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Micro QuEChERS-based method for the simultaneous biomonitoring in whole blood of 360 toxicologically relevant pollutants for wildlife

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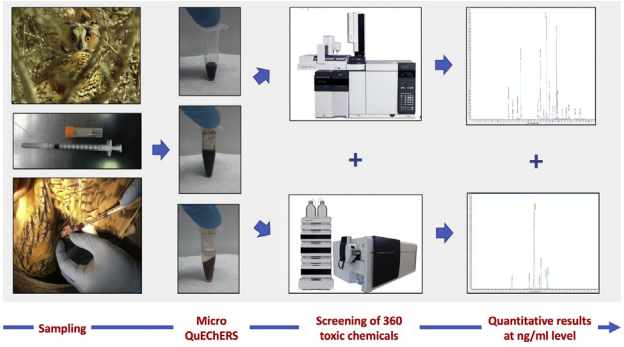
HIGHLIGHTS

- Validation of a method for the simultaneous quantification of 360 toxic chemicals in whole blood
- One-step acetate buffered micro QuEChERS using acidified acetonitrile yielded recoveries >70%.
- Only 250 μl of sample and quantification at the sub-ppb level makes it suitable for biomonitoring.
- Verified in a series of 36 barn owls and 112 common kestrels, which test positive for 3–25 pollutants

GRAPHICAL ABSTRACT



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ABSTRACT

This work presents the optimization, validation, and verification of a miniaturized method for the determination of 360 environmental pollutants that are of toxicological concern for wildlife. The method implies a one-step QuEChERS-based extraction of 250 μl whole blood using acidified acetonitrile, followed by two complementary analyses by LC–MS/MS and GC–MS/MS. The optimized conditions allow the simultaneous determination of the major persistent organic pollutants, a wide range of plant protection products, rodenticides, pharmaceuticals, and a suite of metabolites that can be used as biomarkers of exposure. The method is very sensitive, and 95% of the pollutants can be detected at concentrations below 1.5 ng/ml. The method was applied to a series of 148 samples of nocturnal and diurnal wild raptors collected during field ecological studies in 2018 and 2019. Fifty-one different contaminants were found in these samples, with a median value of 7 contaminants per sample. As expected, five of the six contaminants that were detected in >50% of the samples were persistent or semi-persistent organic pollutants. However, it is striking the high frequency of detection of some non-persistent pollutants, such as 2-phenylphenol, benalaxyl, metaflumizone, diphenylamine, brodifacoum or levamisole, indicating the penetration of these chemicals into the food chains. The toxicological significance of all these findings should be studied in depth in future research. However, the results clearly demonstrated that the approach developed provides reliable, simple, and rapid determination of a wide range of pollutants in wildlife and makes it very useful to obtain valuable data in biomonitoring studies with only small amounts of sample.

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1. Introduction

In environmental chemistry, biomonitoring is the procedure by which the body burden of toxic or potentially toxic chemicals, in living beings, is assessed as a means of exposure assessment. Blood, its fractions, and urine samples are the most common samples, but tissues and other fluids can also be used, such as hair, feathers, or breast milk, among others (Haines et al., 2017; Ibarluzea et al., 2016).

A recent report, has indicated that since 1974 there has been a 60% decrease in wildlife populations worldwide (WWF, 2018). This alarming decrease responds to multiple causes, among which are the change in land uses, the destruction of the habitat of many species, the climate change, but also the penetration of chemistry in ecosystems (Hernout et al., 2011). A clear and very well documented example of the latter, is found in the drastic decline in populations of Asian vultures due to exposure to diclofenac, widely used as a veterinary anti-inflammatory drug, and that in these birds, produces lethal nephropathy (Sathishkumar et al., 2020). The amount and variety of chemical risks that wildlife faces are enormous, and for many of these pollutants, we have limited knowledge of the potential pressures on wildlife (Hernout et al., 2011).

One of the best-studied chemical groups in wildlife is that of the persistent organic pollutants (POPs, mainly organohalogenated compounds), as these compounds have been linked for decades to population declines, diseases or abnormalities in several species, including certain types of fish, birds, and mammals (Luzardo et al., 2014b; Malarvannan et al., 2020). Besides, their monitoring in wildlife seems to be important because certain animal species can also act as sentinels for human health (Bucchia et al., 2015; Elliott et al., 2018; Fox, 2001; Henriquez-Hernandez et al., 2017; Luzardo et al., 2014a; Reif, 2011), and also for identifying trends in levels that can assess the effectiveness of international control measures (Malarvannan et al., 2020).

In addition to all these legacy pollutants, many other chemicals are of concern for wildlife conservation because of their current or recent extensive use, their high toxicity, their stability, and their relatively high half-life. It is the case of some pesticides, pharmaceuticals, or anticoagulant rodenticides, among others. As regards pesticides, most of them are pollutants from the agricultural sector, where dozens of compounds of multiple chemical classes are used massively (Liao et al., 2019). If we take as a reference the list of substances included in the monitoring programs for residues in food for human consumption, at least 200 chemicals should be considered as worrisome in the European Union (EC, 2019a). Unfortunately, the excessive use of these agricultural pesticides causes their penetration into ecosystems and can compromise the health and even survival of many biological species (Encarnacao et al., 2019; Klich et al., 2020; Krief et al., 2017; Plaza et al., 2019). Also, deliberate abuse of pesticides to poison wildlife occurs throughout the world (Bille et al., 2016; Fajardo et al., 2012; Hernandez and Margalida, 2008; Motas-Guzman et al., 2003; Ntemiri et al., 2018; Ogada, 2014; Ruiz-Suarez et al., 2015). Therefore, in wildlife samples, it is also interesting to monitor not only legal but also frequently employed banned compounds (Luzardo et al., 2014c). A special case of pesticides is that of rodenticides, which are employed extensively around the world, leading to unintended exposure of non-target animals, especially raptors (Nakayama et al., 2019; Ruiz-Suarez et al., 2014).

As well as pesticides, pharmaceuticals have the potential to bioaccumulate and transfer through trophic webs and may threaten wildlife health. Wildlife exposure to pharmaceuticals can occur through contaminated water (Obimakinde et al., 2017), agricultural soils, plants and arthropods (Arnold et al., 2014; Bartikova et al., 2015), and through the excreta and carcasses of medicated livestock (i.e., supplementary feeding of threatened avian scavengers) (Blanco et al., 2017; Cuthbert et al., 2014).

The challenge of detecting such a variety of potentially harmful substances in samples from wild animals, is compounded by the fact that the amount of sample available is often small. Blood can be used as a non-lethal simple sampling matrix, but the sample volume is limited by body size, especially in the case of tiny animals such as songbirds or lizards. Therefore, it is desirable to have robust and sensitive analytical methods, and that these are as miniaturized as possible to maximize the information that can be obtained from a field sampling on a wildlife species. Many authors have published methods for the analysis of pesticides in wildlife samples, and some of them are based on the QuEChERS method. However, most of them have been designed for the analysis of a relatively low number of compounds belonging to the same chemical group (Allender and Keegan, 1992; Brown et al., 1996; Brown et al., 2005; Bucchia et al., 2015; Sage et al., 2010; Taliansky-Chamudis et al., 2017). This makes it necessary to use several of these methods in a complementary manner to biomonitoring all the relevant environmental chemicals, which is often impossible due to sample limitation. For this reason, sensitive and specific multi-residue techniques covering a wide spectrum of toxic or potentially toxic environmental pollutants, can substantially contribute to minimizing the costs and maximizing the chance of assessing the exposure of wildlife to most of the relevant chemicals. Thus, some other authors have developed multi-residue and multi-class methods for the determination of drugs (Qie et al., 2019), or of pesticides (Shin et al., 2018; Srivastava et al., 2017), or of POPs in blood (Vijayasarathy et al., 2019), even some using a very small amount of sample (Shin et al., 2018). However, to our knowledge, none has been developed for the simultaneous detection of contaminants for all of these groups together.

We have developed a multi-class multi-residue method comprising a single-step QuEChERS-based extraction of whole blood and two complementary chromatographic analyses coupled to mass spectrometry. The method allows the simultaneous quantification of 360 toxic or potentially toxic chemicals (POPs, agricultural pesticides, pharmaceuticals, and AR) at the sub-part-per-billion level using only 250 μl of whole blood. Additionally, we present data on environmental exposure to pollutants of 148 chicks belonging to two species of birds of prey from the central region of the Iberian Peninsula (common kestrels and barn owls) and discuss their ecological implications.

2. Materials and methods

2.1. Chemicals, reagents, and biological material

Certified standards of all the individual pollutants and deuterated compounds (P-ISs, procedural internal standards) which were initially tested (purity 93.1 to 99.8%), were obtained from Dr. Ehrestorfer (Augsburg, Germany), CPA Chem (Stara Zagora, Bulgaria), A2S – Analytical Standard Solutions (Staint Jean D'Illac, France), Sigma-Aldrich (Augsburg, Germany), Accustandard (New Haven, USA), and European Pharmacopoeia Reference Standards (Strasbourg, France). Salts for extraction based on the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe) were purchased in commercial premixes from Agilent Technologies (Palo Alto, USA) in two formats: QuEChERS Extract Pouch, AOAC Method (6 g de magnesium sulfate and 1.5 g sodium acetate) and QuEChERS Extract Pouch, EN Method (4 g magnesium sulfate, 1 g sodium chloride, 1 g sodium citrate dihydrate, and 0,5 g sodium hydrogencitrate sesquihydrate). QuEChERs dSPE Enhanced Matrix Removal-Lipid (EMR-lipid; Agilent, Palo Alto, USA) was used as a clean-up step in the optimization process. Acetonitrile (ACN, 99.9% purity), methanol (MeOH, 99.9% purity), and formic acid (FA, 98.0% purity) were purchased from Honeywell (Charlotte, USA), and were of LC-MS grade. The water for preparing the mobile phase (18.2 MΩ/cm) was obtained using an Elix Advantage 15UV tandem coupled to a MilliQ A10 Gradient system (Millipore, Molsheim, France). Ammonium acetate and ammonium formate were purchased from Fisher (Fisher Scientific UK, Loughborough, UK), and was of Optima LC-MS grade.

For the development, optimization, and validation of the analytical technique, we employed blood samples obtained from chickens and

goats from the animal housing facilities of the Faculty of Veterinary of the University of Las Palmas de Gran Canaria. All the animals were born in this facility, were healthy and had never been exposed to chemicals (no farms or agricultural facilities in the nearby, and no pharmacological treatments in the last two months), to avoid drug interference. Whole blood was obtained by puncturing the brachial vein (chickens, 23G needle) or the jugular vein (goats, 20G needle), using 3.5 ml-vacutainer tubes with heparin as anticoagulant. Upon arrival at the laboratory, aliquots were homogenized, pooled (by species), and stored at −24 °C until use.

To verify the applicability of the validated method to real samples, we studied a series of 148 blood samples. The samples were obtained from a diurnal and a nocturnal species of raptors (*Falco tinnunculus* and *Tyto alba*) and were collected during an ecological field study on the impact on wildlife of the treatment with rodenticides against a common vole (*Microtus arvalis*) plague. The samples were obtained from nest boxes located in the provinces of Palencia, Salamanca, Burgos, Segovia, Valladolid and Zamora (Castilla-León, Spain). All samples were collected after obtaining the corresponding permits and following the animal welfare protocols during the sampling (Espin et al., 2016).

2.2. Stock solutions, calibration standards and quality controls

Stock solutions of all POPs, pesticides, AR, pharmaceuticals, metabolites, and P-ISs were prepared by dissolving an accurately weighed amount in the suitable solvent (ACN, MeOH, water, acetone) to obtain a concentration of either 1 or 0.5 mg/ml. These stock solutions were stored in aliquots at −32 °C until use (maximum 1 year). Three intermediate working solutions were prepared by combining the individual standards (by groups: pesticides, pharmaceuticals and POPs, to avoid interferences between compounds and solvents), to give a concentration of 1 μg/ml/each and stored at −32 °C. Those solutions were renewed every three months. Deuterated standards were prepared separately in the same way in one mixture for both, GC and LC. Calibration standards were made from independent intermediate solutions of the stock solution and spiked in the chicken-, goat-, or combined blood to obtain 12 calibration standards in the range of 0.1 to 20 ng/ml. These matrix-matched calibration standards were freshly prepared for each experiment (daily). Quality controls (QC) samples were made in the same way to obtain three different levels (0.2, 2, and 10 ng/ml) of all the chemicals. Blank matrix samples were prepared to calculate linearity, matrix effect, carryover, interferences, and stability.

2.3. Instrumental analysis

For the detection and quantification of the 360 analytes finally included in this procedure, it is necessary to perform two complementary chromatographic analyses from the blood extract: a liquid chromatography analysis coupled to triple quadrupole mass spectrometry (LC-MS/MS) and an analysis by gas chromatography coupled to triple quadrupole mass spectrometry (GC–MS/MS).

2.3.1. LC-MS/MS

An Agilent 1290 UHPLC tandem coupled to an Agilent 6460 mass spectrometer (Agilent Technologies, Palo Alto, USA) was employed for the analysis of 234 chemicals. The chromatographic separations were performed using an InfinityLab Poroshell 120 (2.1 mm × 100 mm, 2.7 μm). Agilent 1290 Infinity II Inline Filter with 0.3 μm SS frit, and Agilent InfinityLab Poroshell 120 UHPLC Guard column (2.1 mm × 5 mm, 2.7 μm) were used to protect the column. The mobile phase A consisted on 2 mM ammonium acetate and 0.1% FA in ultrapure water, while the mobile phase B consisted on 2 mM ammonium acetate in MeOH. The mobile phase A gradient was: 95% - 0.5 min; 80% - 1 min; 60% - 2.5 min; 15% - 8 min; 0% - 10 to 14 min; 95% - 14.01 min. The flow rate was set at 0.4 ml/min. The injection volume was 8 μl. The column oven temperature was set at 50 °C. Total run time was 18 min. The mass spectrometer was operated in the dynamic multiple reaction monitoring (dMRM) mode. The optimized operating conditions of the mass spectrometer analyses, in positive and negative, electrospray ionization (Agilent Jet Stream Electrospray Ionization Source, AJS-ESI) were the following: gas temperature 190 °C; nebulizer gas flow 11 l/min; nebulizer pressure 26 psi; sheath gas temperature 330 °C; sheath gas flow 12 l/min; capillary voltages 3900 V (positive), 2600 V (negative); cycle time 800 ms; dwell time 8–60 ms. Nitrogen provided by Zefiro 40 nitrogen generator (F-DGSi, Evry, France) was used as drying and desolvation gas. Nitrogen 6.0 (99.9999% purity, Linde, Dublin, Ireland) was used as collision gas.

