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1 **Development of a simple multiresidue extraction method for the quantification of**
2 **a wide polarity range list of pesticides and transformation products in eggs by**
3 **liquid chromatography and tandem mass spectrometry**

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31

32

33 Abstract

34 Waterfowl populations have been decreasing in Europe for the last years and pollution appears to be
35 one of the main factors. This study was conducted to develop a single sensitive and robust analytical
36 method for the monitoring of 2 fungicides, 15 herbicides, 3 insecticides and 24 transformation
37 products in wild bird eggs. One of the major challenges addressed was the characterization of
38 chemicals with large logP range (from -1.9 to 4.8). A total of 11 different extraction parameters were
39 tested in triplicate to optimize the extraction protocol, on generic parameters, buffer addition and use
40 of clean-up steps. Quantification was based on matrix-match approach with hen eggs as reference
41 matrix (34 analytes with $r^2 > 0.99$). Particular attention was paid to matrix effects (72 % on average),
42 quantification limits (0.5 to 25 ng.g^{-1} dry mass / 0.2 to 7.5 ng.g^{-1} fresh mass) and extraction yields
43 (46 to 87 % with 25 analytes up to 70 %) to ensure the relevance of the method and its compatibility
44 with ultra-trace analysis. It led to a simple solid/liquid low temperature partitioning extraction
45 method followed by LC-MS/MS. Analysis of 29 field samples from 3 waterfowl species revealed
46 that eggs were slightly contaminated with pesticides as only one egg presented a contamination
47 (terbutryn, herbicide, 0.7 ng.g^{-1}) and confirmed the relevance of the method.

48

49 **Keywords:** eggs; pesticides; multiresidue; SLE-LTP; LC-MS/MS; polar transformation products

50

51

52 **1. Introduction**

53 Nowadays we assist to the decline of bird population and diversity across the world [1,2], and plant
54 protection products (PPP) are identified as one of the main factor of this decline [3–5], affecting
55 pollination, seed dispersal, pest control and, thus, economy [6]. The analysis of wild bird eggs could
56 allow to acquire more impregnation data by PPP while preserving reproductive capacities of sampled
57 species and considering a highly vulnerable stage of development. Egg represents an important
58 analytical challenge: it contains ≥ 10 % of both proteins and lipids [7] which are well known to cause
59 matrix effects in extractions and analysis of organic chemicals [8–11], affecting sensitivity,
60 robustness and reliability of methods. Moreover, as their contamination depend on the ability of
61 pesticides to cross different biological membranes [12], contamination is expected to be low and the
62 final protocol should adress trace levels ($\text{ng}\cdot\text{g}^{-1}$).

63 Several works already focus on the characterisation of PPP in eggs. Most [7,12–17] are using a
64 QuEChERS (quick, easy, cheap, effective, rugged, and safe) approach, firstly developed by
65 Anastassiades and coworkers [18]. They monitored between 10 and >100 different pesticides based
66 on LC-MS/MS or GC-MS/MS, with limits of quantification (LOQ) from 0.5 to $10 \text{ ng}\cdot\text{g}^{-1}$. However,
67 nature of solvent, buffer salt and clean-up step can vary a lot between studies, according to the nature
68 of monitored PPP. On the other side, Luo et al. [19] successfully developed a solid-liquid
69 extraction with low temperature partitionning (SLE-LTP) that have to be mentioned as it allow the
70 quantification of 325 pesticides and some transformation products (TP) with most of LOQ below 1
71 $\text{ng}\cdot\text{g}^{-1}$ without using the salting-out effect.

72 Despite the very good performances of the mentioned studies, 3 major limits can be put forward.
73 First, most of characterized analytes are relatively hydrophobic as they are often organochlorines,
74 organophosphorines, permethrines, triazines or chloracetanilides, with $\log P$ close or up to 3. The
75 only exception is the characterization of neonicotinoids that are much more polar ($2 > \log P > -1$

76 according to [20]). Indeed, if less-polar pesticides are more incline to accumulate in organisms,
77 especially via trophic chain, they tend to be ban and replace with more polar ones which can impact
78 aquatic ecosystems and wildlife in general and, thus, have to be monitored. Secondly, recent studies
79 showed the importance of not only considering PPP in environmental investigations, but also their
80 TP as they may have lowest predicted no effect concentrations than active substances [21].
81 Moreover, they often are more polar than the active substance (weak acids or basis), which reinforce
82 the first argument of disposing from a method for the characterization of large polarity range analytes.
83 In studies mentioned above, no TP were monitored. Zhang et al. [22] integrated TP in their analyse
84 but they represent less than 10 % of the analytes. Finally, all previous protocols were based on the
85 analysis of mixed fresh eggs stored at 4 °C or -20 °C. In some cases this is not the easiest way to
86 store them over the long term as they deteriorate at room temperature and necessitate both
87 temperature control and energy source. This makes it difficult to store large series of samples over
88 the long term until they are analysed, while at the same time ensuring their proper conservation.

89 As a consequence, it is necessary to dispose of a quick and reliable method for the monitoring of
90 traces of large polarity range PPP and TP in complex matrices, and with an appropriate long term
91 storage. This study focuses on the development of a sole extraction method for 44 chemicals in
92 freeze-dried eggs: 20 active substances (herbicides, fungicides, and insecticides) but also 24 highly
93 polar and ionic TP. The method, based on QuEChERS extraction and a matrix-matched
94 quantification, should allow to quantify these chemicals at traces levels ($<10 \text{ ng.g}^{-1}$ dry mass), with
95 good extraction recoveries (ER, comprised between 80 % and 120 %) and least possible matrix
96 effects, taking in account the samples complexity. It was tested on a cohort of 29 eggs of 3 different
97 waterfowl species obtained from 4 different ponds in Sologne region (France) to be sure to provide a
98 functional tool for evaluating the impregnation of bird eggs by PPP and TP.

100 **2. Materials and methods**

101 *2.1. Standards and reagents*

102 Ultrapure water (Fisher Chemical, Geel, Belgium), acetonitrile (Honeywell, Seelze, Germany) and
103 heptane (Merck, Darmstadt, Germany) were of LC-MS quality. QuEChERS citrate buffer (4 g
104 magnesium sulfate, 1 g sodium chloride, 1 g sodium citrate dihydrate, 0.5 g sodium hydrogen
105 citrate), acetate buffer (6 g magnesium sulfate, 1.5 g sodium acetate) and NaCl buffer (6 g
106 magnesium sulfate, 1.5 g sodium chloride), and dispersive solid phase extraction salts EMR-lipids
107 (Enhanced Matrix Removal-Lipid) were purchased from Agilent (Santa Clara, USA). Dispersive
108 solid phase extraction reagent PSA (primary-secondary amine) was obtained from Chromebond
109 (Düren, Germany) and magnesium sulfate (MgSO_4) from Sigma-Aldrich (Saint Louis, USA). Formic
110 acid (FA) and ammonium sulfate were purchased from Biosolve-chemicals (Dieuze, France).
111 Zirconium oxide grinding balls (diameter 3 mm) were obtained from Fisher Scientific (Illkirch,
112 France).

113 Analytical standards were purchased from A2S (Martignas-sur-Jalle, France), Honeywell (Seelze,
114 Germany), HPC standards (Cunnersdorf, Germany), LGC group (Teddington, UK), Sigma-Aldrich
115 (Saint-Louis, USA) and TCI Europe (Anvers, Belgium). All were high purity level ($> 96\%$), with
116 most $>99\%$. Individual solutions made in acetonitrile (ACN) ($100\text{ mg}\cdot\text{L}^{-1}$) were stored at $-18\text{ }^\circ\text{C}$.
117 They were mixed to constitute a $5\text{ }\mu\text{g}\cdot\text{L}^{-1}$ solution used to develop and validate the extraction
118 protocol. Mix solutions were regularly characterized to check their stability over time and data were
119 compiled in a control chart. Stored at $18\text{ }^\circ\text{C}$, they were stable at least for 6 months.

120

121 *2.2. Sampling strategy*

122 Egg sampling was carried out in accordance with the country's legislation (Interministerial decree
123 NOR: DEVP0650375A, Environmental Code L424-11, [23]). It was performed during spring 2018
124 in the Sologne region (France). The prospection was done in four ponds to increase the probability to

125 find nests and to improve representativeness of studied sites. The sampled species were typical water
126 birds found in some French regions with different ecological behaviours: *Anas platyrhynchos*
127 (Mallard duck, Anatidae), *Aythya ferina* (Common pochard, Anatidae) and *Fulica atra* (Eurasian
128 coot, Rallidae). Only eggs incubated for less than 7 days were sampled with gloves. In the
129 laboratory, each egg was weighed before and after being broken. The content was roughly mixed and
130 frozen at -20 °C in aluminum tray. Then, it was freeze-dried (Reacti-Vap from PIERCE, Bellafonte,
131 USA), grinded and stocked at room temperature.

132 Eggs from hens kept in battery were used as reference matrix for protocol development, protocol
133 blanks, or positive controls in extraction batches when spiked with analytical standards. They were
134 pretreated as field samples.

