shown as a suitable
301 methodology in the analysis of foodstuff samples. It was also of relevance the high sensitivity and
302 selectivity showed by the QqQ analyzer for the pesticide residues and PCBs studied, providing in some
303 cases LODs and LOQs at ng/kg level.
304
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311
312 **References**
313 [1] A. Covaci, J. de Boer, J.J. Ryan, S. Voorspoels, P, Schepens, Anal. Chem. 74 (2002) 790.
314 [2] The e-Pesticide Manual, 2005-2006 (13th Edition, Version 3.2), The British Crop Protection
315 Council.
316 [3] E. Mutch, F.M. Williams, Toxicology 224 (2006) 22.
317 [4] E. Van den Steen, A. Covaci, V.L.B. Jaspers, T. Dauwe, S. Voorspoels, M. Eens, R. Pinxten,
318 Environ. Int. 33 (2007) 257.
319 [5] A. Smeds, P. Saukko, Chemosphere 44 (2001) 1463.
320 [6] http://www.pops.int
321 [7] N. Fidalgo-Used, E. Blanco-González, A. Sanz-Medel, Anal. Chim. Acta 590 (2007) 1.

322 [8] J. Verreault, R.J. Norstrom, M.A. Ramsay, M. Mulvihill, R.J. Letcher, Sci. Total Environ. 370
323 (2006) 580.
324 [9] A. Garrido Frenich, J.L. Martínez Vidal, A.D. Cruz Sicilia, M.J. González Rodríguez, P. Plaza
325 Bolaños, Anal. Chim. Acta 558 (2006) 42.
326 [10] V.I. Valsamaki, V.I. Boti, V.A. Sakkas, T.A. Albanis, Anal. Chim. Acta 573-574 (2006) 195.
327 [11] F. Hernández, T. Portolés, E. Pitarch, F. J. López, J. Betrán, C. Vázquez, Anal. Chem. 77 (2005)
328 7662.
329 [12] Regulation (EC) No 396/2005 of 23 February 2005. Official Journal of the European Union, L
330 70/1.
331 [13] Council Directive (EEC) No 86/363 of 24 July 1986. Official Journal of the European Union, L
332 221.
333 [14] Commission Regulation (EC) No 1883/2006 of 19 December 2006. Official Journal of the
334 European Union, L 364/32.
335 [15] Commission Regulation (EC) No 1881/2006 of 19 December 2006. Official Journal of the
336 European Union, L 364/5.
337 [16] Commission Recommendation 2006/88 of 6 February 2006. Official Journal of the European
338 Union, L 42.
339 [17] V. Jaspers, A. Covaci, J. Maervoet, T. Dauwe, S. Voorspoels, P. Schepens, M. Eens, Environ.
340 Pollut. 136 (2005) 81.
341 [18] T.A. Albanis, V. Goutner, I.K. Konstantinou, K. Frigis, Environ. Pollut. 126 (2003) 245.
342 [19] S. Chu, C.S. Hong, B.A. Rattner, P.C. McGowan, Anal. Chem. 75 (2003) 1058.
343 [20] J.L. Fernández Moreno, F.J. Arrebola Liébanas, A. Garrido Frenich, J.L. Martínez Vidal, J.
344 Chromatogr. A 1111 (2006) 97.
345 [21] L. Ramos, E.M. Kristenson, U.A.Th. Brinkman, J. Chromatogr. A 975 (2002) 3.
346 [22] N. Carro, I. García, M.C. Ignacio, M Llompart, M.C. Yebra, A. Mouteira, Anal. Bioanal. Chem.
347 374 (2002) 574.
348 [23] C. Sparr Eskilsson, E. Björklund, J. Chromatogr. A 902 (2000) 227.
349 [24] K. Patel, R.J. Fussel, M. Hetmanski, D.M. Goodall, B.J. Keely, J. Chromatogr. A 1068 (2005)
350 289.
351 [25] A. Garrido Frenich, P. Plaza Bolaños, J.L. Martínez Vidal, J. Chromatogr. A 1153 (2007) 194.
352 [26] F.J. Schenck, D.J. Donoghue, J. Agr. Food Chem. 48 (2000) 6412.
353 [27] Y. Picó, M. Fernández, M.J. Ruiz, G. Font, J. Biochem. Biophys. Methods 70 (2007) 117.
354 [28] S. Bogialli, A Di Corcia, J. Biochem. Biophys. Methods 70 (2007) 163.