2.3.2. GC–MS/MS

An Agilent 7890B gas chromatographer equipped with an Agilent 7693 automatic sampler and tandem coupled to an Agilent 7010 mass spectrometer (Agilent Technologies, Palo Alto, USA) was employed for the analysis of 126 chemicals. A 1.5 μl aliquot of the sample extract was injected on an ultra-inert glass wool inlet liner in pulsed splitless mode. Inlet temperature was set at 250 °C. The chromatographic separations were performed using two fused silica ultra-inert capillary columns Agilent J&W HP-5MS (crosslinked 5% phenyl-methylpolysiloxane, Agilent Technologies), each with a length of 15 m, 0.25 mm i.d., and a film thickness of 0.25 μm. The use of two 15-m columns allowed the use of the backflushing technique. Both columns were connected by a Purged Ultimate Union (PUU; Agilent Technologies). Helium (99.999%) was set in constant flow mode as carrier gas, and the flow was adjusted by the retention time lock feature using chlorpyrifos methyl as a reference (reference time = 9.143 min). Nitrogen 6.0 (99,9999% purity, Linde, Dublin, Ireland) was used as collision gas. The oven temperature program was programmed as follows: (a) 80 °C held for 1.8 min; (b) increase to 170 °C at a rate of 40 °C/min; (c) increase to 310 °C at a rate of 10 °C/min to 310 °C; (d) 3 min hold time at 310 °C. The final run time was 21.05 min. Post-run backflush was set at −5.8 ml/min, 315 °C for 5 min. The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode, using 24-time segments. The optimized operating conditions of the mass spectrometer analyses were the following: electron impact (EI) ionization source temperature 280 °C; collision gas flow 1.5 ml/min; transfer line temperature 280 °C; solvent delay 3.7 min; cycle time between 300 and 600 ms; dwell time between 15 and 40 ms.

2.4. Sample preparation

The optimized extraction protocol consisted of the modification and miniaturization of the QuEChERS method (Anastassiades et al., 2003). For the simultaneous extraction of 360 chemicals, the final extraction protocol was as follows: whole blood samples (250 μl) were placed into a 2 ml Eppendorf tube. At this point, the fortification of blank matrix samples for a matrix-matched 12-point calibration curve, was done using different volumes of intermediate fortification solutions for each calibration point. Ten microliters of the mixture of P-ISs, which included compounds used for both, GC (acenaphthene-d10, chlorpyrifos-d10, chrysene-d12, diazinon-d10, PCB 200, and phenanthrene-d10) and LC (atrazine-d5, carbendazim-d3, cyromazine-d4, diazinon-d10, linuron-d3, and pirimicarb-d6) were added to all samples and calibration points to yield a final concentration of 1 ng/ml. The samples were vortex-mixed for 30 s, and placed in an orbital shaker for 1 h, to ensure the adequate dispersion and homogenization of the analytes with the blood components. After that, 500 μl of acidified acetonitrile (1% FA) were added, and the tubes were well vortexed for 30 s. Then, the tubes were placed in an ultrasonic bath (Selecta, Barcelona, Spain) at room temperature for 20 min. After that, anhydrous magnesium sulfate (150 mg) and sodium acetate (37.5 mg) were added to each tube and thoroughly mixed using vortex for 30 s, and then, vigorous-manually shaken for 1 min. Finally, the samples were microcentrifuged (4200 rpm, 5 min) using an ALC 4214 microcentrifuge (A.L.C.

International SRL, Cologno Monzese, Italy). The supernatant (approximately 400 μl) was collected with a 1-ml syringe, passed through a 0.2 μm Chromafil PET-20/15 MS syringe filter (polyester, HPLC certified, Macherey-Nagel, Düren, Germany), and placed in an amber inserted chromatographic vial. This vial was used directly in two consecutive analyses by GC–MS/MS and LC-MS/MS, without the need for further clean-up, dilution or solvent change steps.

2.5. Assay validation procedures

The main objective of the validation was to demonstrate the reliability and performance of the method, developed and applied to the whole blood matrix. Initially, chicken and goat blood were tested separately. Still, since there were no significant differences in the performance of the extraction procedures, the complete validation was done with a mixture of the two types of blood, as recommended (EC, 2019b; SWGTOX, 2013). The method validation was performed using the following parameters: identity, selectivity, linearity (as a working range), accuracy (as bias and precision), carryover, interferences and LOQ. The assessment of the matrix effect was also carried out. In general, we followed the recommendations contained in the SANTE guide (EC, 2019b). Since this guide is mainly aimed at the analysis of pesticides in food and feed samples, we have also taken into account the recommendations contained in the guide of Standard Practices for Method Validation in Forensic Toxicology (SWGTOX, 2013), mainly with regard to the particularities of working with the whole blood matrix and pharmaceuticals. All validation assays involve adding known concentrations of analytes to the matrix. However, given the enormous amount of substances included in the method, the whole blood was not completely free of 100% of the chemicals, in particular the POPs. Therefore, the response of the white matrix sample was subtracted from calibration standards and QC to calculate the response of the analyte added externally. All the details of the final method validation are generally described in the Results and Discussion section. Still, the data for each compound are summarized in Table 1 of the accompanying Data in Brief article entitled "*Supporting dataset on the method validation of micro QuEChERS-based method for the simultaneous biomonitoring in whole blood of 360 toxicologically relevant pollutants for wildlife and results in 148 real samples (Falco tinnunculus and Tyto alba)*".

2.6. Statistical analysis

Both, within-run and between-run precisions, were calculated using the one-way ANOVA approach with the run number (usually $n = 5$) as the grouping variable. The ANOVA calculations were done using the GraphPad Prism v6.0 (GraphPad Software, CA, USA).

3. Results and discussion

3.1. Optimization of MS/MS conditions and chromatography

The mass spectrometry conditions were optimized for the detection and quantification of 360 compounds (234 by LC-MS/MS and 126 by GC–MS/MS).

3.1.1. LC-MS/MS

For the mass spectrometry optimization of each compound analyzed by LC-MS/MS, individual chromatographic vials with a concentration of around 100–200 ng/ml were prepared. The mobile phase conditions were established based on the literature and methodologies previously developed in our laboratory (Luzardo et al., 2015; Luzardo et al., 2013; Luzardo et al., 2014c; Ruiz-Suarez et al., 2015; Ruiz-Suarez et al., 2014). A stainless steel zero dead volume union was used to replace the chromatographic column. The optimization of the precursor ion signal and the product ion signal was conducted manually, as well as the optimization of the fragmentation and collision energy. The best combination of two MRM transitions was selected for each compound (Table 1). Once the list of transitions for all target compounds and P-ISs was completed, the optimization steps for gas temperature, gas flow, nebulizer gas pressure, sheath gas flow and temperature, capillary voltage (+/−), and nozzle voltage for the AJS-ESI, were performed sequentially using the Mass Hunter Source Optimizer software (Agilent Technologies, Palo Alto, USA).

During the early stage of the chromatographic method development, the suitability and performance of the two different columns were assessed. Both columns are reversed-phased but with slightly different specifications. The first column tested was the ZORBAX Eclipse Plus C18 (2.1 mm × 50 mm, 1.8 μm), that produced a very broad peak with severe peak tailing for many target analytes. The second column, which we routinely use, the Agilent InfinityLab Poroshell 120 (2.1 mm × 100 mm, 2.7 μm), showed an exceptional narrow peak for nearly all target analytes based on the shape of a Gaussian peak. In addition, we tested two different chromatography conditions (mobile phase and gradient) based on our previous experience on the analysis of pesticides and ARs by LC-MS/MS. Condition A: 0.1% FA and 2 mM ammonium formate in both, water and methanol; condition B: 2 mM ammonium acetate in both, water and methanol. We observed that FA was not optimum for ARs, which performed better in the absence of acid. However, FA proved to be necessary for the analysis of many pesticides, so a compromise was adopted, and we continue to use the acid. The different experiments showed that a sufficient degree of ionization for the analysis of all the compounds (positive/negative), was obtained at 2 mM ammonium acetate (water and methanol), with an optimal percentage of 0.1% formic acid in water, nor in methanol. In the last step, a series of experiments were conducted to select the optimal volume of injection, which was finally set in 8 μl.

3.1.2. GC–MS/MS

Acquisition method for GC–MS/MS compounds was initially supplied by Agilent, but it was further optimized in our laboratory. A sequence of injections was programmed to determine the optimal collision energies in increments of 5 eV (range from 5 to 60 eV); the results of this optimization are also reflected in Table 1, where the analytical parameters of the complete list of chemicals (in alphabetical order) has been summarized. Dwell time and cycle time were also optimized. In GC, no optimization was made in relation to the column type or the temperature program, since our group had previous experience on the separation of many of these compounds or very similar combinations (Bucchia et al., 2015; Luzardo et al., 2015; Luzardo et al., 2014c).

However, since ACN extracts are injected in this method, we did carry out a series of experiments to optimize the solvent delay time, the initial temperature of the oven (60 to 90, in increments of 10 °C), the temperature of the injector (range of 230 to 300 °C, in increments of 10 °C), the temperature of the ionization source (range of 250 to 320 °C, in increments of 10 °C), the temperature of the transfer line (from 270 to 320 °C, in increments of 10 °C), and the injection volume (from 0.8 to 1.8 μl, in increments of 0.2 μl). These experiments were performed by injecting a mixture of all the analytes in acetonitrile at two concentrations (1 ng/ml and 50 ng/ml), and the parameters that gave the best shape and peak intensity for most of the compounds were selected.

3.2. Optimization of sample preparation

The previous experience of our laboratory in the development and application of multi-residue methods made us opt for the QuEChERS method. Two widely available modifications of this method were compared (3 replicates at two concentrations (2–20 ng/ml), analyzed in duplicate): the AOAC Official Method 2007.01 (Lehotay et al., 2010); and the UNE-EN 15662:2019 Official Method (EN, 2019). Both methodologies employ acetonitrile as the extraction solvent. We decided not to test others as acetonitrile has proved to be the solvent with the highest

Table 1
List of compounds analyzed in whole blood together with the category of use, legal status, the technique employed, and the instrumental conditions of the optimized methods.

