Analytical development for the assessment of pesticide contaminations in blood and plasma of wild birds: the case of grey partridges (*Perdix perdix*).

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Abstract

In this study, blood and plasma of grey partridges (*Perdix perdix)* were analyzed to assess their potential contamination by plant protection products (PPP) and especially pesticide compounds.

The group of pesticides selected is composed of a huge variety of compounds. Therefore, in this study, two methods were optimized and validated to analyze 104 compounds including herbicides, insecticides, fungicides and photoprotectors or synergists.

Various extraction methods found in the literature were compared and adapted for the extraction of pesticides from blood and plasma. After extraction, samples were concentrated then injected for quantification simultaneously in LC-MS/MS and ATD-GC/MS/MS with an automatic thermal desorption step (ATD).

Both LC-MS/MS and ATD-GC/MSMS analyses were performed using the MRM mode with 2 mass transitions for each compound.

For both analytical methods, calibrations in triplicate were done by spiking within clean matrices using deuterated internal standard as ISTD. Evaluation of reproducibility, repeatability and determination of extraction yields were also performed with satisfactory results for most compounds then LOD and LOQ were determined with good sensibility results. LODs varied from 0.005 to 0.035 pg mg^{-1} and LOQs from 0.017 to 0.116 pg mg^{-1} for both matrices and methods.

The two distinct analytical methods were then successfully applied to 70 blood samples and 35 plasma samples.

Keywords

Biomonitoring, pesticides, birds, blood, plasma.

1. Introduction

The constant research of efficiency and rentability in our agriculture and food production has made the use of chemical pesticides more and more trendy because of their high crop yields and economic benefits [1]. Moreover, the European Commission has agreed there are many environmental impacts from using pesticides and recognized the urge of reduce pesticide use [2,3] whereas their number increases exponentially [4].

In this environmental context, many studies have showed the importance of biomonitoring as an evaluation tool to assess environmental contamination by pesticides in agrosystems and to determinate the exposition of animals living inside this ecosystem [5–8]. The doses absorbed by animals are often very low resulting in an exposure that does not necessarily lead to an increase in mortality in the short term but could have sublethal effects on individuals in the long term [9]. Therefore, quantifying the exposure of wild animal is a necessary challenge especially since risk assessment studies require data on exposure of organisms [10,11]. Very often, the pesticide exposure of animals is determined after death by analyzing specific organs [12]. However, analyzing the amount of pesticides in cadavers is certainly not a good picture of the real exposure of an entire population because it can be assumed that death may be due to pesticide overload, dose that is certainly not representative of all live animals. In addition, it is no longer acceptable to sacrifice animals to determine their exposure to pesticides and there is an urgent need for efficient analytical means to measure their pesticide exposure in matrices that can be easily collected and that do not cause the death of individuals [12]. Blood and plasma of wild birds seem to be good candidates because it is easily to collect once the bird is trapped and can represent a recent pesticide contamination allowing to follow in real time the contamination of environments [12,13].

The grey partridge is an iconic European farmland bird, currently declining and exposed to phytosanitary products throughout their lives, as they live in crops and feed with seeds and

65 insects from cultivated fields [14]. Thus, the grey partridge fulfils all the requirements to be a
66 valuable biosentinel of farmlands despite little attention has been paid to its field ecotoxicology
67 [14]. So far, phytosanitary products have been found in wild grey partridges' eggs with 15
68 different compounds detected [14] or in the liver of hunted birds (n.b. only neonicotinoid
69 clothianidin was sought [15]). Investigating the potential presence of such compounds in bird
70 blood and plasma required the development of specific analytical procedures.