355 [29] E.M. Kristenson, L. Ramos, U.A.Th. Brinkman, Trends Anal. Chem. 25 (2006) 96.
356 [30] S.A. Barker, in J.L. Martínez-Vidal, A. Garrido-Frenich (Editors), Methods in Biotechnology,
357 Pesticide Protocols, vol. 19, Humana Press, Totowa, NJ, 2006, p. 285.
358 [31] C. Ferrer, M.J. Gómez, J.F. García-Reyes, I. Ferrer, E.M. Thurman, A.R. Fernández-Alba, J.
359 Chromatogr. A 1069 (2005) 183.
360 [32] C. Matos Lino, C.B. Ferreira Azzolini, D. S. Valente Nunes, J.M. Rocha Silva, M.I. Noronha da
361 Silveira, J. Chromatogr. B 716 (1998) 147.
362 [33] A. Martínez, M. Ramil, R. Montes, D. Herranz, E. Rubí, I. Rodríguez, R. Cela Torrijos, J.
363 Chromatogr. A 1072 (2005) 83.
364 [34] J.L. Gómez-Ariza, M. Bujalance, I. Giradles, A. Velasco, E. Morales, J. Chromatogr. A 946
365 (2002) 209.
366 [35] P. Plaza Bolaños, J.L. Fernández Moreno, D.D. Shtereva, A. Garrido Frenich, J.L. Martínez Vidal,
367 DOI: 10.1002/rcm.3091.
368 [36] N.H. Snow, in R.L. Grob, E.F. Barry (Editors), Modern practice of gas chromatography, Wiley-
369 Interscience, Hoboken, NJ, 2004, p. 461.
370 [37] F.J. Arrebola, A. Garrido Frenich, M.J. González Rodríguez, P. Plaza Bolaños, J.L. Martínez
371 Vidal, J, Mass Spectrom. 41 (2006) 822.
372 [38] Maštovská K, Lehotay S.J., J. Chromatogr. A 1000 (2003) 153.
373 [39] Comission Decission implementing Council Directive No 96/23/CE of 12 August 2002. Official
374 Journal of the European Union, L 221/8.
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379 **Figures**

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381 Fig. 1. Total ion chromatogram (TIC) of a blank egg sample spiked at 50 μg/kg extracted with: a) C18
382 + acetonitrile saturated in *n*-hexane, b) C18 + ethyl acetate and c) aminopropil + acetonitrile saturated
383 in *n*-hexane.
384 Fig. 2. TIC and SRM chromatograms of five compounds co-eluted in segment 5 in a spiked egg sample
385 at 50 μg/kg.
386 Fig. 3. Spectra of PCB 77 in: a) full scan; b) product ion scan of precursor ion *m/z* 220 at a collision
387 energy (CE) of 30 eV, c) *m/z* 290 at CE = 20 eV and d) *m/z* 292 at CE = 20 eV. In all cases the
388 selected product ion is pointed.
389 Fig. 4. MS/MS chromatogram of methamidophos acquired with a scan time of: a) 0.15 s, b) 0.25 s, c)
390 0.35 s and d) 0.45 s.
391 Fig. 5. SRM chromatograms and spectra of: a) *p,p'*-DDE and b) endosulfan sulphate in a real sample
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397 Fig. 1
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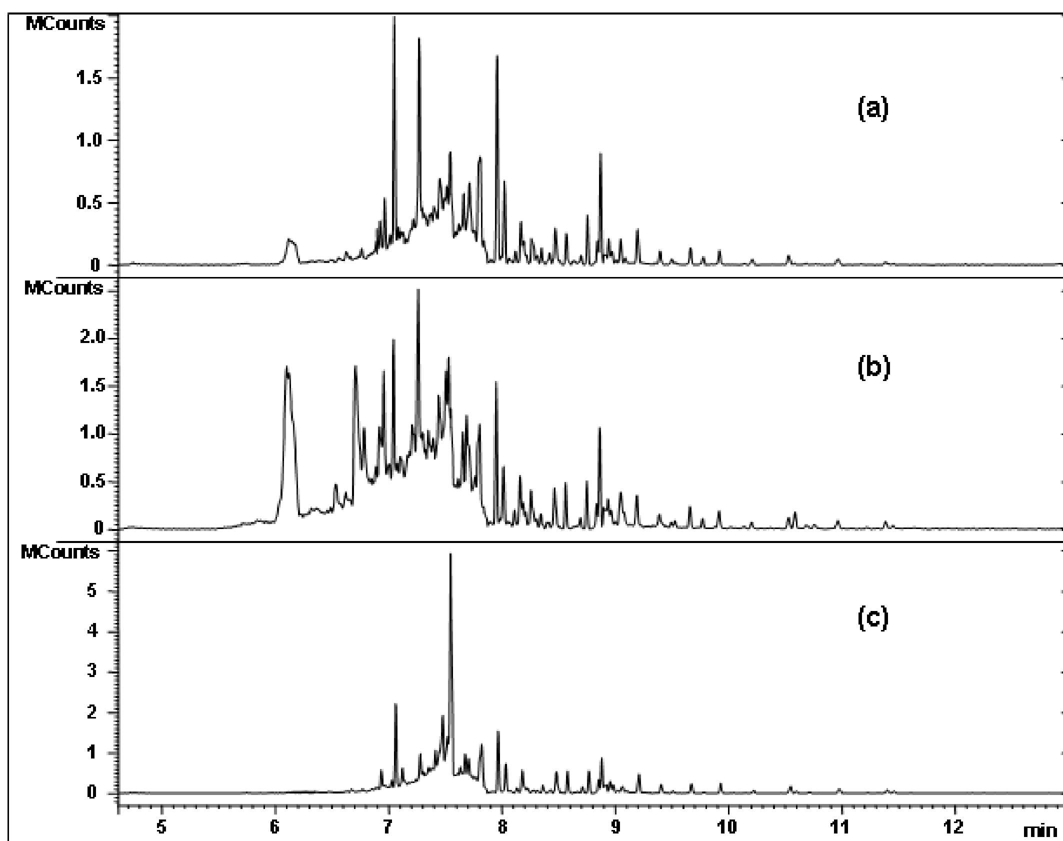