No.	Compound	Category[a]	Legal status in the EU[b]	Subjected to MRL[c]	Technique[d]	Retention time (min)	LOQ (ng/ml)	Polarity	Quantification		Confirmation		Fragmentor voltage (V)
									MRM transition (*m/z*)	Collision energy (eV)	MRM transition (*m/z*)	Collision energy (eV)	
1	2-Phenylphenol	F	Approved	Yes	GC	6.28	0.20	Positive	169.0 → 115.0	30	169.0 → 141.0	15	70
2	4,4′-Dichlorobenzophenone (metabolite of dicofol)	Met	–	No	GC	9.99	0.80	Positive	250.0 → 139.0	15	250.0 → 215.0	5	70
3	Abamectine	I, A, AH	Approved	Yes	LC	10.99	4.00	Positive	890.5 → 567.1	10	895.5 → 751.4	45	160
4	Acenaphthene	POP	–	No	GC	6.15	0.20	Positive	153.0 → 152.0	25	153.0 → 151.0	35	70
5	Acenaphtylene	POP	–	No	GC	5.94	0.20	Positive	152.0 → 151.0	25	152.0 → 126.0	30	70
6	Acephate	I	Not approved	Yes	LC	1.64	2.00	Positive	184.0 → 143.0	15	143.0 → 95.0	15	70
7	Acetaminophen (paracetamol)	V, NSAID	Approved	–	LC	2.71	1.20	Positive	152.1 → 65.0	40	152.1 → 93.0	20	150
8	Acetamiprid	I	Approved	Yes	LC	4.43	0.40	Positive	223.1 → 126.0	27	223.1 → 90.0	45	140
9	Acrinathrin	I, A	Approved	Yes	GC	10.70	1.20	Positive	559.0 → 208.0	10	559.0 → 181.0	30	70
10	Albendazole	V, AH	Approved	–	LC	7.14	0.10	Positive	266.1 → 234.1	16	266.1 → 191.0	32	155
11	Aldicarb	I	Not approved	Yes	LC	5.11	0.10	Positive	208.0 → 116.0	10	116.0 → 89.1	4	100
12	Aldicarb-sulfone	Met	–	Yes	LC	3.21	0.40	Positive	240.1 → 76.0	16	223.1 → 86.1	13	75
13	Aldicarb-sulfoxide	Met	–	Yes	LC	2.75	1.60	Positive	207.1 → 131.9	10	207.1 → 89.1	10	86
14	Aldrin	POP	–	Yes	GC	9.90	0.40	Positive	255.0 → 220.0	25	263.0 → 228.0	10	70
15	Anthracene	POP	–	No	GC	8.40	0.80	Positive	178.0 → 176.0	35	178.0 → 152.0	30	70
16	Atrazine	H	Not approved	No	LC	6.73	0.10	Positive	216.0 → 173.9	15	216.0 → 103.8	30	130
17	Azinphos-methyl	I	Not approved	Yes	LC	7.27	0.20	Positive	318.0 → 132.1	8	340.0 → 160.0	10	60
18	Azoxystrobin	F	Approved	Yes	LC	7.59	0.10	Positive	404.1 → 372.1	8	404.1 → 344.1	24	110
19	BDE-28	POP	–	No	GC	12.22	0.20	Positive	406.0 → 246.0	20	406.0 → 167.0	25	70
20	BDE-47	POP	–	No	GC	14.31	0.20	Positive	326.0 → 138.0	45	484.0 → 324.0	25	70
21	BDE-85	POP	–	No	GC	17.08	0.10	Positive	564.0 → 404.0	25	566.0 → 406.0	25	70
22	BDE-99	POP	–	No	GC	16.27	0.10	Positive	566.0 → 406.0	25	564.0 → 404.0	30	70
23	BDE-100	POP	–	No	GC	15.85	0.10	Positive	566.0 → 406.0	25	564.0 → 404.0	25	70
24	BDE-153	POP	–	No	GC	18.04	0.20	Positive	644.0 → 484.0	25	486.0 → 377.0	30	70
25	BDE-154	POP	–	No	GC	17.47	0.10	Positive	644.0 → 484.0	25	486.0 → 377.0	30	70
26	BDE-183	POP	–	No	GC	20.12	0.20	Positive	561.6 → 454.7	40	563.6 → 454.7	40	70
27	Benalaxyl	F	Approved	No	LC	8.96	0.10	Positive	326.2 → 148.0	20	326.2 → 208.0	12	90
28	Bendiocarb	I	Not approved	No	LC	5.88	0.10	Positive	224.1 → 166.9	8	224.2 → 108.9	15	120
29	Bendiocarb metabolite (2, 2-dimethylbenzo-1, 3-dioxol-4-ol)	Met	–	No	GC	4.84	1.20	Positive	166.0 → 151.0	10	166.0 → 126.0	20	70
30	Benfuracarb	I, AH	Not approved	No	LC	9.73	0.10	Positive	411.2 → 190.0	13	411.2 → 252.0	15	110
31	Benzo[*a*]anthracene	POP	–	No	GC	13.95	0.80	Positive	228.0 → 226.0	40	228.0 → 202.0	35	70
32	Benzo[*a*]pyrene	POP	–	No	GC	16.89	0.10	Positive	252.0 → 250.0	45	252.0 → 248.0	60	70
33	Benzo[*b*]fluoranthene	POP	–	No	GC	16.30	0.80	Positive	252.0 → 248.0	60	252.0 → 226.0	35	70
34	Benzo[*ghi*]perylene	POP	–	No	GC	19.61	0.40	Positive	276.0 → 274.0	50	276.0 → 272.0	60	70
35	Benzo[*k*]fluoranthene	POP	–	No	GC	16.29	0.40	Positive	252.0 → 250.0	45	252.0 → 224.0	40	70
36	Bifenthrin	I	Not approved	Yes	GC	11.25	0.20	Positive	440.0 → 181.0	5	440.0 → 165.0	60	94
37	Bitertanol	F	Not approved	Yes	LC	9.23	0.40	Positive	338.2 → 70.0	4	338.2 → 269.2	5	100
38	Boscalid (formerly nicobifen)	F	Approved	Yes	GC	7.84	0.10	Positive	3434.0 → 272.0	30	343.0 → 140.0	45	100
39	Brodifacoum	R	Not approved	No	LC	10.78	0.80	Negative	521.3 → 79.0	50	523.3 → 135.0	45	220
40	Bromadiolone	R	Approved	No	LC	9.75	0.40	Negative	525.3 → 250.0	40	527.3 → 250.0	40	200
41	Bromopropylate	A	Not	Yes	GC	13.87	0.20	Positive	341.0 → 183.0	15	341.0 → 157.0	45	70

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Table 1 (*continued*)

No.	Compound	Category[a]	Legal status in the EU[b]	Subjected to MRL[c]	Technique[d]	Retention time (min)	LOQ (ng/ml)	Polarity	Quantification		Confirmation		Fragmentor voltage (V)
									MRM transition (*m*/*z*)	Collision energy (eV)	MRM transition (*m*/*z*)	Collision energy (eV)	
			approved										
42	Bromuconazole (two isomers)	F	Approved	No	GC	13.81/14.24	0.20	Positive	295.0 → 173.0	10	295.0 → 175.0	10	70
43	Bupirimate	F	Approved	Yes	GC	11.78	0.20	Positive	273.0 → 108.0	15	273.0 → 193.0	5	70
44	Buprofezin	I	Approved	Yes	LC	9.83	0.10	Positive	306.1 → 201.0	12	306.1 → 116.0	12	140
45	Cadusafos (ebufos)	I, AH	Not approved	No	LC	9.39	0.10	Positive	271.1 → 159.0	16	271.1 → 131.0	22	100
46	Carbaryl	I	Not approved	Yes	LC	6.21	0.10	Positive	202.1 → 145.1	4	202.1 → 127.1	28	95
47	Carbendazim (azole)	F	Not approved	Yes	LC	2.90	0.40	Positive	192.1 → 160.1	4	202.1 → 127.1	28	90
48	Carbofuran	I, AH	Not approved	Yes	LC	5.91	0.10	Positive	222.1 → 123.1	20	222.1 → 165.1	30	80
49	Carbofuran-3-hydroxy	Met	–	Yes	LC	4.27	0.40	Positive	238.1 → 163.1	10	238.1 → 181.1	10	110
50	Carbosulfan	I, AH	Not approved	No	LC	11.03	0.40	Positive	381.2 → 160.2	12	381.2 → 76.1	36	120
51	Cefuroxima axetil (two isomers)	V, MB	Not approved	–	LC	5.13	0.80	Positive	533.0 → 447.0	15	533.0 → 386.0	20	160
52	Chloramphenicol	V, MB	Approved	–	LC	4.63	2.00	Negative	321.0 → 152.1	4	323.0 → 152.1	4	113
53	Chlorantraniliprole	I	Approved	Yes	LC	7.32	0.20	Positive	483.9 → 452.9	16	483.9 → 285.9	8	105
54	Chlorfenapyr	I, A	Not approved	Yes	GC	12.01	1.20	Positive	247.0 → 200.0	30	247.0 → 227.0	15	70
55	Chlorfenvinphos	I	Not approved	No	LC	9.09	0.20	Positive	361.1 → 98.9	34	358.9 → 155.1	8	105
56	Chlorobenzilate	A	Not approved	No	GC	12.14	0.40	Positive	251.0 → 111.0	40	251.0 → 139.0	15	70
57	Chlorophacinone	R	Not approved	No	LC	8.88	0.80	Negative	373.2 → 201.0	20	375.2 → 203.0	20	160
58	Chlorpropham	H	Not approved	Yes	GC	7.13	0.20	Positive	213.0 → 127.0	15	153.0 → 90.0	25	70
59	Chlorpyrifos	I	Not approved	Yes	GC	9.93	0.80	Positive	314.0 → 258.0	15	314.0 → 286.0	5	70
60	Chlorpyrifos methyl	I	Not approved	Yes	GC	9.12	0.40	Positive	286.0 → 93.0	25	286.0 → 271.0	15	70
61	Chlorthal dimethyl	H	Not approved	No	GC	10.02	0.20	Positive	300.9 → 166.9	55	300.9 → 222.9	25	70
62	Chrysene	POP	–	No	GC	13.86	0.80	Positive	228.0 → 226.0	40	228.0 → 227.0	25	70
63	Clindamycin	V, MB	Approved	–	LC	5.33	0.40	Positive	425.2 → 126.1	20	425.2 → 377.2	20	150
64	Clofentezine	A	Approved	Yes	LC	9.19	0.40	Positive	303.1 → 138.0	12	303.1 → 102.0	40	120
65	Clothianidin	I	Not approved	Yes	LC	3.91	1.20	Positive	250.0 → 169.0	8	250.0 → 131.9	8	100
66	Cloxacillin	V, MB	Approved	–	LC	6.86	1.60	Positive	436.1 → 160.0	8	436.1 → 277.0	12	126
67	Cortiscosterone	V, GC	Not approved	–	LC	7.89	0.80	Positive	389.1 → 329.0	13	389.1 → 371.0	13	80
68	Coumachlor	R	Not approved	No	LC	8.63	0.20	Positive	343.1 → 162.8	15	342.1 → 285.0	15	120
69	Coumaphos	I, A	Not approved	No	LC	8.98	0.10	Positive	363.0 → 227.0	30	363.0 → 306.9	15	120
70	Coumatetralyl	R	Not approved	No	LC	8.31	0.40	Negative	291.1 → 141.0	30	291.1 → 247.0	20	140
71	Cyazofamid	F	Approved	Yes	LC	8.49	0.80	Positive	325.0 → 108.0	20	325.0 → 261.1	15	90
72	Cyflufenamid	F	Approved	Yes	LC	9.18	0.20	Positive	413.1 → 223.1	33	413.1 → 295.1	23	70
73	Cyfluthrin (sum of four isomers)	I	Not approved[e]	Yes	GC	16.07/16.19/16.25/16.32	1.20	Positive	226.0 → 206.0	25	198.9 → 170.1	25	70

74	Cyhalothrin (lambda isomer)	I	Approved	Yes	LC	10.49	2.00	Positive	467.0 → 225.0	10	467.0 → 141.0	46	66
75	Cymoxanil	F	Approved	Yes	LC	4.67	0.40	Positive	199.1 → 128.0	4	199.1 → 110.9	12	90
76	Cypermethrin (sum of four isomers)	I	Approved[f]	Yes	GC	16.34/16.44/16.52/16.63	4.00	Positive	163.0 → 109.0	20	163.0 → 127.0	5	70
77	Cyproconazole (two isomers)	F	Approved	Yes	LC	8.14	0.40	Positive	292.2 → 70.2	18	292.2 → 125.1	24	100
78	Cyprodinil	F	Approved	Yes	LC	8.46	0.20	Positive	226.0 → 93.0	33	226.0 → 108	25	100
79	Cyromazine	I, A	Not approved	Yes	LC	1.23	2.00	Positive	167.1 → 85.0	16	167.1 → 125.0	20	120
80	Danofloxacin	V, MB	Approved	–	LC	4.04	1.20	Positive	358.2 → 340.1	20	358.2 → 82.1	50	159
81	Dazomet	I, A, AH, F, H	Approved	No	GC	7.80	1.60	Positive	161.9 → 44.0	28	161.9 → 89.0	5	70
82	Deltamethrin	I, A	Approved	Yes	LC	10.65	0.80	Positive	523.0 → 281.0	10	523.0 → 506.0	5	100
83	Demeton-*S*-methyl	I, A	Not approved	No	LC	5.97	0.10	Positive	230.9 → 88.9	5	230.9 → 61.0	30	50
84	Demeton-*S*-methyl-sulfone (Dioxydemeton)	I, A	Not approved	No	LC	3.31	0.40	Positive	263.0 → 169.0	24	263.0 → 109.0	12	120
85	Dexamethasone	V, GC	Approved	–	LC	7.16	0.40	Positive	393.2 → 373.2	2	393.2 → 355.2	6	103
86	Diazinon	I	Not approved	Yes	GC	8.29	0.40	Positive	137.1 → 54.0	20	304.0 → 179.0	15	70
87	Dibenzo[*a*,*h*]anthracene	POP	–	No	GC	19.15	0.40	Positive	278.0 → 276.0	40	278.0 → 250.0	60	70
88	Dichlorodiphenyldichloroethane (p,p′ DDD)	POP	–	Yes	GC	12.31	0.10	Positive	235.0 → 165.0	20	235.0 → 199.0	15	70
89	Dichlorodiphenyldichloroethylene (p,p′ DDE)	POP	–	Yes	GC	11.58	0.10	Positive	318.0 → 176.0	60	318.0 → 248.0	30	70
90	Dichlorodiphenyltrichloroethane (p,p′ DDT)	POP	–	Yes	GC	12.84	1.20	Positive	235.0 → 165.0	40	235.0 → 199.0	15	70
91	Diclofenac	V, NSAID	Approved	–	LC	8.73	0.80	Positive	296.0 → 215.1	16	296.0 → 214.1	48	103
92	Dicloran	F, MB, WP	Not approved	Yes	GC	7.80	0.10	Positive	206.0 → 176.0	10	206.0 → 148.0	25	70
93	Dicloxacillin	V, MB	Not approved	–	LC	7.24	1.20	Positive	470.0 → 160.0	8	470.0 → 310.8	10	106
94	Dieldrin	POP	–	Yes	GC	11.66	1.20	Positive	263.0 → 228.0	15	277.0 → 241.0	15	70
95	Diethathyl ethyl	H	Not approved	No	LC	8.71	0.20	Positive	312.2 → 238.1	15	312.2 → 162.0	30	120
96	Diethofencarb	F, MB, WP	Approved	Yes	LC	7.57	0.10	Positive	268.2 → 226.1	5	268.2 → 152.0	20	110
97	Difenacoum	R	Not approved	No	LC	10.38	0.40	Negative	443.2 → 135.0	40	443.2 → 293.0	35	200
98	Difenoconazole	F, MB, WP	Approved	Yes	LC	9.41	0.40	Positive	406.1 → 250.9	28	406.1 → 337.0	16	176
99	Difethialone	R	Not approved	No	LC	10.93	0.80	Negative	537.3 → 79.0	50	537.3 → 151.0	45	220
100	Difloxacin	V, MB	Not approved	–	LC	3.86	0.80	Positive	400.2 → 382.1	20	400.2 → 356.1	16	149
101	Diflubenzuron	I	Approved	Yes	LC	8.63	1.20	Positive	311.0 → 158.0	8	311.0 → 141.0	32	90
102	Diflufenican	H	Approved	No	LC	9.51	0.10	Positive	395.1 → 266.0	24	395.1 → 246.0	36	150
103	Dimethenamid-P (and its R-isomer)	H	Approved	No	LC	7.68	0.10	Positive	276.1 → 244.1	10	276.1 → 168.1	20	125
104	Dimethoate	I	Not approved	Yes	LC	4.21	0.40	Positive	230.0 → 125.0	16	230.0 → 198.8	20	70
105	Dimethomorph (two isomers)	F, MB, WP	Approved	Yes	LC	7.86	0.40	Positive	388.1 → 301.1	20	388.1 → 165.1	32	180
106	Dimethylphenylsulfamide (DMSA, metabolite of dichlofluanid)	Met[h]	–	No	LC	5.21	0.80	Positive	201.1 → 92.1	15	201.1 → 137.1	5	100
107	Diniconazole-M	F, MB, WP	Not approved	Yes	LC	9.34	0.20	Positive	326.1 → 70.0	28	328.1 → 70.0	28	110
108	Dinocap	F, MB, WP	Not approved	No	LC	10.51	0.80	Negative	295.4 → 208.9	30	295.4 → 193.0	35	150
109	Diphacinone	R	Not approved	No	LC	8.60	1.20	Negative	339.1 → 167.0	25	339.1 → 145.0	20	170
110	Diphenylamine	PHP	Not approved	Yes	GC	6.98	0.20	Positive	168.0 → 167.2	15	169.0 → 66.0	15	70
111	Dodine	F, MB, WP	Approved	Yes	LC	9.02	0.40	Positive	228.3 → 43.0	40	228.3 → 57.0	25	150

