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

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Abstract

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

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

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 (ng.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 ng.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 developped 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 ng.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 logP close or up to 3. The
75 only exception is the characterization of neonicotinoids that are much more polar ($2 > \text{logP} > -1$

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.

2. Materials and methods

2.1. Standards and reagents

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

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

2.2. Sampling strategy

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

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

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

2.3. QuEChERS-based extraction

Different conditions were tested to optimize the extraction procedure. The first applied protocol (Ref#1) (Fig. 1) was based on a simple SLE-LTP, aiming to increase its complexity until reaching QuEChERS approaches. Two hundred milligrams of reference matrix were weighted in a 50 mL polypropylene centrifuge tube (VWR, Radnor, USA) and spiked with 50 µL of a mix solution containing all analytes and 75 µL of a mix solution containing all isotope labeled standards, both at nominal concentration 500 µg.L⁻¹. Solution solvent was evaporated under gentle nitrogen flux for 10 min. A volume of 5 mL of ultrapure water was added to the sample, mixed together for 5 min at 16 Hz with Geno/Grinder (SamplePrep 2010 Geno/Grinder from SPEX, Costa Mesa, USA) to rehydrate the sample. Then 10 mL of ACN and 5 mL of heptane were added in the centrifuge tube and mixed together for 5 min at 16 Hz with Geno/Grinder to extract analytes and remove part of non-polar interferents. Samples were then centrifuged for 5 min at 9500 RCF at 20 °C (SIGMA 3K30H, Fisher, Osterode am Harz, Germany). Six milliliters of the ACN/water mix were transferred in new 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

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

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

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

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

2.6. Method application

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

3. Results and discussion

3.1. Optimization of extraction conditions

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

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

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

3.1.1. Preliminary tests

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

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

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

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

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

3.1.2. Determination of a new reference protocol

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

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

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

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

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

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

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

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

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

3.4. Field sample analysis

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

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

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

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

4. Conclusion

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

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

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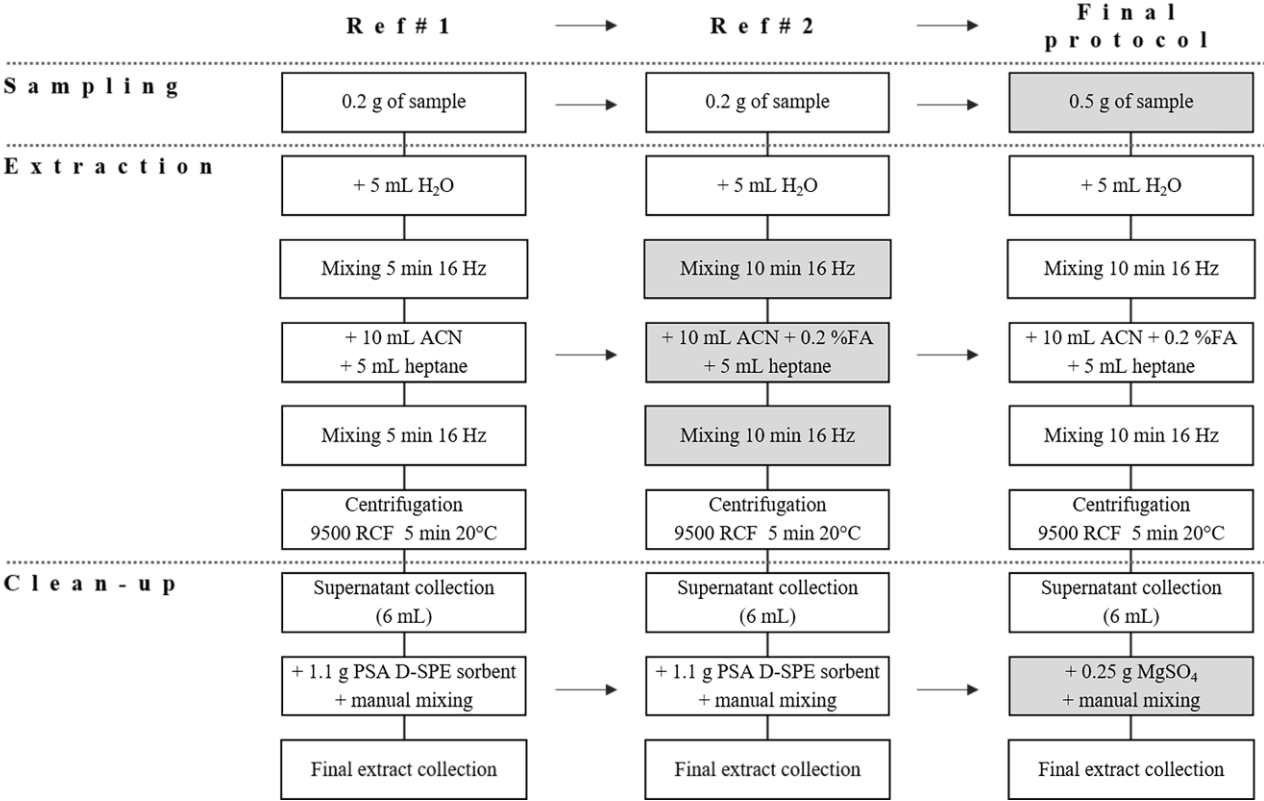
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555