71 Many analytical procedures have already been developed so far for the quantification of
72 contaminants such as perfluoroalkyl substances (PFAs), novel flame retardants (NFRs),
73 neonicotinoids, chlorinated paraffins, parabens, bisphenols but also organochlorine pesticides
74 (OCs), polychlorinated biphenyls (PCBs), neonicotinoids and polycyclic aromatic
75 hydrocarbons (PAHs) in birds of prey, eggs, honey or bees [16–20]. It is already known that,
76 in bird blood and plasma, some compounds are present in high concentrations [15,21–24].
77 However, few studies have reported combined extraction and analysis methods to quantify such
78 a large variety of pesticides and to the best of our knowledge none were reported in grey
79 partridges so far. Basically, Liquid Chromatography coupled to tandem Mass Spectrometry
80 (LC/MSMS) is a reliable method for the quantification of many pesticides at low detection
81 levels [25,26], but does not allow a fine quantification of more volatile and semi-volatile
82 compounds. Therefore, we chose to add a second analysis step using Automated Thermal
83 Desorption Gas Chromatography coupled to tandem Mass Spectrometry (ATD-GC/MSMS) to
84 quantify a wider spectrum of molecules.

85 This study aimed at developing a sensitive analytical methodology to quantify 104 different
86 pesticide compounds among the most used in both blood and plasma of birds using grey
87 partridges (*Perdix perdix)* as model species by optimizing extraction and quantification
88 methods. Pesticides from blood and plasma were extracted by two different liquid-liquid
89 extractions with a mixture of ethyl-acetate and dichloromethane and a mixture of methanol and

acetonitrile for blood and plasma respectively. Analyses were then run by internal standard quantification using both LC/MSMS and ATD-GC/MSMS for detection and quantification. The developed methods were finally applied to 70 blood samples and 35 plasma samples for the quantification of the exposure of grey partridges to pesticides.

2. Material and methods

2.1. Chemicals and solutions

Formic acid, acetonitrile and water for LC/MS were purchased from Sigma-Aldrich (LPCR, France). The ultra-pure water was obtained through a Milli-Q system (18 MΩ cm) from Merck, Germany. Other solvents of HPLC grade quality (ethanol, ethyl ether, methanol, n-hexane) were purchased from VWR Prolabo (Paris, France).

Pesticide high purity standard (>98%) powders were supplied by Sigma Aldrich (l'Isle d'Abeau, France) and/or Dr Ehrenstorfer GmbH (Cluzeau Info Labo, St. Foy la Grande, France) and Riedel de Haën (Sigma Aldrich, St. Quentin Fallavier, France).

Internal standards, trifluralin-d_{14}, nitrophenol-d_4, 2-4-D-d_3, pendimethalin-d_5 diuron-d_6, acetamiprid-d_{11} and carbendazim-d_4, were obtained from Sigma-Aldrich (L'Isle d'Abeau, France) and Cambridge isotope laboratories (Cluzeau Info Labo, St. Foy la Grande, France).

Stock solutions of each standard and internal standard were prepared in acetonitrile (ACN) (HPLC grade supplied by Sigma Aldrich, St. Quentin Fallavier, France) at a concentration of 1 g L^{-1} and stored at -20°C.

The agent of derivatisation, N-*tert*-butyldimethylsilyl-N-methyltrifluoroacetamide (M*t*BSTFA) was purchased from Fluka (Sigma Aldrich, St. Quentin Fallavier, France).

Perkin Elmer® ATD-empty tubes (89 mm x 5 mm i.d.) were purchased from PerkinElmer Corp. (Norwalk, CT, USA).

115

116 *2.2. Blood and plasma materials*

117

118 To validate those methods, 70 seven-month-old captive-born grey partridges were monitored
119 in a commercial game farm in south-western France. All birds were fitted with an alphanumeric
120 metal ring and lived in two large pens (100 m × 10 m × 4 m) equipped with feeders, drinkers
121 and metal sheet for shelter. They were fed a mixture of corn, wheat, faba bean and pea *ad*
122 *libitum* since they were born. Blood samples from all 70 grey partridges were collected in March
123 2021 after five months of exposure to the two different diets; 200 µL of blood were taken when
124 it was possible from the brachial vein using a sterile needle (Ø 0.06 mm) and heparinized micro-
125 capillary tubes. One hundred microliters of these blood samples were directly stored at -20°C.
126 When there was 100 µL of blood left, it was centrifuged immediately after collection (9000
127 rpm. at 20°C for 10 min) to recover the plasma which was then stored in Eppendorf tubes also
128 at -20°C until analysis. For the 70 partridges, a total of 70 blood samples and 35 plasma samples
129 were collected. All experiments complied with French laws on animal experimentation and all
130 experimental protocols were approved by the Deux-Sèvres Committee of Animal
131 Experimentation (APAFIS#9465-201703101551625).