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Table 1 (*continued*)

No.	Compound	Category[a]	Legal status in the EU[b]	Subjected to MRL[c]	Technique[d]	Retention time (min)	LOQ (ng/ml)	Polarity	Quantification		Confirmation		Fragmentor voltage (V)
									MRM transition (*m*/*z*)	Collision energy (eV)	MRM transition (*m*/*z*)	Collision energy (eV)	
112	Endosulfan alfa	POP	–	Yes	GC	11.21	0.80	Positive	241.0 → 206.0	15	195.0 → 160.0	10	70
113	Endosulfan beta	POP	–	Yes	GC	12.21	0.80	Positive	241.0 → 206.0	15	195.0 → 159.0	15	70
114	Endosulfan sulfate	POP	–	No	GC	12.96	0.80	Positive	270.0 → 235.0	15	387.0 → 289.0	5	70
115	Endrin	POP	–	No	GC	12.05	1.60	Positive	263.0 → 193.0	35	245.0 → 173.0	25	70
116	Enrofloxacin	V, MB	Approved	–	LC	3.94	1.20	Positive	360.2 → 316.1	16	360.2 → 245.1	28	144
117	EPN	I, A	Not approved	No	GC	13.90	0.80	Positive	157.0 → 63.0	10	157.0 → 110.0	15	70
118	Epoxiconazole	F	Approved	Yes	LC	8.47	0.20	Positive	330.0 → 120.9	24	330.1 → 100.9	50	120
119	Eprinomectin	V, MB	Approved	–	LC	10.84	0.20	Positive	878.5 → 186.0	15	936.5 → 490.4	60	160
120	Eritromicin	V, MB	Approved	–	LC	6.74	0.20	Positive	734.5 → 158.1	32	734.5 → 576.3	16	172
121	Esfenvalerate	I	Approved	No	GC	17.56	2.00	Positive	167.1 → 125.1	15	167.1 → 89.1	45	70
122	Ethion (diethion)	I, A	Not approved	Yes	LC	10.03	0.10	Positive	385.0 → 199.0	5	385.0 → 171.0	10	100
123	Ethirimol	F, MB, WP	Not approved	Yes	LC	4.80	0.40	Positive	210.2 → 140.1	20	210.2 → 98.1	28	160
124	Ethofumesate	H	Approved	No	GC	9.59	0.80	Positive	286.0 → 207.0	5	286.0 → 161.0	20	70
125	Ethoprophos	I, AH	Not approved	No	LC	8.38	0.20	Positive	243.1 → 97.0	30	243.1 → 130.9	15	90
126	Etofenprox	I, A	Approved	Yes	GC	16.75	0.80	Positive	163.0 → 107.0	20	163.0 → 135.0	10	70
127	Etoxazole	A	Approved	Yes	LC	10.34	0.10	Positive	360.1 → 141.0	26	360.1 → 304.0	16	160
128	Famoxadone	H	Approved	Yes	LC	9.07	1.20	Positive	392.1 → 330.9	5	392.2 → 238.1	12	110
129	Fenamidone	F	Not approved	Yes	LC	9.06	0.10	Positive	392.1 → 330.9	5	392.1 → 238.1	12	110
130	Fenamiphos	I, AH	Approved	Yes	LC	7.72	0.10	Positive	304.1 → 217.1	20	304.1 → 202.0	36	120
131	Fenamiphos sulfone	Met	–	Yes	LC	8.63	0.20	Positive	336.1 → 188.0	31	336.1 → 266.0	23	120
132	Fenamiphos sulfoxide	Met	–	Yes	LC	5.93	0.40	Positive	320.1 → 233.0	20	320.1 → 108.1	44	120
133	Fenarimol	F, MB, WP	Not approved	Yes	GC	15.03	0.20	Positive	139.0 → 75.0	30	139.0 → 111.0	15	70
134	Fenazaquin	A	Approved	Yes	LC	10.73	0.80	Positive	307.2 → 57.1	25	307.2 → 161.1	16	90
135	Fenbendazole	V, AH	Approved	–	LC	8.04	0.10	Positive	300.1 → 268.1	20	300.1 → 159.0	36	156
136	Fenbuconazole	F, V	Approved	Yes	GC	16.17	0.40	Positive	198.0 → 102.0	30	198.0 → 78.0	30	70
137	Fenbutatin oxide	I, A	Not approved	Yes	LC	11.67	0.80	Positive	519.0 → 197.0	55	517.3 → 194.9	60	180
138	Fenhexamid	F	Approved	Yes	LC	8.35	1.60	Positive	302.1 → 97.1	20	302.1 → 55.1	40	130
139	Fenitrothion	I	Not approved	Yes	GC	9.57	0.20	Positive	277.0 → 109.0	15	277.0 → 125.0	15	70
140	Fenoxycarb	I	Approved	Yes	LC	8.69	0.10	Positive	302.1 → 88.0	20	302.1 → 116.1	10	110
141	Fenpropathrin	I, A	Not approved	Yes	LC	10.43	0.40	Positive	367.2 → 125.0	16	350.1 → 125.0	16	72
142	Fenpropidin	F	Approved	Yes	LC	7.13	0.10	Positive	274.3 → 147.0	30	274.3 → 86.0	25	170
143	Fenpropimorph	F	Not approved	Yes	LC	7.37	0.10	Positive	304.3 → 147.1	30	304.3 → 130.0	25	120
144	Fenpyroximate	A	Approved	Yes	LC	10.49	0.40	Positive	422.2 → 366.2	12	422.2 → 135.0	36	160
145	Fenthion	I, A	Not approved	Yes	LC	8.90	0.10	Positive	278.9 → 168.8	18	278.9 → 247.0	8	98
146	Fenthion oxon	Met	–	Yes	LC	7.31	0.10	Positive	263.1 → 231.2	16	263.1 → 216.0	24	120
147	Fenthion oxon sulfone	Met	–	Yes	LC	4.50	0.80	Positive	295.0 → 217.0	15	295.0 → 104.2	24	110
148	Fenthion oxon sulfoxide	Met	–	Yes	LC	4.26	0.20	Positive	279.0 → 264.2	20	279.0 → 104.1	28	110
149	Fenthion sulfone	Met	–	Yes	LC	6.39	0.80	Positive	311.0 → 125.0	22	311.0 → 109.0	28	140
150	Fenthion sulfoxide	Met	–	Yes	LC	6.16	0.40	Positive	295.0 → 108.9	30	295.0 → 280.0	18	140
151	Fenvalerate	I	Not approved	Yes	GC	17.36	2.00	Positive	167.0 → 125.1	22	167.0 → 89.0	30	70
152	Fipronil	I, V	Not	Yes	LC	8.68	0.20	Negative	435.0 → 330.0	12	435.0 → 249.9	26	116

			approved											
153	Fipronil sulfide	Met	–	Yes	GC	10.49	0.80	Positive	351.0 → 255.0	20	420.0 → 351.0	25	70	
154	Flocoumafen	R	Not approved	No	LC	10.44	0.20	Negative	541.3 → 382.0	25	541.3 → 161.0	40	230	
155	Fluazinam	F	Approved	No	LC	10.01	0.20	Negative	462.9 → 416.0	10	462.9 → 398.0	9	140	
156	Flubendiamide	I	Approved	Yes	LC	8.82	2.00	Positive	408.0 → 274.0	15	408.0 → 256.0	30	120	
157	Flucythrinate (two isomers)	I, A	Not approved	No	GC	16.67/16.84	0.80	Positive	156.9 → 107.1	15	199.1 → 107.1	25	70	
158	Fludioxonil	F	Approved	Yes	GC	11.51	0.20	Positive	248.0 → 127.0	30	248.1 → 182.1	10	70	
159	Flufenoxuron	I, A	Not approved	Yes	LC	10.37	0.10	Positive	489.1 → 158.0	20	489.1 → 140.9	56	110	
160	Flumequine	V, MB	Approved	–	LC	6.12	0.10	Positive	262.1 → 244.0	16	262.1 → 202.0	32	116	
161	Flunixin	V, NSAID	Approved	–	LC	8.09	0.20	Positive	297.1 → 279.1	24	297.1 → 264.1	32	141	
162	Fluopyram	F	Approved	Yes	GC	10.61	0.20	Positive	173.0 → 95	35	223.0 → 196.0	40	70	
163	Fluoranthene	POP	–	No	GC	10.66	0.20	Positive	202.0 → 201.0	27	202.0 → 152.0	42	70	
164	Fluorene	POP	–	No	GC	6.81	0.20	Positive	165.0 → 163.0	40	165.0 → 139.0	30	70	
165	Fluquinconazole	F	Approved	Yes	GC	15.81	0.20	Positive	340.0 → 298.0	15	340.0 → 286.0	25	70	
166	Flusilazole	F, MB, WP	Not approved	Yes	LC	8.64	0.20	Positive	316.1 → 247.1	15	316.1 → 165.0	20	160	
167	Flutolanil	F, MB, WP	Approved	No	LC	7.93	0.10	Positive	324.1 → 262.1	16	324.1 → 242.1	24	130	
168	Flutriafol	F	Approved	Yes	GC	11.26	0.20	Positive	219.0 → 95.0	35	219.0 → 123.0	15	70	
169	Fluvalinate tau	I, A	Approved	Yes	GC	17.56	4.00	Positive	250.1 → 55.1	30	252.0 → 200.0	20	70	
170	Fonofos	I	Not approved	No	GC	8.24	0.40	Positive	246.0 → 109.0	15	246.0 → 237.0	5	70	
171	Formetanate	I, A	Approved	Yes	LC	1.76	0.10	Positive	222.1 → 165.1	12	222.1 → 46.2	28	105	
172	Fosthiazate	AH, V	Approved	Yes	LC	6.50	0.10	Positive	284.0 → 104.0	20	284.0 → 227.8	8	90	
173	Heptachlor	POP	–	Yes	GC	9.31	0.80	Positive	272.0 → 237.0	15	274.0 → 239.0	15	70	
174	Hexachlorobencene	POP	–	Yes	GC	7.77	0.20	Positive	284.0 → 214.0	40	284.0 → 249.0	25	70	
175	Hexachlorocyclohexane (alpha)	POP	–	Yes	GC	7.64	0.40	Positive	219.0 → 109.0	10	219.0 → 183.0	10	70	
176	Hexachlorocyclohexane (beta)	POP	–	Yes	GC	8.02	0.40	Positive	219.0 → 109.0	40	219.0 → 183.0	5	70	
177	Hexachlorocyclohexane (delta)	POP	–	No	GC	8.50	0.20	Positive	219.0 → 109.0	45	219.0 → 183.0	5	70	
178	Hexaclorocyclohexane (gamma, lindane)	POP	–	Yes	GC	8.13	1.20	Positive	291.0 → 109.0	40	219.0 → 183.0	10	70	
179	Hexaconazole (two isomers)	F, MB, WP	Not approved	Yes	LC	8.49	0.80	Positive	314.1 → 70.1	20	316.0 → 70.1	20	95	
180	Hexaflumuron	I	Not approved	No	LC	9.58	0.40	Negative	458.8 → 439.0	8	458.8 → 175.0	30	100	
181	Hexythiazox	A	Approved	Yes	LC	10.18	0.10	Positive	353.1 → 227.9	8	353.1 → 168.1	24	120	
182	Imazalil (enilconazole)	F, MB, WP, V	Approved	Yes	LC	6.53	0.40	Positive	297.1 → 159.0	20	297.1 → 69.1	18	100	
183	Imidacloprid	I	Approved	Yes	LC	3.93	0.80	Positive	256.0 → 175.0	12	256.0 → 209.0	12	110	
184	Indeno [1,2,3-cd] pyrene	POP	–	No	GC	19.08	0.40	Positive	276.0 → 274.0	50	276.0 → 272.0	60	70	
185	Indoxacarb	I	Approved	Yes	LC	9.49	0.20	Positive	528.1 → 293.1	10	528.1 → 202.8	48	140	
186	Iprodione	F, MB, WP	Not approved	Yes	GC	13.67	4.00	Positive	314.0 → 56.0	20	314.0 → 245.0	10	70	
187	Iprovalicarb	F	Approved	Yes	LC	8.18	0.20	Positive	321.2 → 119.0	15	321.2 → 202.9	20	110	
188	Isocarbophos	I	Not approved	Yes	GC	10.04	1.60	Positive	230.0 → 155.0	25	230.0 → 198.0	10	70	
189	Isofenphos methyl	I	Not approved	No	GC	10.38	0.40	Positive	199.0 → 121.0	10	241.0 → 121.0	25	70	
190	Isoprothiolane	F, MB, WP	Not approved	Yes	LC	7.94	0.10	Positive	291.1 → 189.0	30	291.1 → 145.0	36	100	
191	Ivermectin B1a	V, AH, A	Approved	–	LC	11.52	1.60	Positive	897.5 → 753.5	50	897.5 → 329.3	60	160	
192	Josamycin	V, MB	Not approved	–	LC	7.40	0.40	Positive	860.5 → 173.9	40	860.5 → 108.9	40	200	
193	Ketoprofen	V, NSAID	Approved	–	LC	7.34	0.40	Positive	255.1 → 209.1	8	255.1 → 77.1	48	123	
194	Kresoxim methyl	F	Approved	Yes	GC	11.78	1.20	Positive	116.0 → 89.0	15	206.0 → 131.0	10	70	
195	Leptophos	I	Not approved	No	GC	14.58	0.80	Positive	171.0 → 77.1	15	377.0 → 362.0	20	70	