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.

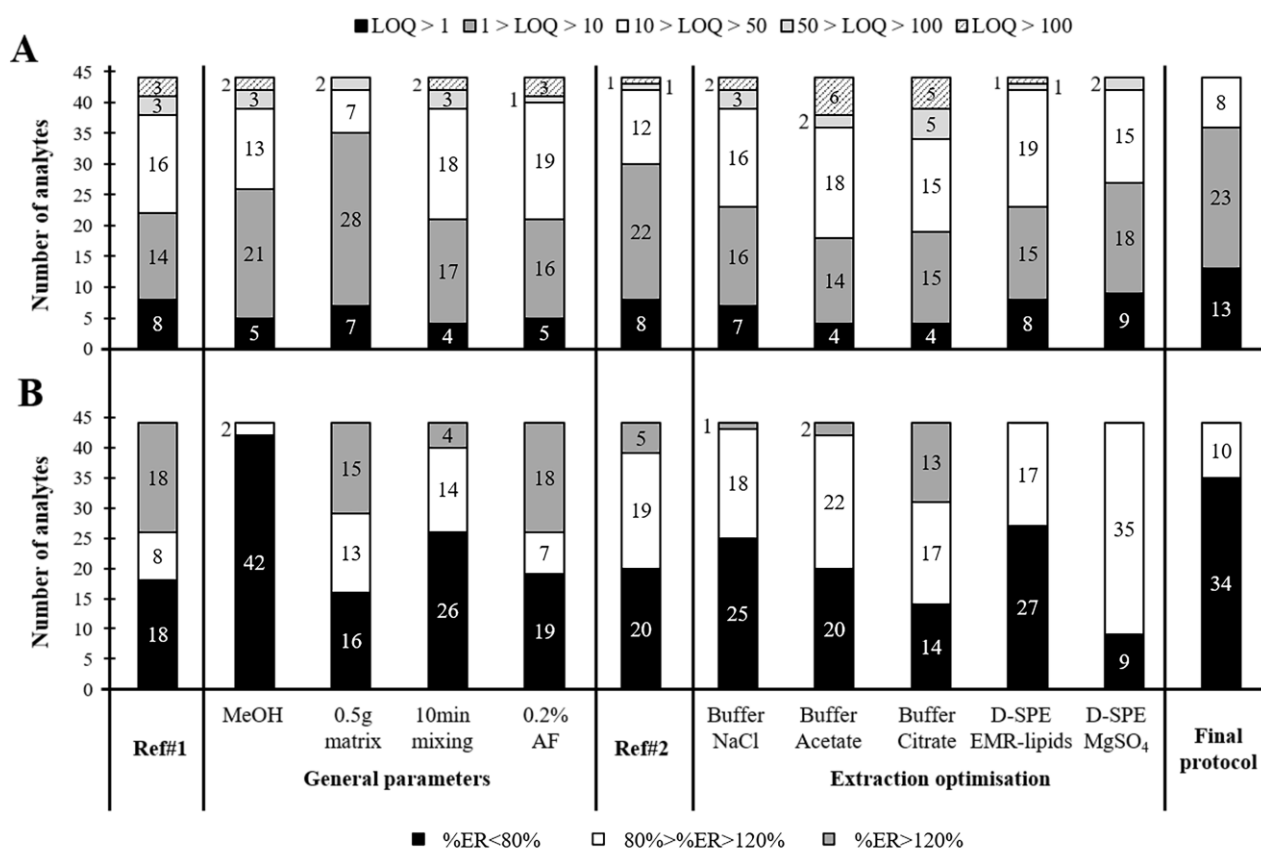
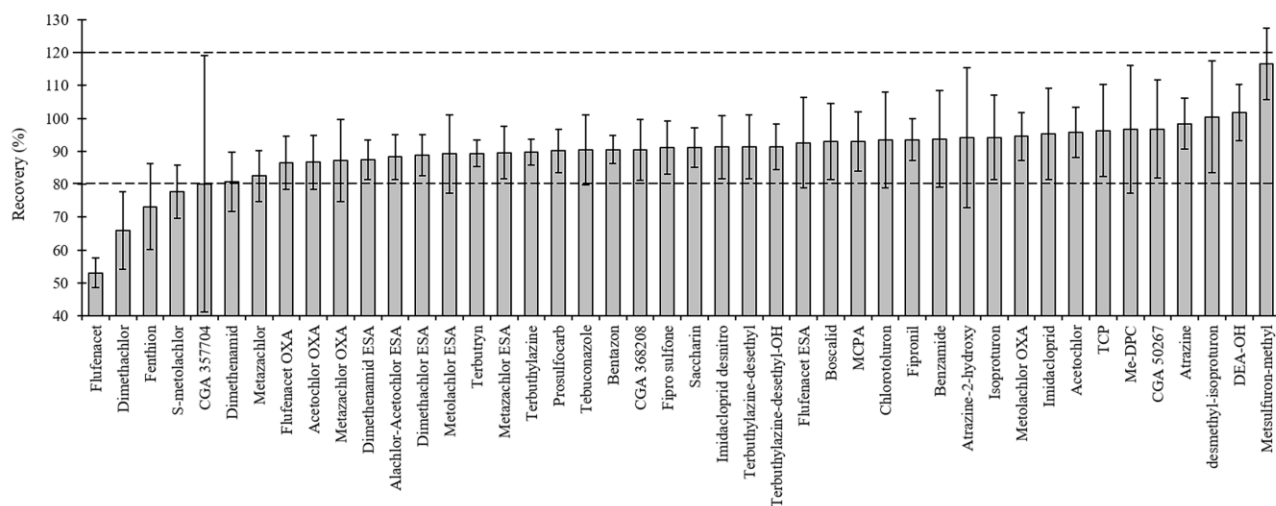


Fig. 2. Evaluation of global LOQ in ng.g⁻¹ (A) and Extraction Recoveries (ER) in % (B) for each tested parameter (n=3), with Ref#1: the first protocol tested, and Ref#:2 the protocol built from general parameters' optimization.



563

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

565 **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:
566 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

567

568

569 **Table S1.** Analytes and analytical parameters associated, (t_R: retention time; DP: declustering potential; EP: entrance potential; CE: collision energy; CXP: collision cell exit
570 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 ($\alpha = 0.005$).