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133 *2.3. Plasma extraction*

134 Plasma samples were defrosted and weighted within the Eppendorf. Before extraction, 10
135 µL of a solution of carbendazim-d_4 at 1 g L^{-1} were added to each sample and homogenised by
136 vortex. For the extraction procedure, the protocol of Hao *et al.* [27] was adapted and consisted
137 in a precipitation step of the lipids and then a liquid-liquid extraction. The figure 1 resumes the
138 adapted extraction procedure.

Twenty microliters of methanol and 2,5 µL of a formic acid solution (pH 2.8) were added to plasma samples to precipitate the proteins. After homogenization by vortex, the samples were put under a fume hood for gentle evaporation. After ca. 4 hours of evaporation, 500 µL of a mixture of methanol and acetonitrile (20:80, *v/v*) was added. Samples were then centrifugated 20 minutes at a speed of 4000 rpm for elimination of the proteins' residues.

Finally, 500 µL of the supernatant were collected and stored at -20°C until further analyses by ATD-GC/MSMS and LC/MSMS. The empty and dried Eppendorf tubes were weighted again to determinate the exact weight of the samples.

2.4. Blood extraction

Blood samples were defrosted and weighted within the Eppendorf. Before extraction, 10 µL of a solution of carbendazim-d_4 at 1 g L^{-1} were added to each sample and homogenised by vortex. For the extraction procedure, the protocol of Goutner *et al.* [28] was adapted and consisted in a liquid-liquid extraction and then a purification step. The Figure 1 resumes the adapted extraction procedure.

Two millilitres of a mixture of dichloromethane and ethyl acetate (1:1) were added to each sample and homogenised 1 minute by vortex. Extracts were then transferred to a centrifugation tube of 10 mL and sonicated for 10 minutes. This step of extraction was repeated 3 times successively. The supernatant was collected each time and put under a fume hood for gentle evaporation until a volume of 500 µL.

The final extract of 500 µL was collected and stored at -20°C until analyses by ATD-GC/MSMS and LC/MSMS. The empty and dried Eppendorf tubes were weighted again to determinate the exact weight of the samples.

2.5. Analysis by LC/MSMS

A Thermo Scientific TSQ Quantum Access Triple Quadrupole Mass Spectrometer operating in heated positive electrospray ionization mode (HESI+) coupled with a Thermo Accela 1250 pump and a Thermo Combi Pal autosampler were used. The sampler is equipped with a 20 µL injection loop and the samples were kept at a temperature of 5°C. The analysis was performed on a Nucleodur C_{18} Pyramid column (150 mm × 3 mm, 3 µm) at room temperature. Samples were analyzed using a mobile phase water / ACN both containing 0.1% formic acid, at a flow rate of 0.3 mL min^{-1}. The composition of the mobile phase started at 70:30 (*v/v*) then changed to 50:50 in 5 min, then 20:80 in 11 min and 5:95 in 18 min (hold for 6 min) and finally returned at 70:30 in 25 min (hold 8 minutes) for a total run of 33 minutes.

Detection and quantitation of all compounds were performed using multiple reactions monitoring (MRM). The ion source was operated in positive ion mode with a spray voltage of 4,500 V and a vaporizer and capillary temperature of 300°C each. Nitrogen was used for sheath and auxiliary gas pressure (20 and 10 arbitrary units) while argon was used for collision pressure (1.5 arbitrary unit). Two precursors product ion transitions for each analyte and each internal standard were used for quantitation. The selected transitions for MSMS analysis, the collision energy and the retention times are presented in Table A.1 in a subsequent appendix (Appendix A). Data were acquired and processed using Excalibur software.

2.6. Analysis by ATD-GC/MSMS

First, prior to GC–MS/MS analysis, 100µL of the obtained extracts were diluted into 20 mL using briny water (1.5% NaCl) allowing their reconcentration by SPME using a CTC CombiPAL autosampler according to Levy *et al.* [29]. Pesticides were extracted at 60 °C for 40 min using a polyacrylate (PA) 85 µm fiber SUPELCO (Sigma Aldrich, St. Quentin Fallavier, France).