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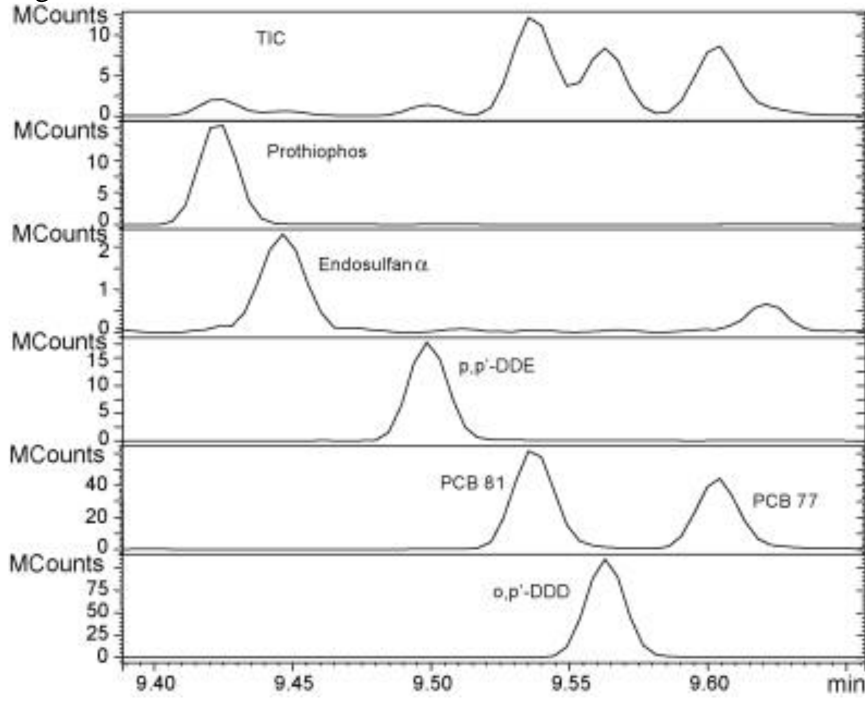
402 Fig 2.