135

136 2.3. *QuEChERS-based extraction*

137 Different conditions were tested to optimize the extraction procedure. The first applied protocol
138 (Ref#1) (Fig. 1) was based on a simple SLE-LTP, aiming to increase its complexity until reaching
139 QuEChERS approaches. Two hundred milligrams of reference matrix were weighted in a 50 mL
140 polypropylene centrifuge tube (VWR, Radnor, USA) and spiked with 50 µL of a mix solution
141 containing all analytes and 75 µL of a mix solution containing all isotope labeled standards, both at
142 nominal concentration 500 µg.L⁻¹. Solution solvent was evaporated under gentle nitrogen flux for 10
143 min. A volume of 5 mL of ultrapure water was added to the sample, mixed together for 5 min at 16
144 Hz with Geno/Grinder (SamplePrep 2010 Geno/Grinder from SPEX, Costa Mesa, USA) to re-
145 hydrate the sample. Then 10 mL of ACN and 5 mL of heptane were added in the centrifuge tube and
146 mixed together for 5 min at 16 Hz with Geno/Grinder to extract analytes and remove part of non-
147 polar interferents. Samples were then centrifuged for 5 min at 9500 RCF at 20 °C (SIGMA 3K3OH,
148 Fisher, Osterode am Harz, Germany). Six milliliters of the ACN/water mix were transferred in new
149 centrifuge tube containing PSA, handshaked for 30 s and centrifuged 2 min 9500 RCF to separate

150 salts from extract. Final extract was aliquoted: 1 mL was transferred in a 2 mL glass injection vial
151 (from Supelco, USA) and stored at -20 °C until analysis. Generic parameter modifications were
152 tested to improve the protocol: replacing ACN with methanol (MeOH), increasing the mass of
153 sample to 0.5 g, increasing the extraction time to 10 min, acidification of ACN (0.2 %FA),
154 introducing QuEChERS buffers (NaCl, acetate or citrate) and replacing PSA d-SPE salts with EMR-
155 lipid salts or MgSO₄. The comparison of samples spiked before or after extraction (in triplicate and
156 by a single operator) allowed to calculate extraction yield and estimate limits of quantification
157 (LOQ) of each tested condition. Twenty microliters of the final extract were diluted in 180 µL of
158 ultrapure water + 0.1 %FA (v:v) before injection.

159 The optimized protocol was used to evaluate if sample preparation (e.g. freeze drying) affected the
160 representativeness of samples. Fresh hen eggs were broken, weighted and mixed and 57 g of it was
161 spiked with 1 mL of the 500 µg.g⁻¹ standards mix solution while 54 g were supplemented with 1 mL
162 of ACN as reference. Both were strongly mixed for 2 min to ensure good samples homogeneity,
163 aliquoted in 3 distinct samples and then freeze dried (Water represented 80 % ± 2 % of the total egg
164 mass; n = 6). Reference samples were spiked with 50 µL of mix solution (taking into account
165 dehydration) and all samples were extracted following final protocol (section 3.2. and Fig. 1).

166

167 2.4. LC-MS/MS

168 The following analytical method was initially developed in the Laboratory of Hydrology of Nancy
169 (ANSES-LHN, Nancy, France) for the quantification of 88 pesticides and transformation products in
170 water bodies, among which the 44 herein targeted chemicals. Analysis was performed with a 1290
171 Infinity UPLC (Agilent Technologies, Massy, France) coupled with 5500 QTRAP (Sciex, Villebon
172 sur Yvette, France) in both positive and negative modes, using electrospray ionization. Separation
173 was performed at 0.4 mL.min⁻¹ at 50 °C over a Xselect® HSS T3 column 2.1x5 mm, 2.5 µm
174 (Waters, Saint Quentin-en-Yvelines, France). The column was protected with a KrudKatcher Ultra

175 (Phenomenex, Le Pecq, France) and a Xselect® HSS T3 precolumn 2.1x5 mm, 2.5 µm (Waters,
176 Saint Quentin-en-Yvelines, France). Ultrapure water + 0.1 %FA (A) and ACN + 0.1 %FA (B) were
177 used as mobile phases in positive mode while 1 mM ammonium acetate in ultrapure water (A) and
178 ACN (B) were used in negative mode. The gradient was the same for both methods, started with 10
179 % B for 1 min, increased to 100 % B in 7 min, held 6 min, back to 10 % B in 1 min and held 4 min.
180 Temperature of the mass spectrometer source, nebulizer gas, heating gas, were respectively settled at
181 550 °C, 40 psi and 50 psi. Ion spray voltages were optimal at 5500 eV for positive ionization and -
182 4500 eV, for negative ionization.

183 Analysis parameters of each compound, including multiple reaction monitoring (MRM) transitions,
184 are summarized in SI Table S1. Analyte infusions were performed in MeOH:H₂O mix (50:50), with
185 formic acid in positive mode and with ammonium acetate in negative mode, to ensure good signal
186 intensity. Transitions were automatically optimized with Analyst 1.6.2 and the 2 most intense and
187 sensitive ones were kept for each analyte. Nature of phases, flow, injection volume and column
188 temperature were tested to ensure stable retention times, good separation, and good intensity on the
189 chromatographic peaks in both modes. The most sensitive transition of each analyte was kept for
190 quantification and the other as confirmation.

191

192 2.5. Method validation

193 The matrix-matched approach was validated over six days with two different operators (three days
194 each), following the experimental organization described by Giroud *et al.* [24] and the SANTE
195 Guidance [25], and changing extraction materials every day. Hen eggs samples spiked with
196 analytical standards from LOQ/2 to 7xLOQ (6 concentration levels) and a matrix blank (reference
197 matrix extracted without standards spiking) were extracted each day for three days by each of the
198 operators to determine regression law for each of the analytes. Each day one of the concentration
199 levels (LOQ, 3xLOQ, 7xLOQ) was extracted in triplicate to document the intermediate precision of

200 all parameters. Matrix effect (ME, in %), extraction recoveries (ER, in %) were calculated as follows
201 (equation 1 and 2):

$$202 \quad ME = \frac{\text{Area of post-extraction spike}}{\text{Area of solution reference}} \times 100 - 100 \quad (1)$$

$$203 \quad ER = \frac{\text{Area of pre-extraction spike}}{\text{Area of post-extraction spike}} \times 100 \quad (2)$$

204 Herein ER were determined by comparison of artificial samples spiked before and after the
205 extraction (LOQ, 3xLOQ, 7xLOQ) on two triplicates for each concentration level. Reference matrix
206 samples spiked after extraction were also compared with the analytical response of a solution of
207 equivalent constitution to calculate ME for each analyte.

208

209 *2.6. Method application*

210 The developed method was applied on 29 waterfowl eggs (section 2.2.) for pesticides quantification,
211 with a six points matrix match calibration curve for each sequence (same concentrations as described
212 in section 2.3.) and following the European Commission recommendations [25]. A blank (to track
213 contamination) and an artificial sample made from reference matrix spiked with analytes at LOQ
214 level (to calculate recoveries and LOQ) were also extracted. To preserve analytical relevance of the
215 results, protocol blanks were taken into account by increasing LOQ at three times the concentration
216 in blanks when a contamination was identified.

217

218 **3. Results and discussion**

219 *3.1. Optimization of extraction conditions*

220 Sequential modifications of the protocol were tested compared to the protocol described in section
221 2.3. of this article. Performances were evaluated with the aim of centering ER between 80 and 120

222 %, and keeping LOQ below 10 ng.g⁻¹ to analyze contaminant traces. All these performances are
223 described in sections below and are summarized in Fig. 2.

224 The nature of the solvent, the mass of matrix extracted, contact times between sample and solvents
225 can all play role in the determination of a performant extraction protocol. A total of 4 different
226 versions of the reference protocol based on generic parameter changes were tested in triplicates to
227 evaluate their impact on LOQ and ER: (1) ACN was replaced with MeOH, (2) the mass of matrix
228 was increased from 0.2g to 0.5g, (3) the mixing time was increased to 10 min, and (4) ACN was
229 acidified with 0.2 % AF.

230

231 3.1.1. Preliminary tests

232 ACN is a commonly used organic solvent for the extraction of polar pesticides from solid matrices
233 and more particularly in QuEChERS approaches [12–14,17,18,24,26] as it is easily separable from
234 water with the adjunction of salts [18]. MeOH was tested in parallel as it is also a polar solvent that
235 may be useful for the extraction of most polar molecules. The use of MeOH instead of ACN
236 decreased the ER by 40 %, and only 2 analytes presented good ER (120 % > ER > 70 %) while 8
237 were concerned for Ref#1. Otherwise, the solvent change did not affect the global LOQ as 26 and 22
238 analytes presented LOQ below 10 ng.g⁻¹ for Ref#1 and MeOH condition respectively. The difference
239 in ER was probably due to matrix effects: (1) MeOH can extract a more important part of the matrix,
240 leading to strong signal extinction and low ER determination, and (2) MeOH is well known to
241 increase the ionization of chemicals in the mass spectrometer source which may have led to more
242 important noise on chromatograms even in MRM acquisition. This solvent may also induce an
243 esterification of some of the acidic molecules as proposed by Dong *et al.* [27] leading to some of the
244 low ER observed.

245 The mass of analyzed sample has to be considered: this parameter increases the quantity of available
246 analytes for extraction and can therefore decrease the LOQ, but it may also increase the quantity of

247 interferences extracted, leading to more matrix effects. In our case, increasing sample mass from 0.2 g
248 to 0.5 g strongly reduced the LOQ with respectively 22 and 35 analytes quantifiable below 10 ng.g⁻¹.
249 Moreover, the number of analytes quantified above 50 ng.g⁻¹ decreased from 4 to 2. At the same
250 time, 5 more molecules presented good ER when matrix mass was increased.

251 Mixing time is also an important parameter: an increase leads to better interaction between solvent
252 and matrix and can contribute to a better ER. Extending the extraction time from 5 to 10 min increased
253 the LOQ by a factor 2 on average with still 21 analytes quantifiable under 10 ng.g⁻¹. However, ER
254 were improved as 14 analytes presented good recoveries and only 4 of them were up to 120 %, with
255 no outlier >200 % which ensure a better quantification. Moreover, compared to the Ref#1, longer
256 mixing time limited the variability of the extraction process as the %RSD on extraction recoveries
257 decreased from 40 % on average to only 11 %.

258 Some of the analyzed chemicals (especially TP) are weak acids that may be bonded to the matrix in the
259 Ref#1 conditions. Acidification at pH close or lower than the pKa of acidic molecules may increase
260 extraction performances by favoring their neutral form, less susceptible to interact with matrix or
261 water and ease the transfer to organic solvent [28]. The acidification of the ACN with 0.2 % of FA
262 (v:v) increased LOQ by a factor 2 on average but 21 chemicals presented LOQ below 10 ng.g⁻¹, but
263 ER were close to Ref#1 with only 7 analytes with ER comprised between 70 % and 120 %.
264 Nevertheless, acidification reduced the outliers as no analyte presented ER up to 200 %. It is also
265 important to mention that acidification induced better separation between the different fractions and
266 a more compact solid deposit after centrifugation, leading to quicker and easier-to-perform clean-up
267 step.