Finally, instead of SPME, a thermal desorption step was added by spiking a Tenax®-TA passive tubes by deposition upside down of 100 µL of extract. For this, conditioned Tenax®-TA clean passive sampling tubes, placed in empty stainless-steel tubes (89 mm × 5 mm i.d.), were used as support for analysis. Ten microliters of a mix of internal standards (trifluralin-d_{14}, nitrophenol-d_4, 2-4-D-d_3 and pendimethalin-d_5) and 10 µL of a derivatization agent, M*t*BSTFA, were deposited. The derivatization agent was selected because of its efficiency and ease of use [30–32]. Moreover, the mass spectra of derived compounds using this agent, possess a characteristic fragment of m/z = M-57 (M corresponding to the molecular ion of the derived molecule) which can be, in the most cases, the most intense fragment, allowing its use in MSMS using an electronic impact source. Figure 2 presents the sialylation reaction of a primary alcohol.

For derived molecules, formation of the derived product, which has a mass [M+114] relative to the mass of the molecular ion of the compound alone was observed. This by-product was fragmented in the mass spectrometer source and then gave several characteristic masses including the mass [M-57] representing the loss of the $C(CH_3)_3$ group of M*t*BSTFA and the mass [M-131] representing the loss of the $OSi(CH_3)_2C(CH_3)_3$ group.

The liquid was then allowed to spread during 5 min before returning the tube and inserting it in the automated thermal desorber (ATD) autosampler. Thermal desorption was carried out by using an automatic thermal desorption system (ATD 350, PerkinElmer Corp.; Norwalk, CT, USA) connected to a Trace 1300 GC coupled to an ITQ 900 mass spectrometer (Thermo Scientific). ATD 350 was coupled to a GC/MSMS system *via* a valve and a transfer line maintained at 280°C and 300°C respectively. The thermal desorption consisted of a two steps desorption. First, a 5 min tube purge with helium (He) at a flow rate of 45 mL min^{-1}; then, a desorption step occurs when the sample tube is heated at 300°C during 30 min under a He flow stream (45 mL min^{-1}). All compounds are refocused in a cold trap maintained at 20°C by Peltier

effect. At the end, the trap is heated to 300°C by induction (temperature rate 20°C s^{-1}) for a flash desorption step.

Compounds from ATD desorption were separated on a Macherey-Nagel OPTIMA XLB capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness) with He as carrier gas with an electronically regulated constant flow of 1.2 mL min^{-1}. The GC oven temperature was initially set at 50°C for 3 minutes, then increased to 160°C at 36.6°C.min^{-1} and then to 206°C at 5.8°C.min^{-1} (held for 2 minutes) and then programmed to 230°C at 1.5°C.min^{-1} and then to 300°C at 6°C.min^{-1} where it was held for 6 minutes for a total analysis time of approximately 50 minutes.

Spectra of compounds were obtained in electron impact (EI) ionization mode at 70 eV electron energy. Transfer line temperature was set up at 300°C and source temperature at 210°C. The most abundant ion of a full scan analysis of each compound was selected as precursor ion for the second ionization step. Collision induced dissociation (CID) was performed in resonant mode. The highest abundant product ions were then selected as characteristic ions for each compound. The selected ions for MSMS analysis, the CID excitation voltage, and the retention times are presented in Table B.2 in a subsequent appendix (Appendix B). Data were acquired and processed using Excalibur software.

2.7. Calibration and uncertainties

For all sample types, calibration was performed with curves obtained by spiking, within clean matrices, increasing amount of all 104 pesticide compounds. The concentration of internal standard solutions was of 1 mg L^{-1} for 10 µL injected in GC and 0.1 mg L^{-1} for 10 µL injected in LC. In LC/MSMS, diuron-d_6, acetochlore-d_{11} and pendimethalin-d_5 were used for calibration and carbendazim-d_4 was used to evaluate the extraction performance. Extraction yields were determined for each sample. In ATD-GC/MSMS, nitrphenol-d_4 was used to evaluate the

derivatization performance and trifluarin-d_{14}, 2,3-D-d_4 and pendimethalin-d_5 were used for calibration. For calibration, multi-compound solutions ranging between 0.2 pg and 0.2 ng for 20 µL injected in LC and 0.01 to 50 ng for 100 µL injected in GC were prepared. In order to evaluate the potential matrix effect, samples with and without matrix were spiked for each concentration range and responses were compared. The analytical procedures as described before were performed three times on each calibration sample. The blank samples were obtained from birds guaranteed that no pesticides contamination has occurred.