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Table 1 (*continued*)

No.	Compound	Category[a]	Legal status in the EU[b]	Subjected to MRL[c]	Technique[d]	Retention time (min)	LOQ (ng/ml)	Polarity	Quantification		Confirmation		Fragmentor voltage (V)
									MRM transition (*m*/*z*)	Collision energy (eV)	MRM transition (*m*/*z*)	Collision energy (eV)	
196	Levamisole	V, AH	Approved	–	LC	3.12	0.20	Positive	205.1 → 178.1	20	205.1 → 123.0	32	141
197	Lincomycin	V, MB	Approved	–	LC	3.50	0.40	Positive	407.2 → 126.1	24	407.2 → 359.2	16	150
198	Linuron	F	Approved	Yes	LC	7.54	0.20	Positive	249.0 → 160.1	20	249.0 → 182.3	8	120
199	Lufenuron	I	Not approved	Yes	LC	10.05	0.40	Negative	509.0 → 339.0	5	509.0 → 326.1	15	90
200	Malaoxon	I	Not approved	No	LC	6.03	0.10	Positive	315.1 → 127.2	12	315.1 → 99.1	36	120
201	Malathion	I	Not approved	Yes	LC	7.93	0.20	Positive	348.0 → 126.7	15	348.0 → 285.0	8	100
202	Mandipropamid	F	Approved	Yes	LC	7.90	0.10	Positive	412.1 → 328.1	8	412.1 → 356.1	4	130
203	Marbofloxacin	V, MB	Approved	–	LC	3.53	2.00	Positive	363.2 → 72.1	25	363.2 → 320.1	15	134
204	Mebendazole	V, AH	Approved	–	LC	6.68	0.10	Positive	296.1 → 264.1	20	296.1 → 77.0	48	151
205	Mefenamic acid	V, NSAID	Not approved	–	LC	9.52	0.40	Positive	242.1 → 209.1	28	242.1 → 180.1	0	108
206	Mefenoxam (metalaxyl-M)	F	Approved	Yes	LC	6.95	0.10	Positive	280.0 → 220.0	10	280.0 → 192.0	15	110
207	Meloxicam	V, NSAID	Approved	–	LC	7.17	0.20	Positive	352.5 → 114.8	20	352.5 → 140.8	20	130
208	Mepanipyrim	F, MB, WP	Approved	Yes	GC	11.13	0.40	Positive	222.0 → 221.0	15	222.0 → 207.0	15	70
209	Mepiquat	H	Approved	Yes	LC	0.64	0.40	Positive	114.0 → 98.0	36	114.0 → 70.0	45	100
210	Metaflumizone	I	Approved	No	LC	9.94	0.20	Negative	505.0 → 302.0	14	541.0 → 302.0	20	90
211	Metaldehyde	M	Approved	No	LC	3.87	4.00	Positive	194.1 → 61.9	5	194.1 → 106.0	5	50
212	Metconazole	F	Approved	No	LC	9.17	0.10	Positive	320.1 → 70.2	33	322.1 → 70.2	24	250
213	Methamidophos (two isomers)	I, A	Not approved	Yes	LC	1.18	1.20	Positive	142.0 → 94.0	12	142.0 → 125.0	12	85
214	Methidathion	I, A	Not approved	Yes	LC	7.12	0.10	Positive	320.1 → 144.8	8	320.1 → 85.0	30	84
215	Methiocarb	I, A, M	Not approved	Yes	LC	7.67	0.10	Positive	226.1 → 169.0	4	226.1 → 121.1	12	90
216	Methiocarb-sulfoxide	Met	–	Yes	LC	4.03	0.80	Positive	242.0 → 185.0	22	242.0 → 122.0	28	90
217	Methomyl	I, A, AH	Nor approved	Yes	LC	3.23	0.40	Positive	163.1 → 88.0	5	163.0 → 106.0	8	80
218	Methomyl oxime	Met	–	Yes	LC	3.25	8.00	Positive	106.2 → 58.1	10	106.2 → 31.2	20	70
219	Methoxyfenozide	I	Approved	Yes	LC	8.00	0.10	Positive	369.2 → 149.0	10	369.2 → 313.1	15	85
220	Metoxychlor	POP	–	No	GC	13.98	0.80	Positive	227.0 → 141.0	20	227.0 → 169.0	15	70
221	Metrafenone	F	Approved	Yes	LC	9.27	0.10	Positive	409.1 → 209.1	8	411.1 → 209.1	12	108
222	Metronidazole	V, MB	Approved	–	LC	2.63	0.80	Positive	172.1 → 128.0	12	172.1 → 82.1	24	98
223	Mevinphos (phosdrin)	I, A	Not approved	No	LC	4.38	0.80	Positive	225.0 → 193.1	15	225.0 → 127.0	12	65
224	Mirex	POP	–	No	GC	5.66	2.00	Positive	237.0 → 143.0	30	274.0 → 237.0	10	70
225	Monocrotophos	I	Not approved	Yes	LC	3.31	0.80	Positive	224.1 → 126.8	12	224.1 → 98.1	15	100
226	Myclobutanil	F, MB, WP	Approved	Yes	LC	8.10	0.10	Positive	289.1 → 70.1	16	289.1 → 125.1	32	110
227	N-(2,4-dimethylphenyl)-*N*′-methylformamidine (DMPF, metabolite of amitraz)	Met[g]	–	No	LC	3.35	0.80	Positive	163.1 → 122.1	15	163.1 → 107.1	15	100
228	*N*,*N*-dimethylformamidine (DMF, metabolite of amitraz)	Met[g]	–	No	LC	5.45	1.20	Positive	150.1 → 77.0	40	150.1 → 105.8	30	100
229	*N*,*N*-dimethyl-N′-p-tolylsulphamide (DMST, metabolite of tolyfluanid)	Met[i]	–	No	LC	6.06	0.20	Positive	215.1 → 106.1	10	215.1 → 151.1	4	90
230	Nafcillin	V, MB	Not approved	–	LC	7.33	0.80	Positive	415.0 → 199.1	8	415.0 → 171.0	36	103
231	Naphtalene	POP	–	No	GC	4.45	0.80	Positive	128.0 → 127.0	15	128.0 → 102.0	25	70
232	Naproxen	V, NSAID	Not approved	–	LC	7.59	1.60	Positive	231.0 → 185.0	10	231.1 → 169.9	21	120

233	Nitenpyram	I	Not approved	No	LC	3.30	2.00	Positive	271.1 → 56.1	36	271.1 → 224.9	12	100
234	Novobiocin	V, MB	Not approved	–	LC	9.69	0.80	Positive	613.2 → 218.1	10	613.2 → 396.1	10	150
235	Nuarimol	F, MB, WP	Approved	No	GC	13.27	0.20	Positive	235.0 → 139.0	15	235.0 → 111.0	40	70
236	Ofurace	F, MB, WP	Approved	No	LC	5.97	0.10	Positive	282.0 → 159.9	20	282.0 → 147.9	30	100
237	Omethoate	I, A	Not approved	Yes	LC	2.80	0.40	Positive	214.1 → 124.8	22	214.1 → 183.0	5	100
238	Oxadixyl	F, MB, WP	Not approved	Yes	LC	5.43	0.20	Positive	279.1 → 219.2	5	279.1 → 132.2	32	110
239	Oxamyl	I, A, AH	Approved	Yes	LC	2.87	0.40	Positive	237.1 → 72.0	12	237.1 → 90.0	5	70
240	Oxfendazole	V, AH	Approved	–	LC	5.61	0.10	Positive	316.1 → 159.0	32	316.1 → 191.1	16	166
241	Oxolinic acid	V, MB	Not approved	–	LC	5.04	0.20	Positive	262.1 → 216.0	32	262.1 → 160.0	36	110
242	Oxydemeton methyl	I	Not approved	Yes	LC	3.01	0.40	Positive	247.0 → 169.0	12	247.0 → 109.0	24	100
243	Oxyfluorfen	H	Approved	No	GC	11.68	0.40	Positive	252.0 → 146.0	40	300.0 → 223.0	15	70
244	Paclobutrazol	H	Approved	Yes	LC	7.89	0.40	Positive	294.1 → 70.1	16	294.1 → 125.2	36	115
245	Paraoxon methyl	I	Not approved	No	GC	9.00	1.60	Positive	230.0 → 106.0	20	230.0 → 136.0	5	70
246	Parathion ethyl	I	Not approved	No	GC	9.95	1.20	Positive	290.9 → 109.0	10	138.9 → 109.0	5	70
247	Parathion methyl	I	Not approved	Yes	GC	9.12	0.80	Positive	263.0 → 109.0	15	263.0 → 79.0	30	70
248	PCB 28	POP	–	Yes	GC	9.01	0.10	Positive	256.0 → 186.0	25	256.0 → 151.0	50	70
249	PCB 52	POP	–	Yes	GC	9.58	0.20	Positive	292.0 → 222.0	25	292.0 → 220.0	25	70
250	PCB 77	POP	–	Yes	GC	11.73	0.20	Positive	292.0 → 220.0	25	292.0 → 222.0	25	70
251	PCB 81	POP	–	Yes	GC	11.56	0.10	Positive	292.0 → 220.0	25	292.0 → 222.0	25	70
252	PCB 101	POP	–	Yes	GC	11.08	0.20	Positive	326.0 → 256.0	30	328.0 → 256.0	30	70
253	PCB 105	POP	–	Yes	GC	12.66	0.10	Positive	326.0 → 256.0	30	328.0 → 256.0	30	70
254	PCB 114	POP	–	Yes	GC	12.38	0.20	Positive	326.0 → 256.0	30	328.0 → 256.0	30	70
255	PCB 118	POP	–	Yes	GC	12.18	0.20	Positive	326.0 → 256.0	30	328.0 → 256.0	30	70
256	PCB 123	POP	–	Yes	GC	12.10	0.40	Positive	326.0 → 256.0	30	328.0 → 256.0	30	70
257	PCB 126	POP	–	Yes	GC	13.23	0.20	Positive	326.0 → 256.0	30	328.0 → 256.0	30	70
258	PCB 138	POP	–	Yes	GC	13.07	0.10	Positive	360.0 → 290.0	25	360.0 → 288.0	25	70
259	PCB 153	POP	–	Yes	GC	12.57	0.10	Positive	360.0 → 290.0	25	360.0 → 288.0	25	70
260	PCB 156	POP	–	Yes	GC	13.96	0.20	Positive	360.0 → 290.0	25	360.0 → 288.0	25	70
261	PCB 157	POP	–	Yes	GC	14.07	0.40	Positive	360.0 → 290.0	25	360.0 → 288.0	25	70
262	PCB 167	POP	–	Yes	GC	13.55	0.10	Positive	360.0 → 290.0	25	360.0 → 288.0	25	70
263	PCB 169	POP	–	Yes	GC	14.61	0.20	Positive	360.0 → 290.0	25	360.0 → 288.0	25	70
264	PCB 180	POP	–	Yes	GC	14.25	0.10	Positive	394.0 → 324.0	30	394.0 → 322.0	30	70
265	PCB 189	POP	–	Yes	GC	15.25	0.10	Positive	394.0 → 324.0	30	394.0 → 322.0	30	70
266	Penconazole	F, MB, WP	Approved	Yes	GC	10.52	0.40	Positive	248.0 → 157.0	30	248.0 → 192.0	15	70
267	Pencycuron	F, MB, WP	Approved	Yes	LC	9.33	0.10	Positive	329.1 → 125.1	24	329.1 → 217.9	12	160
268	Pendimethalin	H	Approved	Yes	GC	10.49	0.80	Positive	252.0 → 162.0	10	252.0 → 191.0	5	70
269	Penicillin G	V, MB	Not approved	–	LC	5.82	2.00	Positive	335.1 → 176.0	10	335.1 → 160.0	4	110
270	Penicillin V	V, MB	Not approved	–	LC	6.47	2.00	Positive	383.2 → 159.9	10	383.2 → 113.9	40	130
271	Permethrin	I, A	Not approved	Yes	GC	15.69	1.20	Positive	183.0 → 128.0	15	183.1 → 153.1	15	70
272	Phenanthrene	POP	–	No	GC	8.40	0.20	Positive	178.0 → 176.0	35	178.0 → 152.0	28	70
273	Phenylbutazone	V, NSAID	Approved	–	LC	8.25	1.60	Positive	309.2 → 160.2	20	309.2 → 77.1	55	140
274	Phosalone	I, A	Not approved	No	LC	9.20	0.20	Positive	385.1 → 182.0	20	385.1 → 110.9	55	80

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Table 1 (*continued*)