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407 Fig. 3
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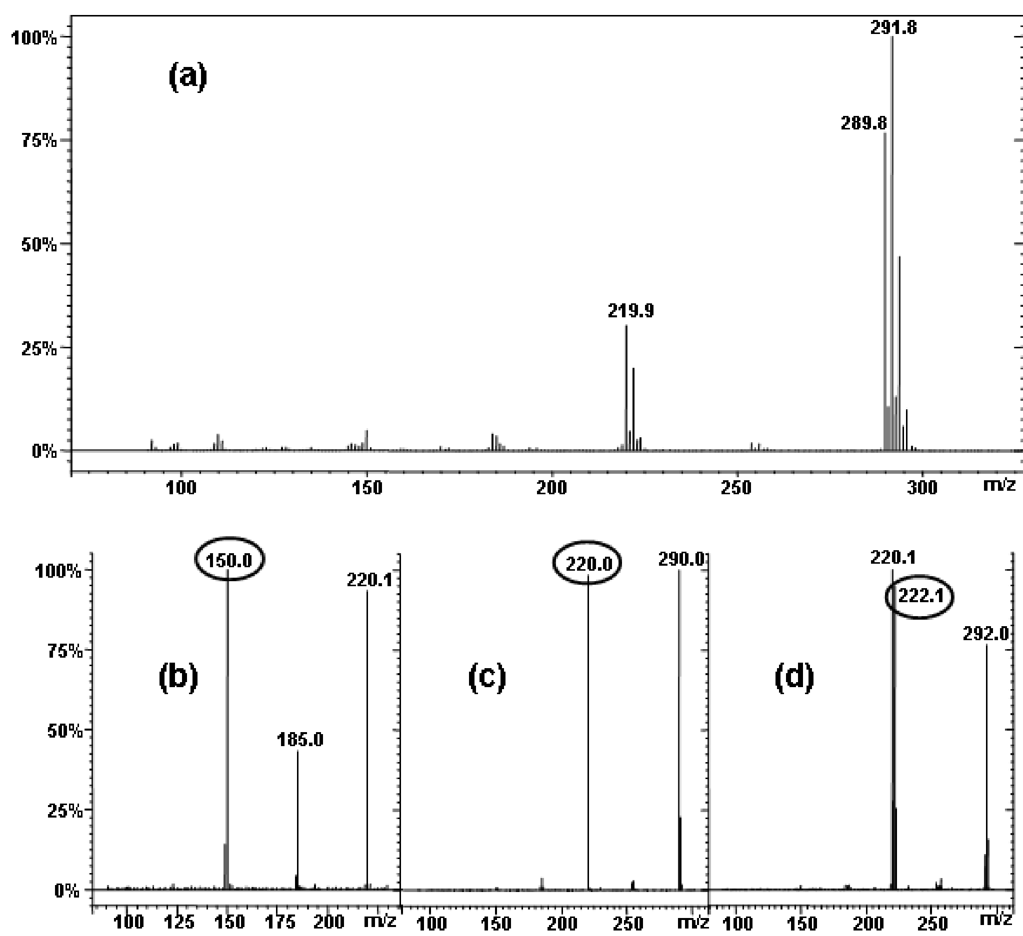

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411 Fig. 4
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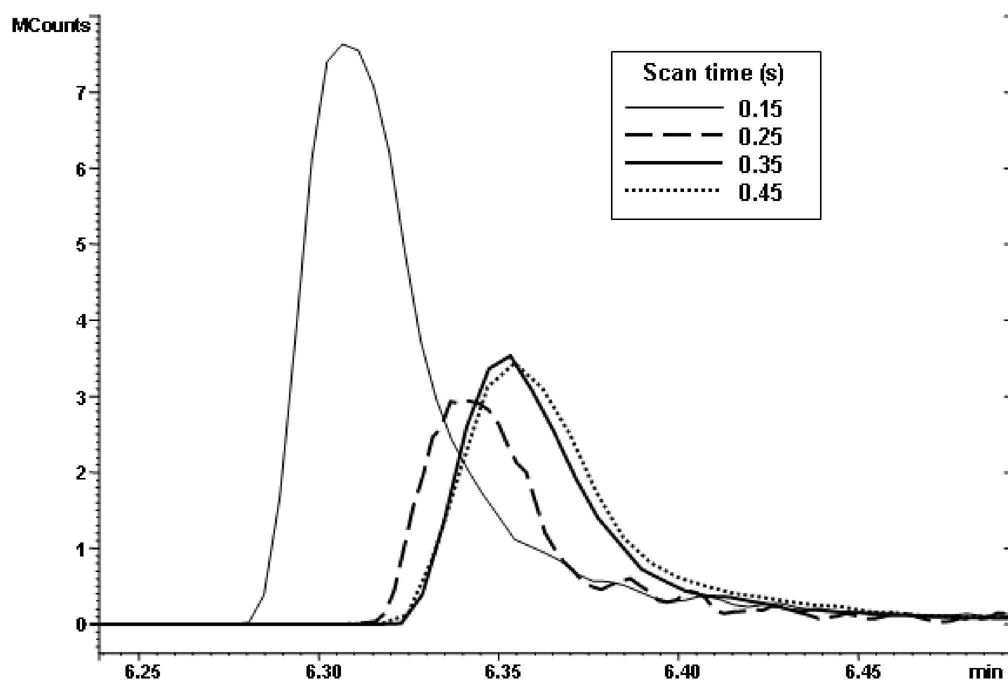