Limits of quantification (LOQs) and detection (LODs) were determined for all compounds as the concentrations giving signal to noise (S/N) ratios of 3 and 10 respectively. Calibrations were done in triplicate using deuterated compounds as internal standards. Standard deviation of the slope was determined and considered acceptable for all compounds (RSD < 15%). Uncertainties were determined for both analytical methods (LC and GC) and each sample types (plasma and blood) by repeating and analyzing three time all calibration levels of all compounds from spiked samples on different days for reproducibility and on the same day for repeatability.

3. Results and Discussion

3.1. Choice of the column

A Nucleodur C_{18} pyramid (150 mm × 3 mm diameter, 3 µm particle size) column was used for separation of non-volatile compounds in LC/MSMS. This column has already shown good results and efficiency in air and biological samples [33,34].

For volatile and semi-volatile compounds analyzed in GC, the method proposed by Sonnette *et al.* [30] was adapted to consider only pesticides. The length of the column was reduced to 30 m permitting to decrease the time of analysis and increase the stability of some pesticides sensible to long period exposure at high temperature. By decreasing the length of the column,

the sensitivity obtained for example for deltamethrin and tebuconazole was increased by a factor of 2 in comparison to a column with 60 m length [35]. Some other molecules, like cymoxanil and metrafenone, which cannot be detected with a 60 m column, were identified with a good sensitivity on the 30 m column.

3.2. Extraction optimization

Different extraction methods were evaluated and two were finally selected, adapted and optimized according to the compounds of interest and the matrices. First, solvent extraction efficiency was evaluated by comparing several solvents or mixture of solvents. According to Goutner *et al.* [28], a mixture of hexane and ethyl ether (1:1, *v/v*) was used to extract PCBs from blood of vultures as previously used for OCs determination in blood [36,37]. Due to poor sensibility for some compounds, efficiency of other extraction solvents was evaluated. For plasma extraction, several solvents were also tested for extraction and reconstitution. The Figure 3 presents an example of the extraction recovery obtained for carbendazim, epoxiconazole, pymetrozine and spinosade-A with different extraction solvents or mixtures for blood and plasma procedures.

Finally, in regard of the extraction yields obtained, a mixture of DCM and ethyl acetate (1:1, *v/v*) was selected for blood extraction with the best average recovery for all compounds of 93.11 %. The combination of methanol with formic acid was conserved for plasma extraction as specified by Hao *et al.* [27] with the best average recovery of 94.33 % but the reconstitution of the sample after evaporation was adapted with a mixture of methanol and ACN (20:80, *v/v*) to avoid water in the final extract and giving the best results for detection of the compounds of interests in this study with the best average recovery of 78.85 % for all compounds of interest (Figure 4).

For blood extractions, the purification step was considered as unnecessary according to the very small amount of sample used for this method (35.80 ± 13.15 mg) compared to the previous studies [28,36,37] and it was decided that the extraction step was sufficient with good extraction yields for most of the compounds.

The rest of the extraction procedures consisting on extraction cycles, centrifugation and evaporation were also tested and conserved as described by Goutner *et al.* [28] and Hao *et al.* [27] given the good results obtained for most of the compounds of interest of this study. All tests were done within blank matrices in triplicate by the same operator within the same day.

3.3. Comparison of SPME and ATD

Prior to GC/MSMS analysis, two different pre-concentration and extraction step were compared. Solid phase micro extraction (SPME) using three different fibers (Polydimethylsiloxane 100 µm, polyacrylate 85 µm and Polydimethylsiloxane/Divinylbenzene 65 µm) was first considered. Based on detection and intensity of signal of each compound, the polyacrylate (PA) fiber was selected. These results were consistent with previous studies [33,38–40]. With this pre-concentration method, extraction had to be adapted because of the non-miscibility of hexane used for blood extraction with water needed for SPME extractions. Indeed, poor sensibility was observed with this extraction solvent due to the non-homogenization of the extract with the SPME saltwater solution. Also, many compounds were non-detectable using SPME. In fact, only 54 compounds out of the 71 of interest were considered well-detected with good signal intensities.