268

269 3.1.2. Determination of a new reference protocol

270 The reference protocol presented higher LOQ and lower ER performances than any of the alternative
271 extraction tested. As a consequence, Ref#1 was modified to integrate key strengths of previously

272 evaluated parameters before testing new buffers and clean-up steps: mixing times were increased to
273 10 min and the ACN was acidified with FA (+0.2 % v:v). As extracts were significantly more
274 pigmented with 0.5 g of sample than with 0.2 g, and as pigmentation could be linked with matrix
275 effects and presence of unwanted molecules, this parameter was temporarily set at 0.2 g for Ref#2 to
276 preserve the analytical system. New protocol presented good quantification limits as 30 chemicals
277 had LOQ inferior to 10 ng.g⁻¹. In parallel ER comprised between 80 % and 120 % concerned 19
278 chemicals and only 5 ones are up to 120 %.

279

280 3.1.3. Impact of buffer addition and selection of d-SPE cleanup

281 All parameters tested in this section were built from the new reference Ref#2 (section 3.1.5.) as it
282 represented a significant improvement of initial protocol (Fig. 1). The addition of salts as in
283 QuEChERS approaches can lead to improve ER of the protocol, especially for the most polar
284 chemicals [30,33,36,40,41,49] because of the salting-out effect that force the transfer of chemicals
285 from the water layer to the organic solvent layer. The buffer used in the original QuEChERS method,
286 NaCl [18], the acetate buffer recommended by the American standard (AOAC) [29] and the citrate
287 buffer used in the European standard EN 15662 [30] were tested. Compared to Ref#2, quantification
288 limits were higher when a buffer was used: the number of molecules with LOQ inferior to 10 ng.g⁻¹
289 for NaCl, acetate and citrate condition was respectively 23, 18 and 19, and ones with LOQ up to 100
290 ng.g⁻¹ concerned 2, 6 and 5 analytes respectively. Concerning ER, protocols had 18, 22 and 17
291 molecules with good rates. Acetate buffer which is recommended in standardized American
292 QuEChERS protocols [29] presented the best performances in terms of ER while NaCl allowed to
293 get the lowest LOQ. However, compared to Ref#2, adjunction of any buffer almost systematically
294 decreased the average extraction recovery of acetochlor ESA /alachlor ESA, acetochlor OXA, CGA
295 357704, CGA 368208, dimetachlor ESA, dimethenamid ESA, flufenacet ESA, flufenacet OXA,
296 MCPA, metazachlor ESA, metolachlor ESA and metolachlor OXA to 15 % on average. The addition

297 of important quantities of salts may increased the pH up to the pKa of these molecules, leading to the
298 dominance of the ionic forms, more strongly bounded to the water layer and more hardly extractible
299 with ACN [28], which may explain the poor observed recoveries. Buffer was removed from final
300 protocol because transformation products were strongly impacted by this phenomenon and because
301 the priority of this study was to monitor these TP in biota as very few data are available.

302 D-SPE step can increase method performance by purifying the extract and removing interferences,
303 leading to less matrix effects, but the use of unsuitable salts can also remove analytes. Lipids are
304 often considered as responsible for matrix effect in biotics matrices and can be partially removed
305 with d-SPE [10]. PSA is one of the most widely used d-SPE cleanup mix for the removal of fatty
306 acids [18,31,32] and was *de facto* integrated into the protocols Ref#1 and Ref#2. EMR-lipids mix
307 was tested as it is specifically designed to remove lipids without affecting extraction recoveries of
308 PPP [32] and presented good results for extraction of PPP in eggs [19]. A condition with only
309 MgSO₄ was also tested as this salt is often used in association with the PSA and EMR-lipids to
310 remove water from samples [18,24,32] and force the transfer of analytes from water to organic
311 solvents. The two new conditions tested respectively had 23 and 27 molecules with LOQ lower than
312 10 ng.g⁻¹. ER were acceptable (80-120 %) respectively for 17 and 35 chemicals and none of the
313 experimental conditions presented ER up to 120 %. If all conditions were comparable in terms of
314 LOQ, the addition of MgSO₄ clearly stands out with both good ER for large range of chemicals,
315 including TP, while those of EMR-lipid were close to Ref#2 with no great improvement, nor
316 degradation of performances apart for benzamide (0 ER, no LOQ definable). In opposition to Luo *et*
317 *al.* [19], it seemed that EMR-Lipid causes the removal of many chemicals causing the diminution of
318 ER by average 24 % compared to Ref#2, probably because we studied a wider range of polarity. On
319 the contrary, the use of MgSO₄ only, forced the transfer of chemicals from water to ACN, even for
320 the most polar ones, and part of interferences may have precipitated as chromatograms showed less
321 background noise and as no perturbation of LOQ was highlighted.

3.2. Impact of freeze drying on extraction yields

As specified in section 2.2., due to logistical limitations, sampled eggs were broken, mixed and freeze-dried in laboratory for appropriate storage. As the analytical method was not developed yet when samples were collected (from April 2018 to July 2018 and analyzed in May 2019), they could not be analyzed fresh as in other studies [13,14,16,17] or after being stored at -20 °C [12,15]. The impact of freeze drying was evaluated as stated in section 2.3. and results are presented in Fig. 3.

Freeze drying did not affect the representativeness of the sample for most contaminants as they presented extraction yields between 80 % and 120 %. However, it led to a loss for flufenacet (53 %), dimetachlor (66 %), fenthion (73 %) and s-metolachlor (78 %). CGA 357704 was also affected as it presented extraction yields of 80 % but with an RSD of 39 % while it was below 20 % for other chemicals. These chemicals may have bounded more strongly with the matrix during the time of freeze-drying (48 h) that was much longer than the usual contact time between the native solution and the matrix for the constitution or artificial samples (10 min). In this case, the developed protocol was not efficient for the characterization of these molecules. Studies on the effect of an increasing contact time between matrix and analytes would confirm these observations to evaluate the role of this parameter in the representativeness of artificial samples toward field samples, as it was identified as an important limit in matrix match approaches [33].

3.3. Analytical performances and validation of the method

Method performance parameters are presented in Table 1.

Linear regression with r^2 up to 0.99 was successfully determined for 34 chemicals. Among the 44 analytes, 33 analytes presented $LOQ \leq 10 \text{ ng.g}^{-1}$ and all were $\leq 25 \text{ ng.g}^{-1}$. Moreover, taking into account the hydration rate of samples, these LOQ are 72 % lower on average when considering the fresh mass concentration which is in the high LOQ trends of Luo *et al.* [19] (0.03 to 23 ng.g^{-1}). Considering matrix effects, only metazachlor ESA (225 %) presented a signal enhancement and only 3 chemicals presented signal extinction: prosulfocarb (34 %), dimethenamid ESA (40 %) and

347 metolachlor ESA (48 %). Indeed, matrix effects were low, with 72 % average, which is consistent
348 with literature as signal extinction seemed to affected most part of chemicals in complex matrices
349 [26,34]. However, matrix effect did not affect the relevance of the analysis as matrix match
350 quantification should take them into account if the reference matrix is well chosen (discussed section
351 3.4.).

352 ER at LOQ were comprised between 46 % (metolachlor OXA) and 87 % (imidacloprid), with 25
353 analytes with ER comprised between 70 % and 120 % as recommended by European Commission
354 [25]. However, the inter-day precision was below or close to 20 % (upper values were 21 % for CGA
355 357704 at LOQ, 22 % for metsulfuron-methyl at LOQ and 22 % for metolachlor OXA at 3xLOQ),
356 assuming that the quantification was precise for the tested chemicals. Definitive LOQ according to
357 SANTE guidelines are referenced Table 1.

358 No signal was detected in blanks for most of the chemicals monitored (Table 1). Contaminations
359 ranged from 0.1 ng to 8.9 ng, corresponding to 0.2 and 17.8 ng.g⁻¹ dry mass respectively. No
360 chemical was systematically quantified in blanks but 7 pesticides and TP presented unique
361 contamination over the 6 extraction days, 2 were quantified twice in blanks, and 4 were quantified in
362 more than half of blanks (fipronil sulfone, prosulfocarb, s-metolachlor and terbutryn).

363

364 *3.4. Field sample analysis*

365 Spiking with isotope labeled standards was used to check if the reference matrix (hen eggs) used for
366 quantification was close enough to field samples, by comparing their respective areas. Average ratio
367 was comprised between 80 % and 120 % for all internal standards except for D5-acetochlor ESA
368 (124 ± 19 %, ESI neg, 4.9 min) and D6-chlorpyriphos methyl (123 ± 45 %, ESI pos, 8.6 min). It
369 showed a good correspondence between the reference matrix and field samples, and so proved the
370 good reliability of the method. PCA (Fig. S1) was performed on these ratios to determine if clusters
371 of samples can be identified taking into account egg species (XLstats by Addinsoft Inc, New York,

372 USA). The PCA was based on Pearson regression ($\alpha = 0.005$), axis 1 (47.2 %) and 2 (29.0 %)
373 explain 76.1 % of the total variability but no clear clusters could be identified. It attested of a
374 relatively homogeneous matrix complexity between different species, but also between all samples.
375 It confirmed the good reliability of the developed protocol with field samples, and so the suitability
376 of this method for the monitoring of wide polarity range of pesticides in eggs.

377 Over the 29 collected and analyzed environmental samples, only 1 coot egg presented a
378 contamination superior to LOQ for terbutryn (3.1 ng.g^{-1} dry mass) for LOQ at 2.4 ng.g^{-1} taking in
379 account contamination as mentioned sections 2.6. and 3.3. Considering the hydration ratio of the
380 sample (78.5 %), it corresponded to 0.7 ng.g^{-1} fresh mass contamination or 22 ng for the whole egg.