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416 Fig. 5
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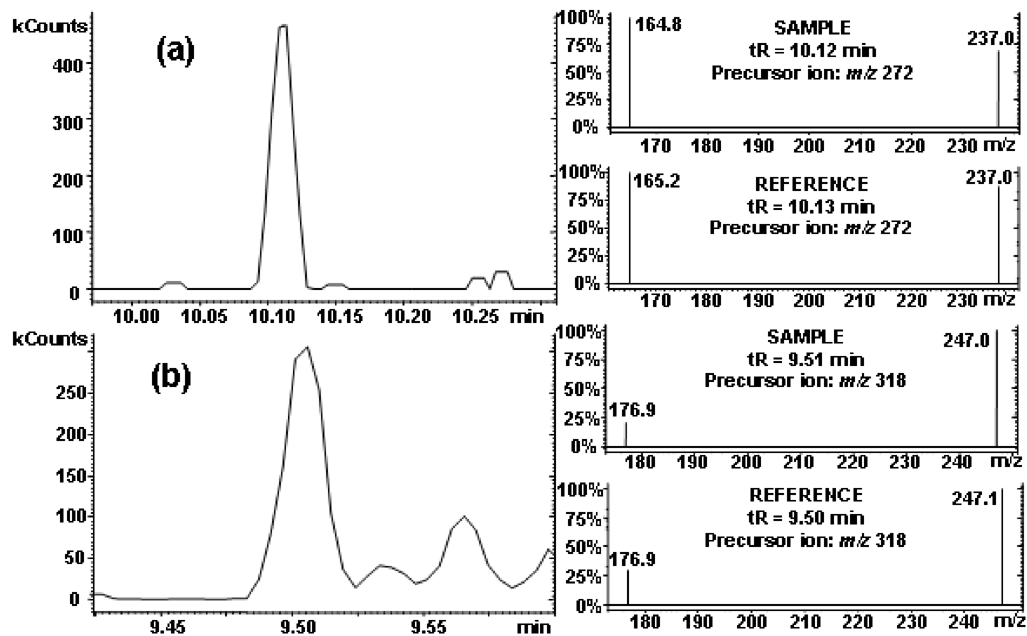