According to these results, thermo desorption (ATD) was then considered. Indeed, this method has already been used for analysis of pesticides in dust and air samples with high efficiency [30]. This method shown good efficiency and recoveries results in both spiked blood

and plasma samples with lower LODs and LOQs as SPME-GC/MSMS. Method performance characteristics are presented in Table 1.

For plasma samples and considering all 104 compounds of the study, LODs ranged from 0.001 ng mg^{-1} to 0.078 ng mg^{-1} in LC/MSMS and from 0.005 ng mg^{-1} to 0.625 ng mg^{-1} in ATD-GC/MSMS and LOQs from 0.003 ng mg^{-1} to 0.263 ng mg^{-1} in LC/MSMS and from 0.005 ng mg^{-1} to 0.625 ng mg^{-1} in ATD-GC/MSMS. Accordingly, LODs ranged from 0.002 ng mg^{-1} to 0.111 ng mg^{-1} in LC and from 0.004 ng mg^{-1} to 0.188 ng mg^{-1} in GC; LOQs from 0.007 ng mg^{-1} to 0.370 ng mg^{-1} and LOQs from 0.001 ng mg^{-1} to 0.625 ng mg^{-1} for blood samples, in LC/MSMS and ATD-GC/MSMS respectively.

3.4. Choice of the internal standards

In LC/MSMS, four deuterated standards were selected with different chemical characteristics and different retention times covering all run. Nicosulfuron-d_6 (t_R = 7.1 min), diuron-d_6 (t_R = 10.21 min), acetochlore-d_{11} (t_R = 12.46 min), and pendimethalin-d_5 (t_R = 17.22 min).

Carbendazim-d_4 (t_R = 2.22 min) was used as a recovery standard for extraction and was added to each sample at a concentration of 1 mg L^{-1} prior to extraction. Table 2 presents the extraction recovery observed in each sample according to the carbendazim-d_4 concentration determined.

Trifluarine-d_{14} (t_R = 11.51 min) and pendimethalin-d_5 (t_R = 19.29 min) were used for quantification of most of the molecules in ATD-GC/MSMS, whereas 2,4-D-d_3 (t_R = 16.88 min) was used for the quantification of derived molecules. In these standards, 4-nitrophenol-d_4 (t_R = 13.19 min) was added as an efficiency standard for the derivatization reaction.

3.5. *Calibrations*

The calibration in LC/MSMS were done in triplicate by spiking within a blank matrix and without matrix to quantify 33 non-volatile compounds. For plasma and blood samples, a good linearity was observed for the responses with correlation coefficients ≥ 0.98, using the linear regression model. The matrix effect was evaluated by comparing the standard deviation of the slope for both calibrations with and without the matrix and no variation of more than 15% was observed.

About the ATD-GC/MSMS method, the same manipulation was conducted to quantify the 71 volatiles or semi-volatiles compounds. Here again, calibrations with a good correlation coefficient were obtained. However, for some fewer volatile compounds, calibration lines of the quadratic type were observed. This can be explained by the desorption kinetics of the compounds (in ATD as in SPME) because for most volatile compounds a curve that is close to a linear regression was obtained. Therefore, for these compounds, a quadratic regression model was used to minimize this deviation. The correlation coefficients were still ≥ 0.98.