No.	Compound	Category[a]	Legal status in the EU[b]	Subjected to MRL[c]	Technique[d]	Retention time (min)	LOQ (ng/ml)	Polarity	Quantification		Confirmation		Fragmentor voltage (V)
									MRM transition (*m*/*z*)	Collision energy (eV)	MRM transition (*m*/*z*)	Collision energy (eV)	
275	Phosmet	I, A	Approved	Yes	LC	7.34	0.20	Positive	318.0 → 159.9	16	318.0 → 133.0	40	90
276	Phosmet oxon	Met	–	Yes	LC	5.36	0.20	Positive	302.0 → 160.0	10	302.0 → 133.0	38	60
277	Piperacillin	V, MB	Not approved	–	LC	5.68	0.40	Positive	518.2 → 143.0	16	518.2 → 160.0	4	121
278	Pirimicarb	I	Approved	Yes	LC	5.11	0.10	Positive	239.1 → 72.1	20	239.1 → 182.1	12	100
279	Pirimiphos ethyl	I, A	Not approved	No	GC	10.26	0.10	Positive	318.0 → 166.0	15	318.0 → 182.0	15	70
280	Pirimiphos methyl	I, A	Approved	Yes	LC	9.13	0.10	Positive	306.1 → 164.0	20	306.1 → 108.1	32	100
281	Prochloraz	F, MB, WP	Approved	No	LC	9.08	0.10	Positive	376.0 → 308.0	10	376.0 → 70.1	20	100
282	Procymidone	F, MB, WP	Not approved	Yes	GC	10.80	1.60	Positive	283.0 → 67.0	40	283.0 → 68.0	25	70
283	Profenofos	I, A	Not approved	Yes	LC	9.75	0.10	Positive	375.0 → 305.0	20	373.0 → 303.0	20	100
284	Propamocarb	F	Approved	Yes	LC	2.85	0.40	Positive	189.2 → 102.0	12	189.2 → 144.0	8	110
285	Propargite	A	Not approved	Yes	LC	10.37	0.10	Positive	368.2 → 231.1	4	368.2 → 175.0	12	88
286	Propiconazole	A	Not approved	Yes	LC	9.01	0.40	Positive	342.0 → 69.0	21	342.0 → 159.0	39	90
287	Propoxur	I	Not approved	No	LC	5.83	0.10	Positive	210.1 → 168.1	35	210.1 → 65.1	40	70
288	Propyzamide (pronamide)	H	Approved	Yes	LC	7.92	0.10	Positive	256.1 → 190.0	16	256.1 → 173.0	25	90
289	Proquinazid	F	Approved	Yes	GC	13.32	0.20	Positive	288.0 → 245.0	15	288.0 → 217.0	30	70
290	Prothioconazol	F	Approved	Yes	GC	11.85	0.40	Positive	186.0 → 49.0	20	186.0 → 53.0	25	70
291	Prothiophos	F	Not approved	No	GC	11.45	0.40	Positive	266.9 → 221.0	35	162.0 → 63.1	30	70
292	Pymetrozine	I	Not approved	Yes	LC	2.74	0.80	Positive	218.1 → 105.0	20	218.1 → 78.0	52	120
293	Pyraclostrobin	F	Approved	Yes	LC	9.15	0.10	Positive	388.1 → 193.8	8	388.1 → 163.1	28	120
294	Pyrazophos	F, MB, WP	Not approved	No	LC	9.22	0.10	Positive	374.1 → 222.1	23	374.1 → 194.0	32	100
295	Pyrene	POP	–	No	GC	11.13	0.20	Positive	202.0 → 201.0	27	202.0 → 200.0	45	70
296	Pyridaben	I, A	Approved	Yes	LC	10.75	0.10	Positive	365.2 → 309.0	8	309.1 → 147.0	16	168
297	Pyridaphenthion	I, A	Not approved	No	LC	8.11	0.20	Positive	341.0 → 189.0	22	341.0 → 205.0	34	100
298	Pyrimethanil	F	Approved	Yes	GC	8.27	0.20	Positive	198.0 → 118.0	40	198.0 → 158.0	20	70
299	Pyriproxifen	I	Approved	Yes	LC	10.07	0.10	Positive	322.2 → 96.0	12	322.2 → 184.9	24	80
300	Quinalfos	I, A	Not approved	No	LC	8.72	0.20	Positive	299.1 → 96.9	30	299.1 → 147.1	20	130
301	Quinoxyfen	F	Not approved	Yes	LC	10.13	0.10	Positive	308.0 → 197.0	32	308.2 → 161.8	55	120
302	Rifampicin	V, MB	Not approved	–	LC	7.89	0.80	Positive	823.5 → 791.4	15	823.5 → 399.1	25	160
303	Rotenone	I, R	Not approved	No	LC	8.64	0.40	Positive	395.1 → 213.1	20	395.1 → 192.1	25	150
304	Roxithromycin	V, MB	Not approved	–	LC	7.67	0.80	Positive	838.5 → 158.1	40	838.5 → 116.1	55	200
305	Sarafloxacin	V, MB	Not approved	–	LC	4.16	4.00	Positive	386.1 → 342.1	16	386.1 → 299.1	28	144
306	Simazine	I	Not approved	No	LC	5.81	0.20	Positive	202.4 → 68.1	30	202.4 → 68.1	20	120
307	Spinosad (two isomers)	I, V	Approved	Yes	LC	9.10/9.43	0.10	Positive	732.4 → 142.0	22	732.4 → 98.0	60	130
308	Spiramycin (two isomers)	V, MB	Approved	–	LC	4.58/4.90	0.40	Positive	439.1 → 101.1	20	439.1 → 88.0	50	70

309	Spirodiclofen	A	Approved	Yes	LC	10.50	0.80	Positive	411.1 → 71.2	15	411.1 → 313.0	5	110
310	Spiromesifen	I	Approved	Yes	LC	10.27	0.20	Positive	388.0 → 273.0	25	273.0 → 187.0	15	110
311	Spiroxamine	F	Approved	Yes	LC	7.55	0.10	Positive	298.3 → 144.1	16	298.3 → 100.1	32	120
312	Strychnine	R	Not approved	No	LC	3.00/3.61	0.80	Positive	335.1 → 184.0	45	335.1 → 156.0	40	105
313	Sulfacetamide	V, MB	Not approved	–	LC	2.13	0.40	Positive	215.3 → 155.9	10	215.3 → 92.0	20	90
314	Sulfachloropiridacine	V, MB	Not approved	–	LC	3.77	0.80	Positive	285.0 → 156.0	12	285.0 → 92.1	28	101
315	Sulfadiacine	V, MB	Approved	–	LC	2.80	0.80	Positive	251.0 → 92.0	28	251.0 → 156.0	12	111
316	Sulfadimetoxine	V, MB	Approved	–	LC	4.81	0.10	Positive	311.0 → 92.0	32	311.0 → 156.0	16	139
317	Sulfadoxine	V, MB	Approved	–	LC	4.12	0.10	Positive	311.1 → 92.0	32	311.1 → 156.0	16	126
318	Sulfameracine	V, MB	Not approved	–	LC	3.26	0.20	Positive	265.0 → 92.0	28	265.0 → 156.0	12	126
319	Sulfametacine	V, MB	Not approved	–	LC	3.44	0.20	Positive	279.1 → 186.0	12	279.1 → 92.0	32	134
320	Sulfametizole	V, MB	Not approved	–	LC	3.37	0.80	Positive	271.0 → 92.0	28	271.0 → 155.9	8	103
321	Sulfametoxazole	V, MB	Approved	–	LC	3.93	0.40	Positive	254.0 → 92.0	28	254.0 → 156.0	12	111
322	Sulfametoxipiridacine	V, MB	Not approved	–	LC	3.45	0.40	Positive	281.0 → 155.9	12	281.0 → 92.1	28	121
323	Sulfamonomethoxine	V, MB	Not approved	–	LC	4.11	1.20	Positive	281.1 → 156.0	14	281.1 → 92.1	32	120
324	Sulfapyridine	V, MB	Not approved	–	LC	2.82	0.40	Positive	250.0 → 156.0	12	250.0 → 92.0	28	126
325	Sulfaquinoxaline	V, MB	Approved	–	LC	4.99	0.40	Positive	301.0 → 156.0	12	301.0 → 92.1	32	159
326	Sulfatiazole	V, MB	Not approved	–	LC	2.98	0.40	Positive	256.0 → 92.0	28	256.0 → 156.0	12	106
327	Sulfisoxazole	V, MB	Not approved	–	LC	4.12	0.80	Positive	268.0 → 156.0	8	268.0 → 92.1	24	106
328	Tebuconazole	I, A	Approved	Yes	LC	8.92	0.80	Positive	308.2 → 70.2	22	308.2 → 125.1	53	120
329	Tebufenocide	I	Approved	Yes	LC	8.66	0.10	Positive	353.1 → 132.9	22	353.1 → 297.1	20	90
330	Tebufenpyrad	A	Approved	Yes	LC	9.88	0.10	Positive	334.2 → 117.0	47	334.2 → 145.0	37	180
331	Teflubenzuron	I	Not approved	Yes	LC	10.01	1.20	Negative	379.0 → 339.0	15	379.0 → 196.0	25	100
332	Tefluthrin	I	Approved	Yes	GC	8.42	0.10	Positive	177.0 → 127.0	15	177.0 → 87.0	15	70
333	Telodrin (isobenzan)	I	Not approved	No	GC	10.14	0.80	Positive	310.8 → 240.8	25	310.8 → 274.8	5	70
334	Terbufos	I, AH	Not approved	No	GC	8.15	0.20	Positive	231.0 → 97.0	20	231.0 → 129.0	15	70
335	Terbuthylazine	H	Approved	Yes	GC	8.12	0.40	Positive	214.0 → 104.0	20	214.0 → 132.0	10	70
336	Tetrachlorvinphos	I	Not approved	No	LC	8.72	0.40	Positive	367.0 → 127.0	16	365.0 → 127.0	16	110
337	Tetraconazole	F, H	Approved	Yes	GC	10.04	0.20	Positive	336.0 → 204.0	35	336.0 → 218.0	20	70
338	Tetradifon	A	Not approved	No	GC	14.36	0.40	Positive	158.9 → 111.0	20	354.0 → 159.0	10	70
339	Tetramethrin	I	Not approved	No	GC	13.87	1.60	Positive	164.0 → 77.0	30	164.0 → 107.0	15	70
340	Thiabendazole	AH, V	Approved	Yes	LC	3.50	0.20	Positive	202.0 → 175.0	24	202.0 → 131.0	36	170
341	Thiacloprid	I	Approved	No	LC	4.80	0.20	Positive	253.0 → 126.0	16	253.0 → 90.0	40	140
342	Thiamethoxam	I	Not approved	Yes	LC	3.59	0.80	Positive	292.0 → 211.1	8	292.0 → 132.0	22	80
343	Thiophanate methyl	I	Approved	Yes	LC	5.87	0.20	Positive	343.0 → 151.0	20	343.0 → 93.0	46	90
344	Tolclofos methyl	F, MB, WP	Approved	Yes	GC	9.21	0.10	Positive	265.0 → 93.0	30	265.0 → 220.0	25	70
345	Tolfenamic acid	V, NSAID	Not approved	–	LC	9.80	0.40	Negative	260.0 → 216.1	8	260.0 → 35.1	20	108
346	Triadimefon	F, MB, WP	Not approved	Yes	LC	8.03	0.40	Positive	294.1 → 69.3	20	294.1 → 197.2	15	100

(continued on next page)

Table 1 (*continued*)

No.	Compound	Category[a]	Legal status in the EU[b]	Subjected to MRL[c]	Technique[d]	Retention time (min)	LOQ (ng/ml)	Polarity	Quantification		Confirmation		Fragmentor voltage (V)
									MRM transition (*m*/*z*)	Collision energy (eV)	MRM transition (*m*/*z*)	Collision energy (eV)	
347	Triadimenol	F, MB, WP	Not approved	Yes	LC	8.22	0.40	Positive	296.1 → 70.0	10	298.1 → 70.0	10	80
348	Triazophos (hostathion)	I, A	Not approved	Yes	LC	8.18	0.10	Positive	314.1 → 162.0	19	314.1 → 118.9	35	100
349	Trichlorfon	I, AH, V	Not approved	No	LC	4.06	1.20	Positive	256.9 → 109.0	12	258.9 → 109.0	12	170
350	Trifloxystrobin	F	Approved	Yes	LC	9.50	0.10	Positive	409.1 → 186.0	12	409.1 → 145.0	52	110
351	Triflumizole	F	Approved	No	LC	9.53	0.10	Positive	346.1 → 278.0	4	345.9 → 73.0	15	80
352	Triflumuron	I	Approved	Yes	LC	9.19	0.40	Positive	359.0 → 156.0	8	359.0 → 139.0	32	120
353	Trifluralin	H	Not approved	No	GC	7.27	0.20	Positive	264.0 → 160.0	15	306.0 → 264.0	5	70
354	Trimethoprim	V, MB	Approved	–	LC	3.45	0.80	Positive	291.2 → 123.0	24	291.2 → 230.1	20	162
355	Triticonazole	F	Approved	No	LC	8.38	0.40	Positive	318.1 → 70.1	33	320.1 → 70.1	16	110
356	Tylmicosin	V, MB	Approved	–	LC	5.52	1.60	Positive	869.6 → 174.1	48	869.6 → 696.4	44	294
357	Tylosin	V, MB	Approved	–	LC	6.76	0.80	Positive	916.5 → 174.1	40	916.5 → 772.4	28	210
358	Vinclozolin	F, MB, WP	Not approved	Yes	GC	9.10	0.20	Positive	212.0 → 145.0	25	212.0 → 109.0	50	70
359	Warfarin	R	Not approved	No	LC	7.86	0.10	Negative	307.1 → 161.1	20	307.1 → 250.1	20	140
360	Zoxamide	F	Approved	No	LC	9.03	0.40	Positive	336.0 → 187.1	25	187.1 → 88.9	40	200
	Acenaphthene-d10	P-IS	–	–	GC	6.16	–	Positive	164.0 → 162.0	18	164.0 → 160.0	35	70
	Atrazine-d5	P-IS	–	–	GC	7.95	–	Positive	205.1 → 127.1	14	205.1 → 105.0	14	70
	Atrazine-d5	P-IS	–	–	LC	6.74	–	Positive	221.2 → 179.0	15	221.2 → 69.1	50	90
	Carbendazim-d3	P-IS	–	–	LC	2.91	–	Positive	195.1 → 160.1	15	195.1 → 131.9	30	100
	Chorpyrifos-d10	P-IS	–	–	GC	9.94	–	Positive	324.0 → 260.0	35	324.0 → 195.0	55	70
	Chrysene-d12	P-IS	–	–	GC	13.86	–	Positive	240.0 → 238.0	20	240.0 → 236.0	38	70
	Cyromazine-d4	P-IS	–	–	LC	1.24	–	Positive	171.0 → 129.0	15	171.0 → 86.0	15	100
	Diazinon-d10	P-IS	–	–	GC	8.29	–	Positive	314.0 → 199.0	5	314.0 → 183.0	15	70
	Diazinon-d10	P-IS	–	–	LC	8.93	–	Positive	315.2 → 170.1	20	315.2 → 154.3	20	100
	Linuron-d3	P-IS	–	–	LC	7.54	–	Positive	255.1 → 185.0	15	255.1 → 159.8	15	100
	PCB 200	P-IS	–	–	GC	14.51	–	Positive	429.8 → 359.8	30	427.8 → 357.8	30	70
	Phenanthrene-d10	P-IS	–	–	GC	8.40	–	Positive	188.0 → 186.0	20	188.0 → 184.0	35	70
	Pirimicarb-d6	P-IS	–	–	LC	5.12	–	Positive	245.2 → 185.0	5	245.2 → 78.2	30	70