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426 Table 1

427 Retention time (RT), retention time windows (RTW) and MS/MS conditions

Compound	Segment	RT (min)	RTW (min)	Precursor ion (*m/z*)	Product ion, *m/z* (Collision energy, eV)
Dichlorvos	1	6.28	6.27- 6.30	185	93 (30), 109 (40)
Methamidophos	1	6.30	6.28- 6.33	141	79 (30), 94 (10)
Sulfotep	1	7.76	7.75- 7.77	322	146 (30), 174 (20), 202 (20)
Hexachlorobenzene	2	8.06	8.05- 8.07	284	177 (50), 214 (40), 249 (20)
Quintozene	2	8.21	8.20- 8.22	297	239 (20), 267 (10)
Etrimfos	2	8.27	8.26- 8.28	292	125 (50), 153 (30), 181 (10)
PCB 18	2	8.27	8.26- 8.28	221	150 (50)
				256	186 (40), 221 (20)
Caffeine	3	8.49	8.47- 8.50	194	109 (20)
Chlorpyriphos methyl	3	8.56	8.54- 8.57	286	208 (10), 241 (40), 271 (30)
PCB 28F	3	8.56	8.55- 8.57	274	204 (35)
Vinclozoline	3	8.57	8.55- 8.58	285	145 (40), 198 (30), 212 (10)
PCB 28 / PCB 31	3	8.60	8.59- 8.61	186	150 (40)
				256	150 (50), 186 (40)
Parathion methyl	3	8.61	8.60- 8.63	263	109 (10), 153 (1), 246 (1)
Pirimiphos methyl	3	8.67	8.66- 8.68	305	125 (50), 180 (10), 290 (20)
Heptachlor	3	8.73	8.71- 8.74	272	141 (50), 165 (50), 237 (20)
Malathion	3	8.75	8.74- 8.76	173	99 (20), 127 (10), 145 (10)
PCB 52	3	8.81	8.79- 8.82	257	222 (10)
				292	222 (50), 257 (30)
Chlorpyriphos ethyl	3	8.84	8.82- 8.85	314	258 (20), 286 (30)
Metolachlor	3	8.85	8.83- 8.86	238	133 (40), 145 (50), 162 (10)
Fenthion	3	8.88	8.86- 8.89	278	109 (20), 125 (40), 169 (40)
Parathion ethyl	3	8.90	8.88- 8.93	291	81 (30), 109 (10), 137 (10)
Pyrimiphos ethyl	3	8.91	8.89- 8.92	318	109 (20), 166 (20), 182 (20)
PCB 44	3	8.95	8.93- 8.96	220	185 (20)
				292	222 (30), 257 (20)
Aldrin	3	8.97	8.95- 8.99	291	185 (50), 221 (20), 256 (20)
Bromophos methyl	3	9.00	8.99- 9.01	331	210 (50), 285 (40), 316 (10)
Isophenphos	4	9.08	9.07- 9.10	213	121 (10), 185 (5)
Chlorfenvinphos	4	9.11	9.09- 9.12	324	159 (50), 267 (20), 296 (10)
Heptachlor epoxide Exo/Endo	4	9.23	9.21- 9.25	353	217 (40), 253 (30), 289 (10)
Bromophos ethyl	4	9.27	9.25- 9.28	359	239 (40), 303 (20), 331 (10)
Tetrachlorvinphos	4	9.31	9.29- 9.32	329	109 (30), 129 (40), 286 (50)
PCB 101	4	9.37	9.35- 9.38	256	186 (40)
				326	256 (30), 291 (20)
Fenamiphos	4	9.38	9.36- 9.39	303	153 (40), 195 (10), 260 (20)
Prothiophos	5	9.46	9.45- 9.47	309	189 (50), 205 (20), 239 (10)
Endosulfan α	5	9.49	9.47- 9.50	241	133 (40), 170 (40), 206 (30)
p,p'-DDE	5	9.54	9.52- 9.55	318	177 (50), 247 (20)
PCB 81	5	9.57	9.56- 9.59	290	185 (35), 220 (25)
				292	222 (15)
o,p'-DDD	5	9.60	9.59- 9.62	235	165 (20), 199 (20)
PCB 77	5	9.64	9.63- 9.65	220	150 (30)
				290	220 (20)
				292	222 (20)
Ethion	6	9.75	9.73- 9.76	231	129 (30), 175 (20), 185 (10)
Chloropropylate	6	9.71	9.70- 9.73	251	111 (20), 139 (20)
PCB 123/ PCB 118	6	9.75/ 9.78	9.74- 9.79/9.75- 9.82	254	184 (40)
				326	254 (25), 256 (25)
o,p'-DDT + p,p'-DDD	6	9.82	9.81- 9.84	236	165 (50), 201 (10)
PCB 114	6	9.86	9.84- 9.87	254	184 (30)
				326	254 (25), 256 (25)
PCB 153	6	9.91	9.89- 9.92	360	289 (25), 290 (30)
				362	292 (30)
Famphur	6	9.94	9.93- 9.96	218	93 (10), 109 (12)
PCB 105	6	9.97	9.95- 9.98	326	184 (50), 254 (40)
				328	256 (30)
PCB 138	6	10.11	10.19- 10.22	290	220 (40)
				360	290 (30), 325 (20)
Endosulfan sulphate	6	10.14	10.13- 10.16	272	165 (50), 237 (10)
PCB 167	7	10.33	10.31- 10.34	360	288 (15), 290 (15)
				362	292 (30)
EPN	7	10.45	10.44- 10.47	157	110 (10)
				169	77 (20), 141 (10)
PCB 156/ PCB 157	7	10.52/10.56	10.50- 10.54/10.55- 10.57	360	218 (50)
				362	290 (25), 292 (25)
PCB 180	7	10.63	10.62- 10.65	324	254 (50)