3.6. Methods performance characteristics

To evaluate the performance characteristics of those methods, number of interest compounds analyzed, LODs and extraction recoveries obtained were compared with previous extraction methods optimized in this work. To our knowledge, no previous studies investigated the quantification of all those compounds of interest in grey partridges. Even if several previous studies proposed methods for determining pesticide contaminations in other birds, only a few of them included both plasma and blood analyses for such a large number and variety of compounds. However, Goutner *et al.* analyzed 7 PCBs and 16 OCs in blood samples of vultures obtained similar recoveries (between 72–94%) and similar LODs ranged from 0.01 to 0.05 ng g^{-1} [28]. The main difference was the amount of blood extracted, which was about 50 mg in this

study but 2 g in the previous study, giving better sensitivity to this method and allowed to eliminate the purification step. In the study of Hao *et al.*, 8 neonicotinoid compounds and 1 metabolite were monitored in songbirds' plasma samples with higher LODs ranged from 2.3 to 177.7 pg mL^{-1} for a similar amount of plasma of 50 µL and with similar recoveries (between 58–101%) [27]. This present study led to develop more sensitive analytical methods for more compounds with similar or lower LODs. In terms of repeatability and extraction recovery, this work is in the same range than most of the other studies.

4. Application to blood and plasma samples

The developed methods were successfully applied to the analysis of 35 plasma samples and 70 blood samples collected in birds. For LC/MSMS analyses, all the measurements were confirmed by the qualification transition, with a deviation of the (product ion 1/ product ion 2) ratio within an accepted tolerance of 20%. In ATD-GC/MSMS analyses, a percentage of fragmentation of minimum 90% was necessary to confirm detection. The Table 3 resumes principal results. The concentrations ranged between 1.552 to 255.7 pg mg^{-1} for LC and GC analyses of plasma with a positivity ratio of 7.10 to 12.31 % respectively. In contrast, for blood samples, the concentrations were lightly superior and ranged from 7.012 to 369.5 pg mg^{-1} with a lower positivity ratio of 4.46 to 7.34%.

Indeed, lower concentrations were monitored in plasma samples with concentrations sometimes close to the LOQs in both LC and GC. This can be explained by the smaller amount of material used for plasma extraction (m = 35.8 ± 13.2 mg) than for blood extraction (m = 44.2 ± 6.8 mg).

However, although the concentrations found were consistent with previous studies [19,27,28,41], they were still lower. This may probably be due to the origin of the samples. In

fact, blood and plasma samples were collected from young captive-born grey partridges. They have likely not been subjected to many sources of contamination so far.

5. Conclusions and perspectives

In this project, two distinct analytical methods which showed very good analytical characteristics were developed and validated. Both analytical methods achieved a good linearity for the calibration responses in plasma and blood. Methods allowed sensitive detection and quantification in complex biological matrices such as plasma and blood in both LC and GC. For plasma samples and considering all 104 compounds of the study, the average LOD was 0.005 ng mg^{-1} in LC/MSMS and 0.035 ng mg^{-1} in ATD-GC/MSMS and the average LOQ was 0.017 ng mg^{-1} and 0.116 ng mg^{-1} in LC/MSMS and ATD-GC/MSMS respectively. Accordingly, the average LOD for blood samples was 0.011 ng mg^{-1} in LC and 0.028 ng mg^{-1} in GC whereas the average LOQ was 0.038 ng mg^{-1} and 0.094 ng mg^{-1} in LC/MSMS and ATD-GC/MSMS respectively.

Those methods were successfully applied to real samples with an average contamination level of 122.35 ng.mg^{-1} for a positivity ratio of 9.12% in plasma and 216.08 ng mg^{-1} in blood samples for a positivity ratio of 5.35%. Results were consistent to previous studies even though these analyses were restricted to assess contaminations to specific compounds. In this sense, this study aimed to propose a simple and efficient methodology for the analysis of contamination with a wild spectrum of pesticides from different chemical groups with two complementary analytical techniques for eco-toxicological studies.

The choice of several complementary matrices such as blood and plasma allowed a good assessment of environmental contaminations for a selected population of birds. This study also showed blood and plasma from grey partridges were appropriated matrices for biomonitoring

and more accurate evaluations of environmental pollution. However, blood seems to be a better matrix than plasma, allowing higher levels of detection with easier sampling to obtain a larger amount of sample. The scope of this technique is substantial and has the potential for application to testing of a wider range of birds and with other vertebrates in natural populations thus providing a useful tool for monitoring programs in environmental studies. In addition, as this monitoring technique is not destructive, it can be applied to the same animal over a period of time to follow the dynamics of their contamination. Therefore, this technique is promising and opens new dimensions for future studies and applications for ecological risk assessment.

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