381 This molecule presented a contamination induced by the extraction protocol (estimated to $0.13 \pm$
382 0.02 ng.g^{-1} dry mass in the extraction batch of the considered sample). It was maybe due to the actual
383 use of terbutryn as a biocide in some paintings, for the protection of building materials against
384 moisture or microalgae [35–37] which can create contamination in laboratories. However, the
385 quantity found in blanks was quite constant and their area was systematically removed for
386 quantification. Moreover, the LOQ was increased at 3 times the concentration of terbutryn found in
387 blanks, so the communicated results were reliable. The quantification of terbutryn in coot egg
388 confirmed that the protocol developed is adapted to quantification of pesticides in waterfowl eggs
389 with good sensitivity and ER, and with acceptable matrix effects even if the contamination was
390 below the quantification limit in most cases.

391

392 **4. Conclusion**

393 We successfully developed a sensitive, robust, accurate and reliable extraction protocol for the
394 characterization of 20 PPP and 24 of their TP in eggs, based on matrix matched quantification. About
395 80 % of LOQ were below 10 ng.g^{-1} dry mass and more than 50 % of extraction yields were
396 comprised between 70 % and 100 %, which makes it compatible with trace monitoring in complex

397 matrices. Special attention was paid to matrix effect as they were evaluated on artificial samples for
398 method validation, but also directly in samples with the survey of isotope labeled standards signals.
399 The application of the developed method to 29 real waterfowl eggs sampled in the French region
400 Sologne, revealed very low contamination for the monitored PPP and TP in this biotic matrix. Only
401 one egg presented a concentration higher than the LOQ of terbutryn.

402

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411

412 **References**

- 413 [1] E. Pennisi, Billions of North American birds have vanished, *Science*. 365 (2019) 1228–1229.
414 <https://doi.org/10.1126/science.365.6459.1228>.
- 415 [2] Y. Si, Y. Xu, F. Xu, X. Li, W. Zhang, B. Wielstra, J. Wei, G. Liu, H. Luo, J. Takekawa, S.
416 Balachandran, T. Zhang, W.F. de Boer, H.H.T. Prins, P. Gong, Spring migration patterns,
417 habitat use, and stopover site protection status for two declining waterfowl species wintering
418 in China as revealed by satellite tracking, *Ecol. Evol.* 8 (2018) 6280–6289.
419 <https://doi.org/10.1002/ece3.4174>.
- 420 [3] A.M. Calvert, C.A. Bishop, R.D. Elliot, E.A. Krebs, T.M. Kydd, C.S. Machtans, G.J.
421 Robertson, A Synthesis of Human-related Avian Mortality in Canada, *Avian Conserv. Ecol.* 8

- 422 (2013) art11. <https://doi.org/10.5751/ACE-00581-080211>.
- 423 [4] P. Mineau, M. Whiteside, Pesticide Acute Toxicity Is a Better Correlate of U.S. Grassland
424 Bird Declines than Agricultural Intensification, *PLoS One*. 8 (2013) e57457.
425 <https://doi.org/10.1371/journal.pone.0057457>.
- 426 [5] R.L. Stanton, C.A. Morrissey, R.G. Clark, Analysis of trends and agricultural drivers of
427 farmland bird declines in North America: A review, *Agric. Ecosyst. Environ.* 254 (2018) 244–
428 254. <https://doi.org/10.1016/j.agee.2017.11.028>.
- 429 [6] K. V. Rosenberg, A.M. Dokter, P.J. Blancher, J.R. Sauer, A.C. Smith, P.A. Smith, J.C.
430 Stanton, A. Panjabi, L. Helft, M. Parr, P.P. Marra, Decline of the North American avifauna,
431 *Science*. 366 (2019) 120–124. <https://doi.org/10.1126/science.aaw1313>.
- 432 [7] F. Hildmann, C. Gottert, T. Frenzel, G. Kempe, K. Speer, Pesticide residues in chicken eggs –
433 A sample preparation methodology for analysis by gas and liquid chromatography/tandem
434 mass spectrometry, *J. Chromatogr. A*. 1403 (2015) 1–20.
435 <https://doi.org/10.1016/j.chroma.2015.05.024>.
- 436 [8] B. Holmes, A. Dunkin, R. Schoen, C. Wiseman, Single-Laboratory Ruggedness Testing and
437 Validation of a Modified QuEChERS Approach To Quantify 185 Pesticide Residues in
438 Salmon by Liquid Chromatography– and Gas Chromatography–Tandem Mass Spectrometry,
439 *J. Agric. Food Chem.* 63 (2015) 5100–5106. <https://doi.org/10.1021/jf5055276>.
- 440 [9] P. Kaczyński, B. Łozowicka, M. Perkowski, J. Szabuńko, Multiclass pesticide residue analysis
441 in fish muscle and liver on one-step extraction-cleanup strategy coupled with liquid
442 chromatography tandem mass spectrometry, *Ecotoxicol. Environ. Saf.* 138 (2017) 179–189.
443 <https://doi.org/10.1016/j.ecoenv.2016.12.040>.
- 444 [10] P. Kaczyński, Clean-up and matrix effect in LC-MS/MS analysis of food of plant origin for

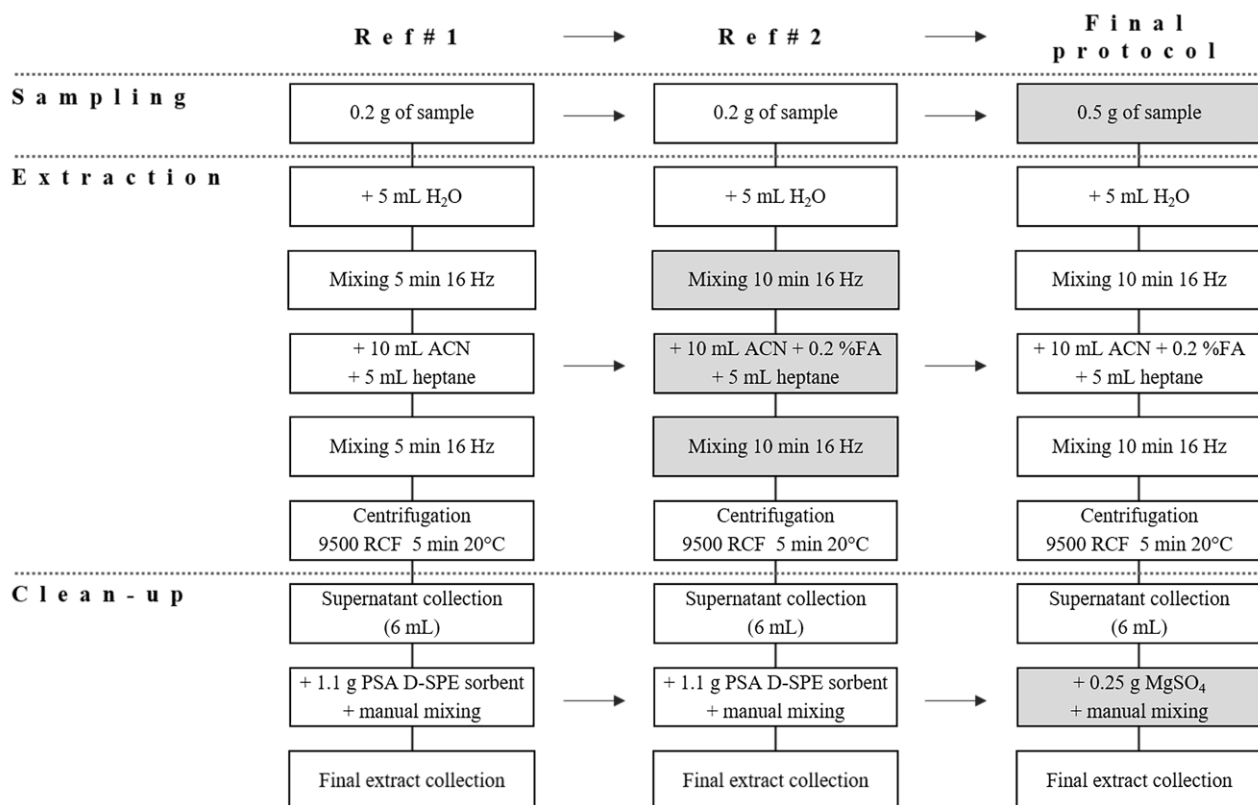
- 445 high polar herbicides, *Food Chem.* 230 (2017) 524–531.
446 <https://doi.org/10.1016/j.foodchem.2017.03.091>.
- 447 [11] R. Romero-González, A. Garrido Frenich, Application of HRMS in Pesticide Residue
448 Analysis in Food From Animal Origin, in: *Appl. High Resolut. Mass Spectrom.*, Elsevier,
449 2017: pp. 203–232. <https://doi.org/10.1016/B978-0-12-809464-8.00007-5>.
- 450 [12] E. Bro, J. Devillers, F. Millot, A. Decors, Residues of plant protection products in grey
451 partridge eggs in French cereal ecosystems, *Environ. Sci. Pollut. Res.* 23 (2016) 9559–9573.
452 <https://doi.org/10.1007/s11356-016-6093-7>.
- 453 [13] K. Lichtmanegger, R. Fischer, F.X. ave. Steemann, H. Unterluggauer, S. Masselter,
454 Alternative QuEChERS-based modular approach for pesticide residue analysis in food of
455 animal origin, *Anal. Bioanal. Chem.* 407 (2015) 3727–3742. [https://doi.org/10.1007/s00216-](https://doi.org/10.1007/s00216-015-8594-5)
456 [015-8594-5](https://doi.org/10.1007/s00216-015-8594-5).
- 457 [14] S. Choi, S. Kim, J.Y. Shin, M. Kim, J.-H. Kim, Development and verification for analysis of
458 pesticides in eggs and egg products using QuEChERS and LC–MS/MS, *Food Chem.* 173
459 (2015) 1236–1242. <https://doi.org/10.1016/j.foodchem.2014.10.143>.
- 460 [15] Y. Li, Z. Chen, R. Zhang, P. Luo, Y. Zhou, S. Wen, M. Ma, Simultaneous Determination of
461 42 Pesticides and Herbicides in Chicken Eggs by UHPLC–MS/MS and GC–MS Using a
462 QuEChERS-Based Procedure, *Chromatographia.* 79 (2016) 1165–1175.
463 <https://doi.org/10.1007/s10337-016-3132-y>.
- 464 [16] X. Xu, X. Xu, M. Han, S. Qiu, X. Hou, Development of a modified QuEChERS method based
465 on magnetic multiwalled carbon nanotubes for the simultaneous determination of veterinary
466 drugs, pesticides and mycotoxins in eggs by UPLC-MS/MS, *Food Chem.* 276 (2019) 419–
467 426. <https://doi.org/10.1016/j.foodchem.2018.10.051>.