[a] POP – persistent organic pollutant; Non persistent pollutants: A – acaricide, MB – microbiocide, AH – anthelminthic, V – veterinary and human pharmaceuticals, F – fungicide, H – herbicide, I – insecticide, R – plant growth regulator, WP – wood preservative, PHP – post-harvest preservative, M – Molluscicide, Met – metabolite, NSAID – nonsteroidal anti-inflammatory drug, GC – glucocorticoid, P-IS – procedural internal standard.

[b] For pesticides and rodenticides the legal status reflecting the EU Pesticide Database was considered (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN), which is valid for the entire EU. For veterinary drugs, the marketing status in Spain is specified, as shown in the Cima vet search engine of the Spanish agency for drugs and health products (https://cimavet.aemps.es/cimavet/publico/home.html).

[c] Pesticide considered in the coordinated multi-annual plan of the EU for the investigation of residues in food of vegetable or animal origin during the years 2020, 2021 and 2022 (Regulation CE/2019/533).

[d] Gas chromatography (GC) or liquid chromatography (LC), both coupled with tandem triple quadrupole mass spectrometry. Some compounds can be detected by both techniques. However, only that technique for which better performance (lower LOQ, best recovery or lower RSD) has been indicated.

[e] Isomer beta (beta-cyfluthrin) is approved until 31 October 2020.

[f] Isomer beta (beta-cypermethrin) has switch to the "not approved" status since September 2017.

[g] The exposure to the acaricide amitraz is evaluated through the presence of these two major metabolites.

[h] The exposure to dichlofluanide is evaluated through the presence of this metabolite.

[i] The exposure to tolyfluanide is evaluated through the presence of this metabolite.

polarity range, while matrix co-eluents extracts the least (Sell et al., 2018) being at the same time suitable for LC and GC chromatography. Both methods have been developed for the analysis of pesticides in foods of plant origin, using 10 g of sample, and involve a clean-up step with secondary primary amine (PSA) for the removal of organic acids, fatty acids, sugars; and C18 for the elimination of lipids and sterols; and/or graphitized carbon (GC) for pigment removal. However, our previous experience demonstrated that these adsorbents retain various polar compounds (for example, a large number of pharmaceuticals and some POPs) that are of interest to this method. Therefore, we decided not to test clean-up with these adsorbents. However, a novel sorbent, the EMR-Lipid, was recently launched for the clean-up of fatty sample extracts, such as whole blood (Agilent, 2015). Therefore, a clean-up step with EMR-lipid was tested at two levels (2 y 20 ng/ml) in triplicate. As different authors have indicated that, when using the EN method, the extraction efficiency of acetonitrile improves in the acidic condition, this method was tested in the presence or absence of 1% FA (EC, 2015). Ten and one grams of whole blood were tested in these initial experiments (3 replicates at two concentrations (2–20 ng/ml), analyzed in duplicate). We did not find differences between them. Therefore, we continued next experiments with 1 g of whole blood instead of 10 g.

We made the decision on which method to choose based on two criteria. First, we chose the method that extracted more compounds above 60% recovery (all quantifications were performed against matrix-matched calibrators). The second criterion was based on the ion abundance. According to these criteria, the clean-up with EMR-lipid was ruled out since 97 and 113 compounds were lost or poorly recovered with the AOAC 2007.01 method and the UNE-EN 15662: 2019 method, respectively. One-step application of QuEChERS gave excellent results with both protocols, with an ability to adequately extract a similar number of compounds (360 with the AOAC method vs. 354 with the EN method). However, ion abundance was better for the majority of compounds with the AOAC method in the presence of 1% FA, so this was the chosen method. In a further step, the possibility of refining the method by modifying the percentage of FA was tested (0.0, 0.2, 0.5, 1, 2.5 and 5%), but no better results than those of 1% FA were found.

One of the most important objectives of this methodological development was to minimize the amount of sample employed. Therefore, several additional experiments were carried out, in which the amount of sample was progressively decreased (1, 0.5, 0.25, 0.1). Obviously, the amount of salts was proportionally decreased. The minimum amount of sample that did not affect the performance of the extraction method was 0.25 g of whole blood, so all validation of the method was performed using these conditions.

3.3. Validation

This method allows the simultaneous quantification of 360 chemicals, including 56 POPs, 205 agricultural pesticides, 11 rodenticides, 67 pharmaceuticals, and 21 metabolites. The detailed list of compounds, together with the specific category of use, and the technique of instrumental analysis, are presented in Table 1. Besides, for agricultural pesticides, the legal status in the EU and whether or not it is included in the coordinated multi-annual plan of the EU for the investigation of residues in food, is also indicated in Table 1. Data obtained during the validation process meet both, the criteria of the SANTE 2017 guidance document and those of the Scientific Working Group for Forensic Toxicology (EC, 2019b; SWGTOX, 2013).

Identity was evaluated through ion qualifier ratio (±30 average ion qualifier ratio of matrix-matched standards from the same sequence), retention time deviation (±0.1 min), peak shape, and signal/noise ratio (s/n > 3 for all ions, peak-to-peak algorithm). At least two transitions per compound were optimized. Transition with higher response and less noise at the lowest calibration point was used as a quantifier and at least one more as a qualifier (confirmation). Whenever possible, the quasimolecular ion was used in the identification of the compounds. The quantification and confirmation transitions that were selected for each compound are shown in Table 1. On the other hand, selectivity, which is the recommended term in analytical chemistry to express the extent of interferences (EC, 2019b), was evaluated by assessing the absence or presence of interfering or co-eluting chromatographic peaks at the retention time of the target analytes in the blank samples extracted by the optimized micro QuEChERS method.

Linearity was assessed within the range of concentrations that were considered appropriate for the purpose of biomonitoring (0.1 to 20 ng/ml). Within this range, 12 matrix-matched calibration points in quintuplicate were evaluated. All compounds showed acceptable linearity, with the lowest correlation coefficient (R^2) values being those of sarafloxacin, naphthalene, benzo[k]fluoranthene, fluvalinate, and fipronil sulfide (R^2 about 0.93). It is noteworthy, however, that the values of R^2 were within the range 0.97–0.99 for nearly 95% of compounds ($n = 342$). Detailed R^2 values for all the compounds are shown in Table 1 of the accompanying Data in Brief article.

The influence of the matrix components on the performance of the method was evaluated by applying the extraction method to a sufficient quantity of whole blood to produce a blank matrix extract, which was subsequently fortified at three levels for the mixture of 360 chemicals (0.2, 2, and 20 ng/ml) and quantified against a calibration curve prepared in the solvent. Matrix effect (ME) was observed both for compounds analyzed by LC–MS/MS and GC–MS/MS. Strong or medium signal suppression was demonstrated for 13.88% of compounds, and enhancement for 29.44% of compounds. For 204 pollutants the ME was considered negligible (−20% < ME < 20%). However, as for the rest 156 chemical, there was a significant ME, and it was concluded that matrix-matched calibration had to be used to compensate matrix interferences. All the detailed data of ME for individual compounds in whole blood are graphically shown in Fig. 1 of the accompanying article in Data in Brief.

The average recovery and precision (at least 5 fortification levels, each in quintuplicate) obtained were satisfactory for 356 pollutants, as they ranged from 76.6 to 119.5% with intraday relative standard deviations (RSD) ranging from 0.1 to 19.6%, and interday RSD ranging from 0.08 to 19.2%. Four pollutants did not strictly meet the validation criteria included in the SANTE guide (recoveries in the range 70–120%, and RSD < 20%). However, due to its importance in biomonitoring studies, we consider it important to include in the method compounds whose recoveries were lower or higher than those established in the recommendations, but which were highly reproducible (RSD < 15%). Thus, marbofloxacin (bias 62%, RSD 14.5%), beta hexachlorocyclohexane (bias 132.6%, RSD 14.5%), spirodiclofen (bias 134.4%, RSD 8.8%), and heptachlor (bias 139.4%, RSD 6.7%) were also included. All detailed validation data for the five levels of fortification are shown in Table 1 of the accompanying article in Data in Brief. For some compounds with higher LOQs, fewer levels are displayed, as no data is included in the table for levels < LOQ.

In the validation process, the possibility of carryover was also assessed. For this, blank matrix extracts were analyzed immediately after injecting the highest point of the calibration curve (also prepared in the matrix). According to the guidelines, it is acceptable for validation if carryover after the highest calibrator does not exceed 10% of the signal of the lowest calibrator, and in our case, this condition was met at 20 ng/ml for all analytes, except for fenbutatin oxide. The carryover effect for this pesticide disappeared completely at the second injection of blank matrix. To assess the need for additional clean-up measures for samples with medium to high levels of contaminants levels, we conducted additional experiments in which the signal from the blank matrix was evaluated after the injection of 100 ng/ml of the chemical mixture in whole blood. In this case, in addition to fenbutatin oxide, a low carryover was observed for brodifacoum, chlorophacinone, danofloxacin, difloxacin, enrofloxacin, flocoumafen, marbofloxacin, and sarafloxacin. However, the signal disappeared completely after the second injection of blank also for these compounds.

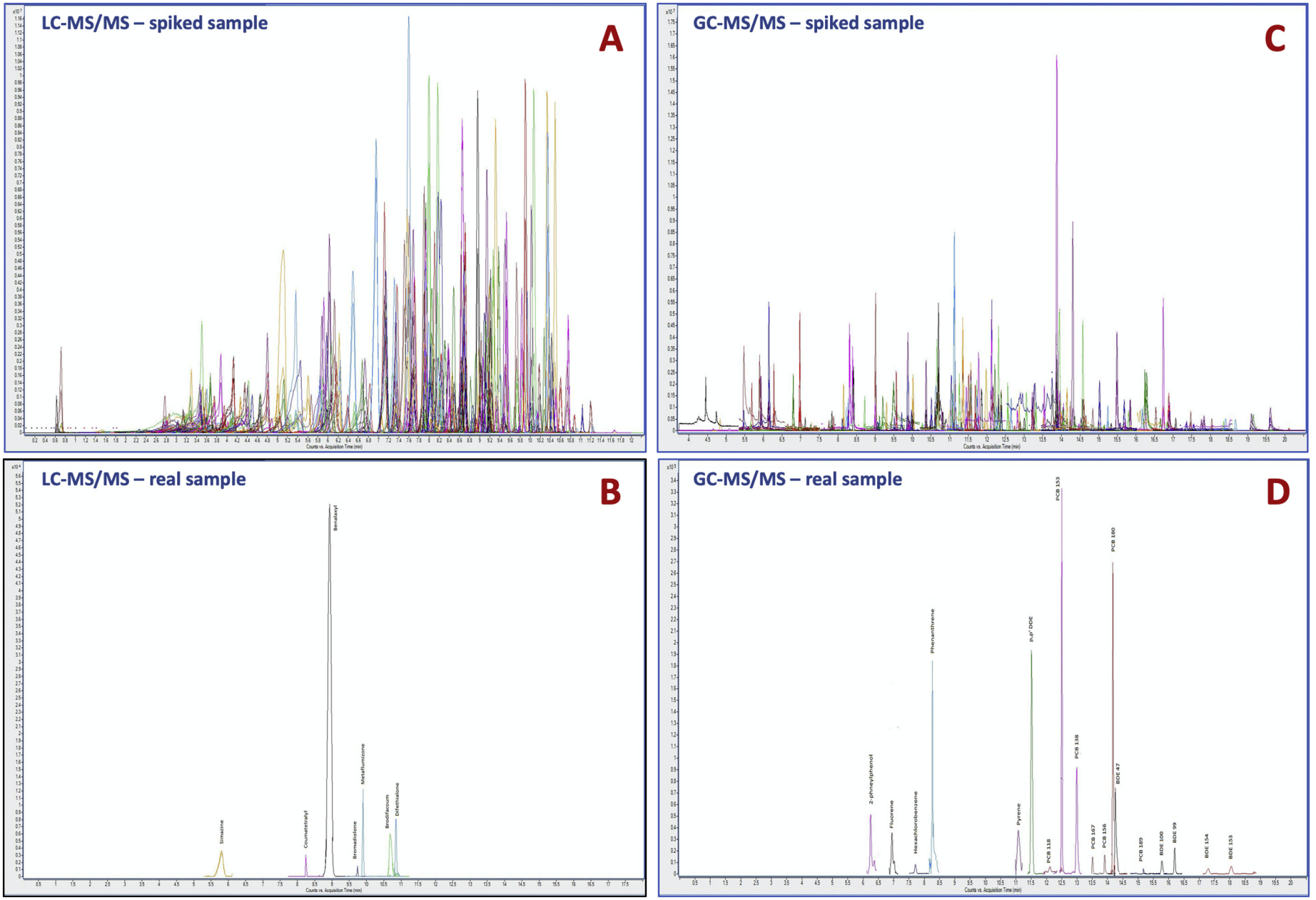

Fig. 1. Top panels. MRM chromatograms of a blank whole blood sample spiked with the mix of 360 chemicals + P-ISs at the level of 20 ng/ml analyzed by LC-MS/MS (A) and GC–MS/MS (C). Bottom panels. MRM chromatograms of the analyses of real samples (barn owl and common kestrel) by LC-MS/MS (B) and by GC–MS/MS (D).