				394	324 (50), 359 (40)
PCB 169	7	10.85	10.84- 10.87	360	218 (50), 290 (35), 292 (40)
Mirex	7	11.18	11.17- 11.20	272	140 (40), 167 (40), 237 (20)

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430 Table 2
431 Validation parameters (n = 5) obtained for the target compounds at two concentration levels in egg
432 matrix
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Compound	15 µg/kg		50 µg/kg				
	Recovery (%)	*R.S.D. (%)*	*Recovery (%)*	*R.S.D. (%)*	*LOD (µg/kg)*	*LOQ (µg/kg)*	R^2
Dichlorvos	110	16	94	12	0,88	1,50	0,9964
Methamidophos	82	17	97	12	0,42	2,39	0,9990
Sulfotep	74	17	93	16	0,02	0,05	0,9991
Hexachlorobenzene	72	14	90	6	0,15	0,17	0,9973
Quintozene	107	15	91	11	0,30	0,48	0,9954
Etrimfos	70	19	94	12	0,40	0,83	0,9994
PCB 18	101	16	90	5	0,03	0,09	0,9995
Chlorpyriphos methyl	75	18	104	9	0,03	0,09	0,9901
Vinclozoline	94	13	81	15	0,03	0,09	0,9922
PCB 28 + PCB 31	78	10	93	9	0,04	0,12	0,9996
Parathion methyl	81	18	83	19	2,20	7,61	0,9894
Pirimiphos methyl	106	20	92	20	0,02	0,07	0,9968
Heptachlor	100	18	85	9	0,08	0,25	0,9944
Malathion	71	14	102	7	2,25	7,78	0,9998
PCB 52	105	5	102	4	0,41	0,67	0,9984
Chlorpyriphos ethyl	83	6	91	14	0,21	0,85	0,9980
Metolachlor	92	14	100	6	0,23	0,25	0,9888
Fenthion	98	7	88	10	0,20	1,67	0,9964
Parathion ethyl	107	3	99	13	0,78	2,84	0,9987
Pyrimiphos ethyl	102	13	89	15	0,85	0,85	0,9986
PCB 44	79	9	76	7	0,37	0,38	0,9917
Aldrin	99	17	83	17	0,05	0,13	0,9969
Bromophos methyl	109	19	100	20	0,88	0,88	0,9999
Isophenphos	85	6	101	14	0,42	0,91	0,9814
Chlorfenvinphos	103	18	106	12	0,53	3,21	0,9986
Heptachlor epoxide (Exo+Endo)	93	15	95	8	0,81	0,81	0,9986
Bromophos ethyl	102	7	87	6	0,12	0,34	0,9864
Tetrachlorvinphos	101	17	99	16	0,17	0,78	0,9877
PCB 101	77	8	88	8	0,15	0,28	0,9998
Fenamiphos	109	9	99	12	0,48	0,50	0,9999
Prothiophos	78	13	88	7	0,14	0,37	0,9995
Endosulfan α	99	15	92	8	0,27	0,91	0,9948
p,p'-DDE	84	16	95	11	0,11	0,36	0,9999
PCB 81	72	17	76	10	0,05	0,17	0,9948
o,p'-DDD	71	14	89	4	0,01	0,02	0,9949
PCB 77	70	11	77	8	0,06	0,20	0,9989
Ethion	102	18	78	7	0,27	0,86	0,9936
Chloropropylate	79	19	88	15	0,17	0,31	0,9955
PCB 118 + PCB 123	70	8	70	5	0,13	0,63	0,9938
o,p'-DDT + p,p'-DDD	71	12	87	7	0,05	0,24	0,9866
PCB 114	84	17	72	8	0,20	0,67	0,9989
PCB 153	83	7	104	9	0,04	0,12	0,9948
Famphur	108	9	80	19	0,12	1,32	0,9975
PCB 105	73	4	72	11	0,06	0,49	0,9973
PCB 138	74	10	92	11	0,07	0,16	0,9975
Endosulfan sulphate	85	19	96	11	0,90	1,40	0,9944
PCB 167	98	6	90	10	0,05	0,21	0,9994
EPN	73	20	76	18	0,02	1,01	0,9909
PCB 156	72	8	70	9	0,07	0,25	0,9881
PCB 157	70	20	73	9	0,08	0,27	0,9811
PCB 180	80	8	72	11	0,30	0,71	0,9965
PCB 169	84	9	92	19	0,08	0,26	0,9807
Mirex	73	20	82	18	0,15	0,50	0,9824

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