- 468 [17] N.-E. Song, J.Y. Lee, A.R. Mansur, H.W. Jang, M.-C. Lim, Y. Lee, M. Yoo, T.G. Nam,
469 Determination of 60 pesticides in hen eggs using the QuEChERS procedure followed by LC-
470 MS/MS and GC-MS/MS, *Food Chem.* 298 (2019) 125050.
471 <https://doi.org/10.1016/j.foodchem.2019.125050>.
- 472 [18] M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, Fast and Easy Multiresidue
473 Method Employing Acetonitrile Extraction/Partitioning and “Dispersive Solid-Phase
474 Extraction” for the Determination of Pesticide Residues in Produce, *J. AOAC Int.* 86 (2003)
475 412–431. <https://doi.org/10.1093/jaoac/86.2.412>.
- 476 [19] P. Luo, X. Liu, F. Kong, L. Tang, Q. Wang, W. Li, W. Xu, S. Wen, L. Chen, Y. Li, Multi-
477 residue determination of 325 pesticides in chicken eggs with EMR-Lipid clean-up by
478 UHPLC–MS/MS and GC–MS/MS, *Chromatographia.* 83 (2020) 593–599.
479 <https://doi.org/10.1007/s10337-020-03876-1>.
- 480 [20] K.A. Lewis, J. Tzilivakis, D.J. Warner, A. Green, An international database for pesticide risk
481 assessments and management, *Hum. Ecol. Risk Assess. An Int. J.* 22 (2016) 1050–1064.
482 <https://doi.org/10.1080/10807039.2015.1133242>.
- 483 [21] Z. Tousova, P. Oswald, J. Slobodnik, L. Blaha, M. Muz, M. Hu, W. Brack, M. Krauss, C. Di
484 Paolo, Z. Tarcai, T.-B. Seiler, H. Hollert, S. Koprivica, M. Ahel, J.E. Schollée, J. Hollender,
485 M.J.F. Suter, A.O. Hidasi, K. Schirmer, M. Sonavane, S. Ait-Aissa, N. Creusot, F. Brion, J.
486 Froment, A.C. Almeida, K. Thomas, K.E. Tollefsen, S. Tufi, X. Ouyang, P. Leonards, M.
487 Lamoree, V.O. Torrens, A. Kolkman, M. Schriks, P. Spirhanzlova, A. Tindall, T. Schulze,
488 European demonstration program on the effect-based and chemical identification and
489 monitoring of organic pollutants in European surface waters, *Sci. Total Environ.* 601–602
490 (2017) 1849–1868. <https://doi.org/10.1016/j.scitotenv.2017.06.032>.
- 491 [22] X. Zhang, Y. Song, Q. Jia, L. Zhang, W. Zhang, P. Mu, Y. Jia, Y. Qian, J. Qiu, Simultaneous

- 492 determination of 58 pesticides and relevant metabolites in eggs with a multi-functional filter
493 by ultra-high performance liquid chromatography-tandem mass spectrometry, *J. Chromatogr.*
494 *A.* 1593 (2019) 81–90. <https://doi.org/10.1016/j.chroma.2019.01.074>.
- 495 [23] Arrêté du 7 juillet 2006 portant sur l'introduction dans le milieu naturel de grand gibier ou de
496 lapins et sur le prélèvement dans le milieu naturel d'animaux vivants d'espèces dont la chasse
497 est autorisée (NOR: DEVP0650375A, Art. L424-11), Code l'Environnement. July 7, 2006.
- 498 [24] B. Giroud, A. Vauchez, E. Vulliet, L. Wiest, A. Buleté, Trace level determination of
499 pyrethroid and neonicotinoid insecticides in beebread using acetonitrile-based extraction
500 followed by analysis with ultra-high-performance liquid chromatography–tandem mass
501 spectrometry, *J. Chromatogr. A.* 1316 (2013) 53–61.
502 <https://doi.org/10.1016/j.chroma.2013.09.088>.
- 503 [25] European Commission DG-SANTE, Analytical Quality Control and Method Validation
504 Procedures for Pesticide Residues Analysis in Food and Feed, N° SANTE/12682/2019, (2019)
505 49.
- 506 [26] A. Lazartigues, L. Wiest, R. Baudot, M. Thomas, C. Feidt, C. Cren-Olivé, Multiresidue
507 method to quantify pesticides in fish muscle by QuEChERS-based extraction and LC-MS/MS,
508 *Anal. Bioanal. Chem.* 400 (2011) 2185–2193. <https://doi.org/10.1007/s00216-011-4945-z>.
- 509 [27] X. Dong, S. Liang, Z. Shi, H. Sun, Development of multi-residue analysis of herbicides in
510 cereal grain by ultra-performance liquid chromatography–electrospray ionization-mass
511 spectrometry, *Food Chem.* 192 (2016) 432–440.
512 <https://doi.org/10.1016/j.foodchem.2015.07.025>.
- 513 [28] M. Anastassiades, E. Scherbaum, B. Tadelén, D. Štajnbaher, Recent Developments in
514 QuEChERS Methodology for Pesticide Multiresidue Analysis, in: H. Ohkawa, H. Miyagawa,
515 P.W. Lee (Eds.), *Pestic. Chem. Crop Prot. Public Heal. Environ. Saf.*, WILEY-VCH,

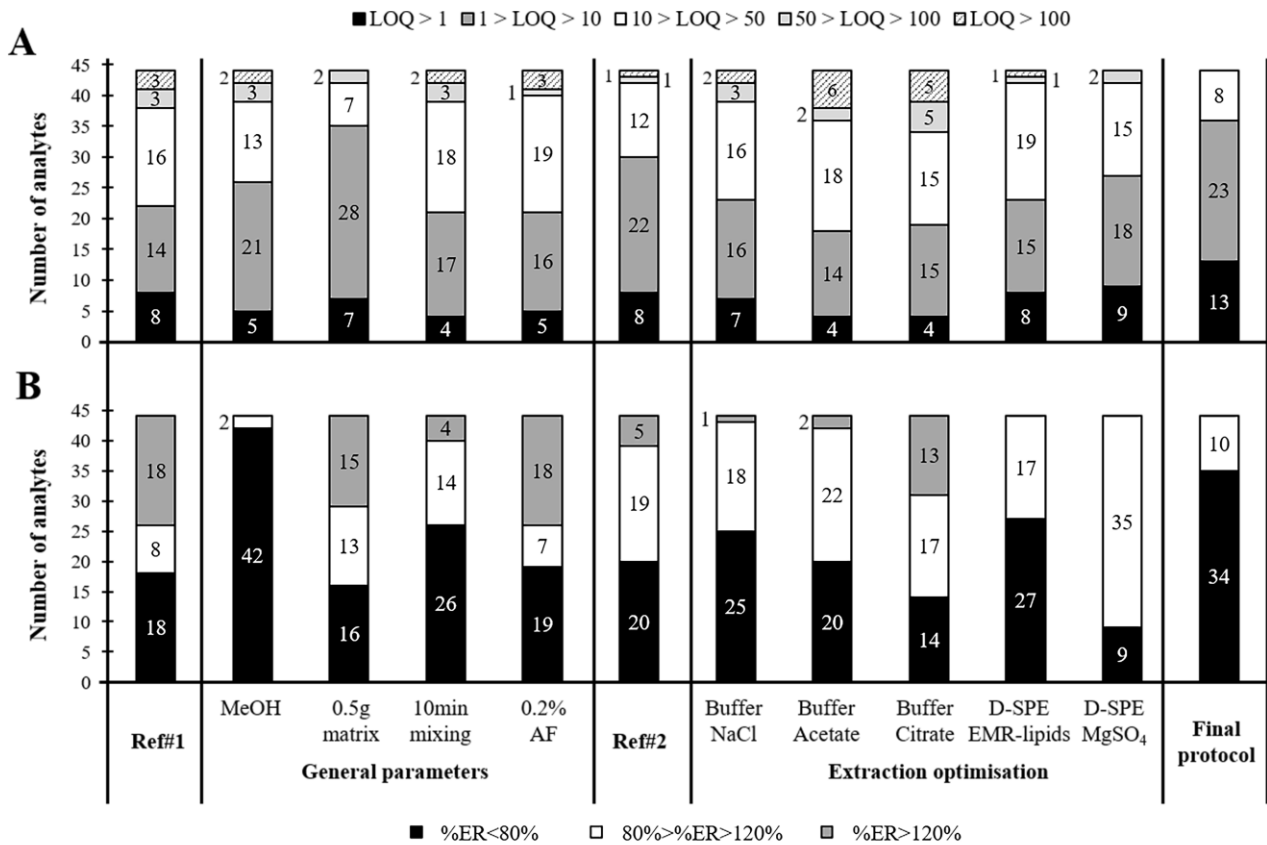
- 516 Cambridge, 2007: pp. 1–30. <https://doi.org/10.1017/CBO9781107415324.004>.
- 517 [29] S.J. Lehotay, M. O’Neil, J. Tully, A.V. García, M. Contreras, H. Mol, V. Heinke, T. Anspach,
518 G. Lach, R. Fussell, K. Mastovska, M.E. Poulsen, A. Brown, W. Hammack, J.M. Cook, L.
519 Alder, K. Lindtner, M.G. Vila, M. Hopper, A. de Kok, M. Hiemstra, F. Schenck, A. Williams,
520 A. Parker, Determination of Pesticide Residues in Foods by Acetonitrile Extraction and
521 Partitioning with Magnesium Sulfate: Collaborative Study, *J. AOAC Int.* 90 (2007) 485–520.
522 <https://doi.org/10.1093/jaoac/90.2.485>.
- 523 [30] NF EN 15662:2018, Aliments d’origine végétale - Multiméthode de détermination des résidus
524 de pesticides par analyse CG et CL après extraction/partition avec de l’acétonitrile et
525 purification par SPE dispersive - Méthode modulaire QuEChERS, France, 2018.
- 526 [31] O. Shimelis, Y. Yang, K. Stenerson, T. Kaneko, M. Ye, Evaluation of a solid-phase extraction
527 dual-layer carbon/primary secondary amine for clean-up of fatty acid matrix components from
528 food extracts in multiresidue pesticide analysis, *J. Chromatogr. A.* 1165 (2007) 18–25.
529 <https://doi.org/10.1016/j.chroma.2007.07.037>.
- 530 [32] J. V. Dias, V. Cutillas, A. Lozano, I.R. Pizzutti, A.R. Fernández-Alba, Determination of
531 pesticides in edible oils by liquid chromatography-tandem mass spectrometry employing new
532 generation materials for dispersive solid phase extraction clean-up, *J. Chromatogr. A.* 1462
533 (2016) 8–18. <https://doi.org/10.1016/j.chroma.2016.07.072>.
- 534 [33] M. Chys, K. Demeestere, A.S. Ingabire, J. Dries, H. Van Langenhove, S.W.H. Van Hulle,
535 Enhanced treatment of secondary municipal wastewater effluent: comparing (biological)
536 filtration and ozonation in view of micropollutant removal, unselective effluent toxicity, and
537 the potential for real-time control, *Water Sci. Technol.* (2017) wst2017207.
538 <https://doi.org/10.2166/wst.2017.207>.
- 539 [34] M.V. Barbieri, C. Postigo, N. Guillem-Argiles, L.S. Monllor-Alcaraz, J.I. Simionato, E. Stella,

- 540 D. Barceló, M. López de Alda, Analysis of 52 pesticides in fresh fish muscle by QuEChERS
541 extraction followed by LC-MS/MS determination, *Sci. Total Environ.* 653 (2019) 958–967.
542 <https://doi.org/10.1016/j.scitotenv.2018.10.289>.
- 543 [35] M. Burkhardt, T. Kupper, S. Hean, R. Haag, P. Schmid, M. Kohler, M. Boller, Biocides used
544 in building materials and their leaching behavior to sewer systems, *Water Sci. Technol.* 56
545 (2007) 63–67. <https://doi.org/10.2166/wst.2007.807>.
- 546 [36] U. Schoknecht, J. Gruycheva, H. Mathies, H. Bergmann, M. Burkhardt, Leaching of Biocides
547 Used in Façade Coatings under Laboratory Test Conditions, *Environ. Sci. Technol.* 43 (2009)
548 9321–9328. <https://doi.org/10.1021/es9019832>.
- 549 [37] U.E. Bollmann, C. Tang, E. Eriksson, K. Jönsson, J. Vollertsen, K. Bester, Biocides in urban
550 wastewater treatment plant influent at dry and wet weather: Concentrations, mass flows and
551 possible sources, *Water Res.* 60 (2014) 64–74. <https://doi.org/10.1016/j.watres.2014.04.014>.
- 552
- 553



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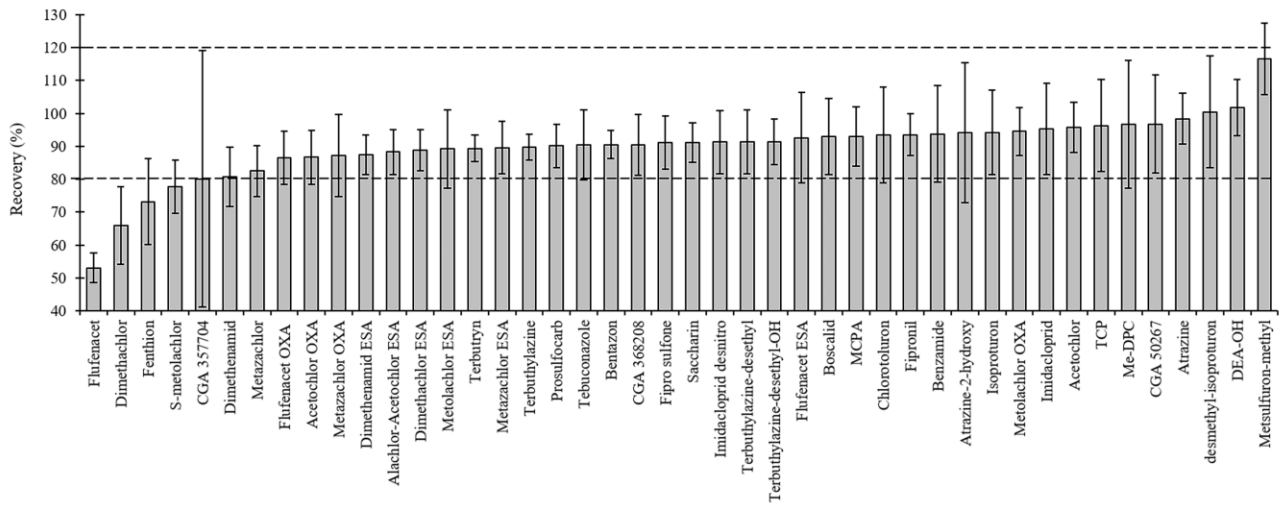
556 **Fig. 1.** Detailed steps of the extractions Ref#1, Ref#2 and the final protocol, with each of the
 557 modified steps in grey.



558

559 **Fig. 2.** Evaluation of global LOQ in ng.g^{-1} (A) and Extraction Recoveries (ER) in % (B) for each
 560 tested parameter ($n=3$), with Ref#1: the first protocol tested, and Ref#:2 the protocol built from
 561 general parameters' optimization.

562



563

564 **Fig. 3.** Impact of freeze drying on the recovery (%) of the monitored chemicals (n=3)

565
566**Table 1.** Analytical performances of the validated method with n=6 unless specified (Range: range of tested concentrations (ng; LOQ: calculated limit of quantification; ME: matrix effect; ER: extraction recovery; QF: quantification frequency)

Chemicals	Range (ng.g ⁻¹)	r ² ± %RSD	LOQ (ng.g ⁻¹)	ME ± SD (%)	ER ± %RSD (%) (n=3)			Inter-day precision (%RSD) (n=3)			Blanks	
					LOQ	3xLOQ	7xLOQ	LOQ	3xLOQ	7xLOQ	Max - Min (ng)	QF (%)
Fungicides												
Boscalid	5 - 100	0.995 (5)	5.0	-30 ± 42	82 ± 22	78 ± 19	85 ± 10	6	6	3	-	-
Tebuconazole	0.5 - 10	0.994 (4)	0.5	-10 ± 17	75 ± 15	75 ± 18	83 ± 12	8	5	3	0.1	17
Herbicides												
Acetochlor	10 - 200	0.994 (7)	25.0	-35 ± 30	67 ± 28	66 ± 30	76 ± 9	13	12	2	-	-
Atrazine	0.5 - 10	0.995 (4)	0.5	-39 ± 30	76 ± 25	71 ± 26	84 ± 7	15	5	2	-	-
Bentazone	0.5 - 10	0.992 (3)	0.5	-26 ± 49	74 ± 38	68 ± 20	78 ± 13	16	5	6	-	-
Benzamide	25 - 500	0.995 (5)	25.0	-35 ± 41	79 ± 20	78 ± 23	87 ± 9	7	7	3	5.6	17
Chlorotoluron	1 - 20	0.996 (3)	5.0	-32 ± 37	82 ± 17	77 ± 21	86 ± 10	8	6	4	-	-
Dimethachlor	5 - 100	0.994 (6)	5.0	-24 ± 10	74 ± 19	74 ± 20	69 ± 27	12	8	2	-	-
Dimethenamid	5 - 100	0.993 (9)	5.0	-35 ± 8	79 ± 17	74 ± 21	71 ± 32	8	6	2	-	-
Flufenacet	0.5 - 10	0.992 (4)	2.5	-26 ± 25	68 ± 22	69 ± 21	69 ± 32	14	9	3	-	-
Isoproturon	0.5 - 10	0.994 (8)	0.5	-42 ± 46	80 ± 14	77 ± 18	91 ± 10	7	7	1	-	-
Metazachlor	0.5 - 10	0.993 (4)	2.5	-39 ± 31	87 ± 32	81 ± 18	75 ± 30	10	7	5	0.4	17
Metsulfuron-methyl	5 - 100	0.99 (5)	25.0	-31 ± 33	68 ± 29	80 ± 24	73 ± 37	22	11	6	-	-
Prosulfocarb	5 - 100	0.991 (11)	5.0	-66 ± 27	39 ± 23	38 ± 24	64 ± 34	9	12	4	4 - 1	50
S-metolachlor	1 - 20	0.993 (7)	1.0	-29 ± 26	80 ± 27	69 ± 27	75 ± 15	14	10	4	0.2	50
Terbuthylazine	0.5 - 10	0.996 (4)	0.5	-37 ± 34	76 ± 20	72 ± 22	80 ± 10	10	8	2	-	-
Terbutryn	0.5 - 10	0.995 (4)	0.5	-43 ± 27	72 ± 20	69 ± 27	78 ± 8	8	9	3	0.4 - 0.1	67
Insecticides												
Fenthion	5 - 100	0.986 (9)	25.0	-36 ± 29	71 ± 25	65 ± 30	76 ± 9	7	11	8	-	-
Fipronil	0.5 - 10	0.992 (8)	0.5	-6 ± 13	75 ± 28	79 ± 23	83 ± 12	11	9	5	0.4 - 0.1	33
Imidacloprid	5 - 100	0.991 (7)	25.0	-29 ± 38	87 ± 27	77 ± 28	92 ± 14	17	10	4	-	-

Transformation products

Acetochlor OXA	5 - 100	0.985 (10)	25.0	-43 ± 33	60 ± 33	51 ± 33	62 ± 14	13	10	7	-	-
Alachlor-Acetochlor ESA	2 - 40	0.985 (7)	10.0	-32 ± 13	51 ± 33	53 ± 23	57 ± 7	14	7	7	-	-
Atrazine-2-hydroxy	1 - 20	0.989 (9)	1.0	-9 ± 27	93 ± 26	78 ± 30	77 ± 7	7	16	6	-	-
CGA 357704	5 - 100	0.987 (7)	5.0	-4 ± 25	49 ± 34	50 ± 25	57 ± 9	21	11	7	1.2	17
CGA 368208	1 - 20	0.978 (15)	1.0	-13 ± 70	61 ± 36	54 ± 22	62 ± 14	24	9	13	-	-
CGA 50267	0.5 - 10	0.995 (5)	0.5	-17 ± 21	84 ± 22	73 ± 20	78 ± 12	9	9	4	-	-
DEA-OH	5 - 100	0.995 (3)	5.0	-50 ± 26	77 ± 20	72 ± 23	69 ± 33	3	5	3	3.5 - 1.8	33
Desmethyl-isoproturon	1 - 20	0.991 (8)	5.0	-31 ± 14	80 ± 23	79 ± 22	80 ± 23	20	8	3	-	-
Dimethachlor ESA	5 - 100	0.992 (5)	25.0	-30 ± 14	56 ± 30	51 ± 24	65 ± 11	13	10	5	-	-
Dimethenamid ESA	5 - 100	0.986 (5)	25.0	-60 ± 32	54 ± 35	47 ± 23	72 ± 15	10	10	7	-	-
Fipronil sulfone	0.5 - 10	0.995 (5)	0.5	-20 ± 38	82 ± 24	77 ± 20	78 ± 23	13	8	1	0.3 - 0.1	83
Flufenacet ESA	5 - 100	0.99 (6)	5.0	-43 ± 29	53 ± 19	48 ± 22	70 ± 17	15	9	5	5.1	17
Flufenacet OXA	10 - 200	0.988 (8)	10	-40 ± 17	52 ± 35	48 ± 25	60 ± 16	18	13	6	-	-
Imidacloprid desnitro	0.5 - 10	0.994 (6)	0.5	-26 ± 49	74 ± 28	78 ± 24	87 ± 11	3	7	3	-	-
MCPA	5 - 100	0.991 (5)	25.0	-36 ± 35	65 ± 43	66 ± 20	79 ± 8	15	8	6	-	-
Me-DPC	5 - 100	0.995 (6)	5.0	-3 ± 32	84 ± 22	74 ± 23	83 ± 18	9	6	2	-	-
Metazachlor ESA	5 - 100	0.989 (7)	5.0	125 ± 359	55 ± 29	52 ± 23	73 ± 17	20	10	4	-	-
Metazachlor OXA	25 - 500	0.994 (7)	25.0	-26 ± 32	55 ± 22	53 ± 24	78 ± 12	5	10	3	5.3	17
Metolachlor ESA	5 - 100	0.99 (9)	5.0	-52 ± 23	53 ± 21	49 ± 21	61 ± 7	6	8	6	-	-
Metolachlor OXA	5 - 100	0.971 (28)	25.0	-46 ± 28	41 ± 26	47 ± 28	49 ± 15	20	22	10	8.9	17
Saccharin	5 - 100	0.99 (5)	25.0	-48 ± 24	55 ± 34	61 ± 23	57 ± 39	17	10	6	-	-
TCP	10 - 200	0.995 (4)	10.0	0 ± 31	68 ± 24	70 ± 21	50 ± 82	9	9	2	-	-
Terbuthylazine desethyl	0.5 - 10	0.993 (7)	0.5	-29 ± 31	86 ± 24	74 ± 20	86 ± 9	9	6	3	-	-
Terbuthylazine-desethyl-OH	5 - 100	0.991 (7)	5.0	-28 ± 30	76 ± 21	71 ± 24	83 ± 8	7	11	4	1.6	17

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570**Table S1.** Analytes and analytical parameters associated, (t_R: retention time; DP: declustering potential; EP: entrance potential; CE: collision energy; CXP: collision cell exit potential; TP: transformation product; IS: isotope labeled standard, MRM1: used for quantification; MRM2: used for confirmation)

Compound	CAS	Uses	Log P	Transition	t _R	DP	EP	CE	CXP	Ion ratio	
Acetochlor ESA + Alachlor ESA*	947601-84-5	Acetochlor and alachlor TP	2.2	MRM 1	314.0→80.0	4.9	-135	-10	-52	-15	2.1
				MRM 2	314.0→120.9	4.9	-135	-10	-30	-13	
Acetochlor OXA*	194992-44-4	Acetochlor TP	2.9	MRM 1	264.1→144.0	4.7	-55	-10	-38	-13	0.4
				MRM 2	264.1→146.0	4.7	-65	-10	-16	-19	
Bentazon*	25057-89-0	Herbicide	-0.5	MRM 1	239.0→132.0	4	-100	-10	-38	-4	2.0
				MRM 2	239.0→175.0	4	-100	-10	-28	-6	
CGA 357704*	1217465-10-5	S-metolachlor TP	1.9	MRM 1	278.0→206.0	2.7	-45	-10	-18	-15	22.8
				MRM 2	278.0→162.0	2.7	-45	-10	-26	-15	
CGA 368208*	1173021-76-5	S-metolachlor TP	1.9	MRM 1	256.0→79.8	4.2	-145	-10	-58	-11	2.0
				MRM 2	256.0→120.8	4.2	-145	-10	-28	-11	
Dimethachlor ESA*	1231710-75-0	Dimethachlor TP	-0.2	MRM 1	300.0→80.0	4.4	-25	-10	-66	-13	1.9
				MRM 2	300.0→120.9	4.4	-25	-10	-30	-15	
Dimethenamid ESA*	205939-58-8	Dimethenamid TP	-0.6	MRM 1	319.9→120.7	4.6	-50	-10	-30	-17	1.5
				MRM 2	319.9→80.0	4.6	-50	-10	-66	-9	
Fipronil sulfone*	120068-36-2	Fipronil TP	4.6	MRM 1	450.8→414.9	8.5	-65	-10	-24	-21	1.2
				MRM 2	450.8→281.9	8.5	-65	-10	-38	-19	
Fipronil*	120068-37-3	Insecticide	3.8	MRM 1	434.9→329.9	8.2	-110	-10	-22	-21	3.1
				MRM 2	434.9→249.9	8.2	-110	-10	-38	-17	
Flufenacet ESA*	947601-87-8	Flufenacet TP	1.2	MRM 1	274.1→121.0	4.5	-25	-10	-28	-11	1.1
				MRM 2	274.1→79.9	4.5	-25	-10	-54	-29	
Flufenacet OXA*	201668-31-7	Flufenacet TP	1.6	MRM 1	224.0→152.0	4.2	-20	-10	-15	-10	1.2
				MRM 2	152.0→136.0	4.2	-90	-8	-17	-10	
MCPA*	94-74-6	Herbicide	-0.8	MRM 1	199.0→141.0	4.5	-65	-10	-20	-12	2.5
				MRM 2	201.0→143.0	4.5	-65	-10	-20	-12	
Metazachlor ESA*	172960-62-2	Metazachlor TP	-0.1	MRM 1	322.0→120.9	4.4	-110	-10	-30	-11	2.2
				MRM 2	322.0→79.9	4.4	-110	-10	-80	-11	
Metolachlor ESA*	947601-85-6	S-metolachlor TP	2.1	MRM 1	328.1→79.8	4.9	-135	-10	-72	-11	2.3
				MRM 2	328.1→121.0	4.9	-135	-10	-30	-13	

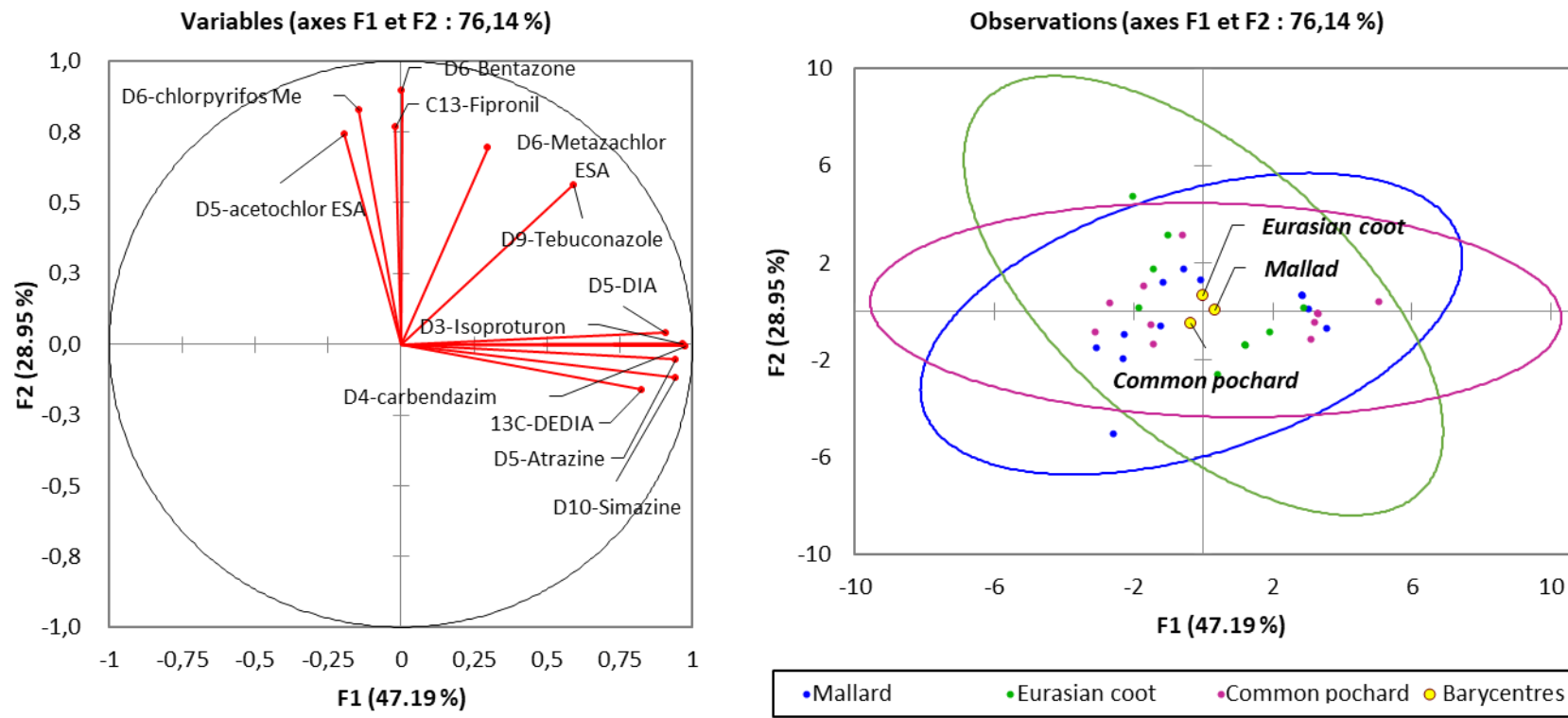
Compound	CAS	Uses	Log P	Transition	t _R	DP	EP	CE	CXP	Ion ratio	
Metolachlor OXA*	152019-73-3	S-metolachlor TP	2.3	MRM 1	278.1→174.1	4.8	-65	-10	-22	-15	7.6
				MRM 2	278.1→206.1	4.8	-65	-10	-14	-13	
Saccharin*	81-07-2	Metsulfuron-methyl TP	0.9	MRM 1	181.9→41.9	2	-150	-10	-88	-19	9.7
				MRM 2	181.9→61.9	2	-115	-10	-26	-9	
13C4-Fipronil*	2140327-54-2	IS	-	MRM 1	438.9→334.0	8.2	-50	-10	-22	-17	-
D5-acetochlor ESA*	-	IS	-	MRM 1	319.0→79.9	4.9	-135	-10	-66	-11	-
D6-Bentazone*	-	IS	-	MRM 1	245.0→131.9	4	-40	-10	-36	-15	-
D6-Metazachlor ESA*	-	IS	-	MRM 1	328.2→120.9	4.4	-115	-10	-32	-13	-
Acetochlor	34256-82-1	Herbicide	4.1	MRM 1	270.1→223.9	8.1	61	10	13	16	1.6
				MRM 2	270.1→148.1	8.1	61	10	27	10	
Atrazine	1912-24-9	Herbicide	2.7	MRM 1	216.1→174.0	6.8	86	4.5	23	4	3.1
				MRM 2	216.1→104.1	6.8	86	4.5	39	4	
Atrazine-2-hydroxy	2163-68-0	Atrazine TP	2.1	MRM 1	198.1→156.0	4.1	71	10	25	12	2.6
				MRM 2	198.1→114.0	4.1	71	10	31	20	
Benzamide	55-21-0	Herbicide	0.6	MRM 1	122.0→77.0	3.8	95	10	33	13	1.6
				MRM 2	122.0→79.0	3.8	95	10	19	13	
Boscalid	188425-85-6	Fungicide	3	MRM 1	343.0→307.0	7.7	111	10	29	20	2.3
				MRM 2	343.0→140.0	7.7	111	10	29	12	
CGA 50267	82508-02-9	S-metolachlor TP	2.4	MRM 1	208.0→135.0	6.4	61	10	22	13	1.1
				MRM 2	208.0→120.0	6.4	61	10	41	13	
Chlorotoluron	15545-48-9	Herbicide	2.5	MRM 1	213.1→72.0	6.6	74	6	33	4	2.6
				MRM 2	213.1→46.2	6.6	74	6	27	4	
DEA-OH	19988-24-0	Atrazine TP	-1	MRM 1	170.0→128.0	1.5	96	10	21	12	1.3
				MRM 2	170.0→86.0	1.5	96	10	29	14	
desmethyl isoproturon	34123-57-4	Isoproturon TP	2.8	MRM 1	193.0→94.0	6.4	40	10	32	13	2.6
				MRM 2	193.0→136.0	6.4	40	10	22	13	
Dimethachlor	50563-36-5	Herbicide	2.2	MRM 1	256.0→224.2	7.2	64	10.5	19	6.5	2
				MRM 2	256.0→148.0	7.2	64	10	33	6	
Dimethenamid	87674-68-8	Herbicide	2.2	MRM 1	275.9→244.1	7.6	16	10	19	16	3.3
				MRM 2	275.9→168.0	7.6	16	10	35	20	

Compound	CAS	Uses	Log P	Transition	t _R	DP	EP	CE	CXP	Ion ratio	
Fenthion	55-38-9	Insecticide	4.8	MRM 1	278.9→169.1	8.3	71	10	23	12	1
				MRM 2	278.9→246.9	8.3	71	10	17	18	
Flufenacet	142459-58-3	Herbicide	3.5	MRM 1	364.0→152.0	8	91	10	27	14	1.1
				MRM 2	364.0→194.1	8	91	10	15	16	
Imidacloprid desnitro	127202-53-3	Imidacloprid TP	-0.8	MRM 1	211.0→126.0	2.6	96	10	33	12	3.7
				MRM 2	211.0→90.0	2.6	96	10	49	8	
Imidacloprid	138261-41-3	Insecticide	0.6	MRM 1	255.9→175.1	5	66	10	29	14	1.0
				MRM 2	255.9→209.1	5	66	10	23	14	
Isoproturon	34123-59-6	Herbicide	2.5	MRM 1	207.2→72.1	6.8	86	4	29	4	5.5
				MRM 2	207.2→165.2	6.8	86	10.5	19	6.5	
Me-DPC	64-55-1	Chloridazon TP	-1.4	MRM 1	160.0→87.9	2.2	61	10	39	10	2.6
				MRM 2	160.0→130.0	2.2	61	10	33	12	
Metazachlor	67129-08-2	Herbicide	2.5	MRM 1	278.1→210.1	7.1	58	3.5	15	4	0.9
				MRM 2	278.1→134.2	7.1	58	3.5	27	4	
Metazachlor OXA	1231244-60-2	Herbicide	1.5	MRM 1	274.0→69.0	4.9	56	10	31	10	1.3
				MRM 2	274.0→134.1	4.9	56	10	29	18	
Metsulfuron-methyl	74223-64-6	Herbicide	-1.9	MRM 1	382.0→167.0	6.2	86	10	23	14	1.4
				MRM 2	382.0→141.0	6.2	86	10	21	10	
Prosulfocarb	52888-80-9	Fungicide	4.5	MRM 1	252.3→91.1	8.9	71	10	35	14	3.5
				MRM 2	252.3→128.1	8.9	71	10	17	14	
S-metolachlor	87392-12-9	Herbicide	3	MRM 1	284.1→252.1	8.1	101	10	21	18	2.8
				MRM 2	284.1→176.2	8.1	101	10	35	14	
TCP	6515-38-4	Chlorpyrifos-ethyl TP	2.2	MRM 1	197.9→107.0	6.8	56	10	43	8	1.9
				MRM 2	197.9→134.0	6.8	56	10	31	10	
Tebuconazole	107534-96-3	Fungicide	3.7	MRM 1	308.1→70.1	7.8	61	10	39	4	8.7
				MRM 2	308.1→125.0	7.8	61	10	51	4	
Terbuthylazine desethyl	30125-63-4	Terbuthylazine TP	2.3	MRM 1	202.0→146.0	6.2	46	10	23	16	5.9
				MRM 2	202.0→103.9	6.2	46	10	37	12	
Terbuthylazine -desethyl-2-OH	66753-06-8	Terbuthylazine TP	-1.2	MRM 1	184.0→128.1	2.2	81	10	21	10	2.3
				MRM 2	184.0→86.1	2.2	81	10	35	10	

Compound	CAS	Uses	Log P	Transition	t _R	DP	EP	CE	CXP	Ion ratio	
Terbuthylazine	5915-41-3	Herbicide	3.4	MRM 1	230.1→174.1	7.4	81	11.5	23	6.5	6.6
				MRM 2	230.1→104.1	7.4	86	7.5	41	4	
Terbutryn	886-50-0	Herbicide and terbuthylazine TP	3.7	MRM 1	242.0→186.1	6.8	41	10	27	14	5.2
				MRM 2	242.0→68.0	6.8	41	10	61	8	
13C-DEDIA	1216850-33-7	IS	-	MRM 1	149.0→105.8	1.7	16	10	27	14	-
D10-Simazine	220621-39-6	IS	-	MRM 1	212.0→137.1	6.1	66	10	29	10	-
D11-Aceto chlor	1189897-44-6	IS	-	MRM 1	281.1→235.0	8.1	71	10	15	14	-
D3-Isoproturon	352438-80-3	IS	-	MRM 1	210.1→75.1	6.8	86	10	25	10	-
D4-carbendazim	291765-95-2	IS	-	MRM 1	196.1→164.2	3.2	60	10	25	12	-
D4-Imidacloprid	1015855-75-0	IS	-	MRM 1	260.0→213.1	5	61	10	23	14	-
D5-Atrazine	163165-75-1	IS	-	MRM 1	221.1→179.1	6.8	121	10	25	16	-
D5-Atrazine-2-hydroxy	2163-68-0	IS	-	MRM 1	203.1→161.4	4.1	126	10	23	10	-
D5-DIA	1189961-78-1	IS	-	MRM 1	179.0→100.9	4.3	101	10	27	10	-
D5-Metazachlor	1246816-51-2	IS	-	MRM 1	284.1→216.1	7.1	58	3.5	15	4	-
D5-Terbutryn	1219804-47-3	IS	-	MRM 1	247.4→190.9	6.8	96	10	23	14	-
D6-chlorpyrifos Me	2083629-84-7	IS	-	MRM 1	327.9→130.9	8.6	76	10	31	10	-
D9-Tebuconazole	1246818-83-6	IS	-	MRM 1	317.1→70.0	7.8	86	10	57	12	-

*: chemicals analyzed in negative electrospray mode, otherwise analyzes were performed in positive mode

572 **Fig. S1.** Representation of isotope labeled standards area ratio between reference and field samples, PCA based on Pearson regression ($\alpha = 0.005$).



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