The LOQ of this method was calculated over five runs of five fortified blank matrix samples, within the low working range (below 5 ng/ml), of three different sources (chicken, goat, and a mixture of both), as recommended (SWGTOX, 2013). The lowest non-zero calibrator approximation was employed to calculate de LOQs. This means that the lowest point of the calibration curve that complied identity, bias and precision criteria was set as the LOQ for a given compound. The expanded method uncertainty (MU) was calculated according to the formula specified in the SANTE guide (1st approach). As this is a new method development, no data of proficiency tests or independent reference materials were available. Therefore, reproducibility RSD was employed, as indicated in the SANTE guide, and an expanded covered factor k = 2 was chosen (EC, 2019b). The MU was below 58% in all cases. As shown in Table 1, the developed method is very sensitive and allows the quantitative analysis at very low levels. Despite the high number of chemicals included, and the small sample volume employed, 95% compounds can be quantified at LOQs < 1.5 ng/ml. This makes the method very appropriate for the biomonitoring of toxic chemicals in wildlife.

3.4. Application of the method to a series of blood samples of wild birds

This method was applied to a serie of 148 samples of whole blood collected in 2018 and 2019 from several species of nocturnal and diurnal raptors belonging to the Group of Rehabilitation of the Native Fauna and its Habitat (GREFA, Majadahonda, Spain). The whole blood samples do not correspond to a homogeneous series of individuals but include birds from 2 different species (*Falco tinnunculus* and *Tyto alba*), including both chickens and adults, and both males and females. These data are presented for the sole purpose of demonstrating the potential of the method for biomonitoring contaminants in wildlife. The individual data obtained for each of the individuals are presented in Tables 2–5 of the accompanying article in Data in Brief.

Fig. 1 shows the typical chromatograms of the spiked at 20 ng/ml whole blood samples obtained by the LC-MS/MS and GC–MS/MS analyses (top panels), and the chromatograms of two different positive samples for 25 pollutants (*Tyto alba* and *Falco tinnunculus*, bottom panels), as an example of the application of the method. Fig. 2 shows the data on the number of contaminants detected per sample. In all the samples, at least three of the contaminants were detected, with a maximum of 25 contaminants detected in the barn owl shown in Fig. 1. The median value of the number of pollutants per sample was 7.

In total, 51 different compounds were detected, which represents 14% of the chemicals included in the method. Contaminants belonging to 4 of the five groups under study (POPs, agricultural insecticides, rodenticides, and pharmaceuticals) were detected. However, none of the metabolites (nor the parent compounds) used as exposure biomarkers were detected in any of the samples. Tables 2 and 3 show the results of the contaminants found, along with the mean, median, percentiles, and detection frequency values throughout the series. As expected, of the six compounds that were detected in >50% of the samples, five were POPs (phenanthrene, pyrene, fluorene, hexachlorobenzene, and p,p′-DDE) (Table 2). However, it is striking that a contaminant that is neither persistent nor semi-persistent, such as 2-phenylphenol (2PHP), appeared in 96% of raptor blood samples (Table 3). 2PHP is a biocide used as preservative and surface disinfectant on fibers and other materials in households, hospitals, and other places, and is recognized as a potential endocrine disruptor (Scientific Committee On Consumer and Bernauer, 2016). Other authors have also reported that 2PHP is a highly prevalent pollutant in biota samples, such as river fish of different species, in which it is found in up to 100% of the samples (Peng et al., 2018). The rest of the POPs that were detected were the ones that have also been reported most frequently in other series of birds of prey (Espin et al., 2018; Garcia-Heras et al., 2018; Jaspers et al., 2013; Luzardo et al., 2014b; Ortiz-Santaliestra et al., 2015).

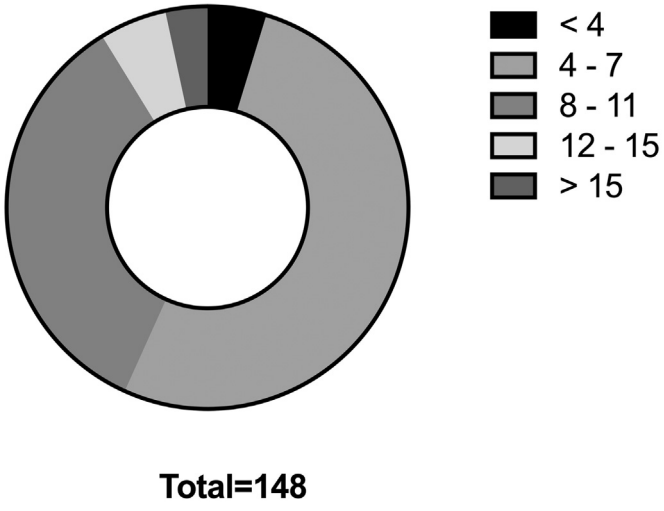

Fig. 2. Occurrence of environmental pollutants in the blood of a series of 148 raptors.

Concerning the rest of the agricultural pesticides, the case of benalaxyl is particularly remarkable, since it was detected in 23% of the individuals analyzed, albeit at low concentrations (Table 3). Benalaxyl is a widely used agricultural fungicide, and both, this agrochemical and, in particular, its metabolites, have been classified as endocrine disruptors endowed with potent anti-estrogenic activity (Ji et al., 2020). However, this pesticide is not routinely included in biomonitoring studies, despite studies on its toxicity to wildlife (Wang et al., 2014). The finding of such a high detection frequency of this fungicide in birds of prey samples indicates that this product has an extensive penetration in the food chains and the ecosystem and also, demonstrates the great utility of the method that we have developed for ecotoxicology studies. On the other hand, diphenylamine was found in 11.5% of the samples. Its principal use has been as a post-harvest preservative (mostly apples and pears), although its use is no longer authorized in the EU, and therefore such a high frequency of detection in wildlife samples is shocking. The percentage of raptor blood samples that tested positive for this residue is very similar to that of positives in fresh vegetables produced in countries where it is still authorized (Mutengwe et al., 2016). To our knowledge, this is the first study in which the presence of diphenylamine in the blood of raptors is reported. However, the presence of diphenylamine has been reported in gray partridge eggs from agricultural ecosystems (Bro et al., 2015). Moreover, the fact that it has also been found in herbs growing in agricultural areas (Malinowska and Jankowski, 2015), suggests that, independently from its origin, this compound is likely to easily penetrate the food chain (plants, arthropods, rodents, etc…), thus possibly reaching raptors. Metaflumizone was also found in a similar percentage of raptors (10.8%). Although it is not possible to know the exact origin, it is a permitted insecticide of great use in agriculture, and it has been reported to have a persistence of several days in soil (Chatterjee and Gupta, 2013). Therefore, it also seems possible that this pesticide penetrates the raptors' trophic chain. Five other agricultural pesticides were detected less frequently (Table 3). Among them, it is worth noting simazine, which is another unauthorized pesticide in the EU, and which is of concern because it is a proven endocrine disruptor (Orton et al., 2009). Simazine was detected in 4.73% of the samples (n = 7 individuals).

Although the penetration of rodenticides into the trophic chain is a known fact (Plaza et al., 2019; Ruiz-Suarez et al., 2014; Sanchez-Barbudo et al., 2012; Seljetun et al., 2019), it is still surprising that residues of at least one of these compounds have been found in almost 15% of the birds in this series. More than one compound was found in 8 of the individuals (from 2 to 4 compounds). It should be noted that the sample used has been the blood of live animals and not liver samples where these compounds tend to concentrate. These results indicate that these animals are constantly exposed to these rodenticides through feeding, even from the time they are in the nest. In addition, there are

Table 2
Persistent and semi-persistent organic pollutants detected in 148 blood s → amples of raptors.

Pollutant	Frequency	Concentrations in samples with residues			
		Mean ± SD	Median	95th percentile	Max
Phenanthrene	95.95	0.75 ± 0.40	0.61	1.46	2.25
Pyrene	95.95	0.23 ± 0.13	0.21	0.45	0.91
Fluorene	91.22	0.55 ± 0.27	0.47	1.12	1.50
Hexachlorobencene	68.92	0.60 ± 0.72	0.38	1.78	5.49
Dichlorodiphenyldichloroethylene (p,p′ DDE)	51.89	0.52 ± 0.87	0.20	2.90	4.40
PCB 153	35.81	0.55 ± 1.01	0.21	3.02	5.17
PCB 138	31.08	0.33 ± 0.56	0.15	1.68	2.87
PCB 180	30.73	0.76 ± 1.64	0.23	5.66	6.88
Fluoranthene	19.59	0.27 ± 0.06	0.26	0.37	0.43
Acenaphtylene	17.57	0.31 ± 0.08	0.33	0.43	0.45
PCB 189	10.14	0.25 ± 0.17	0.18	0.56	0.58
PCB 118	6.76	0.20 ± 0.16	0.13	0.50	0.50
BDE 99	6.08	0.22 ± 0.20	0.10	0.57	0.68
Acenaphthene	5.41	0.29 ± 0.13	0.30	0.37	0.51
PCB 167	5.41	0.20 ± 0.10	0.22	0.33	0.36
BDE 100	4.73	0.14 ± 0.08	0.10	0.26	0.33
PCB 156	4.05	0.23 ± 0.13	0.21	0.42	0.49
BDE 153	3.38	0.20 ± 0.00	0.20	0.20	0.20
BDE 47	2.03	0.20 ± 0.00	0.20	0.20	0.20
Naphtalene	2.03	1.43 ± 1.07	0.83	2.49	2.67
Dichlorodiphenyldichloroethane (p,p′ DDD)	0.68[a]	0.65	0.65	–	–
Hexachlorocyclohexane (alpha)	0.68[a]	0.40	0.40	–	–
Hexaclorocyclohexane (beta)	0.68[a]	5.95	5.95	–	–
PCB 28	0.68[a]	0.10	0.10	–	–
PCB 101	0.68[a]	0.20	0.20	–	–

[a] These compounds were detected in only one individual each.

Table 3
Non persistent pesticides and veterinary drugs detected in 148 blood samples of raptors.

	Category[a]	Legal status in the EU[b]	Subjected to MRL[c]	Frequency	Concentrations in samples with residues			
					Mean ± SD	Median	95th percentile	Max
Agricultural pesticides								
2-Phenylphenol	F	Approved	Yes	95.95	1.02 ± 1.19	0.59	4.19	5.88
Benalaxyl	F	Approved	No	22.97	0.13 ± 0.04	0.10	0.21	0.26
Diphenylamine	PHP	Not approved	Yes	11.49	0.37 ± 0.20	0.31	0.63	0.82
Metaflumizone	I, V	Approved	No	10.81	0.31 ± 0.21	0.20	0.70	0.92
Simazine	I	Not approved	No	4.73	0.25 ± 0.07	0.23	0.36	0.39
Metrafenone	F	Approved	Yes	2.70	0.10 ± 0.01	0.10	0.10	0.10
Thiacloprid	I	Approved	No	2.70	0.83 ± 1.17	0.31	2.25	2.57
Coumaphos	I, A	Not approved	No	1.35	0.15 ± 0.05	0.15	0.18	0.19
Atrazine	H	Not approved	No	0.68[d]	0.12	0.12	–	–
Rodenticides								
Brodifacoum	R	Not approved	No	7.43	4.38 ± 9.55	0.80	19.55	32.73
Difenacoum	R	Not approved	No	3.38	0.40 ± 0.01	0.40	0.41	0.42
Bromadiolone	R	Approved	No	2.03	0.41 ± 0.01	0.40	0.42	0.42
Coumatetralyl	R	Not approved	No	2.03	41.56 ± 60.78	13.02	101.53	111.36
Coumachlor	R	Not approved	No	0.68[d]	5.84	5.84	–	–
Difethialone	R	Not approved	No	0.68[d]	1.77	1.77	–	–
Flocoumafen	R	Not approved	No	0.68[d]	0.20	0.20	–	–
Pharmaceuticals								
Levamisole	V	Approved	–	8.11	0.29 ± 0.10	0.27	0.34	0.52
Fenbendazole	V	Approved	–	2.70	0.10 ± 0.00	0.10	0.10	0.10
Enrofloxacin	V	Approved	–	1.35	1.20 ± 0.00	1.20	1.20	1.20
Eprinomectin	V	Approved	–	1.35	0.32 ± 0.01	0.32	0.32	0.33
Flumequine	V	Approved	–	1.35	0.10 ± 0.00	0.10	0.10	0.10
Sulfadiacine	V	Approved	–	1.35	7.27 ± 9.28	7.27	9.42	13.69
Albendazole	V	Approved	–	0.68[d]	0.10	0.10	–	–
Dexamethasone	V	Approved	–	0.68[d]	0.40	0.40	–	–
Mebendazole	V	Approved	–	0.68[d]	0.25	0.25	–	–
Sulfacloropiridacine	V	Not approved	–	0.68[d]	0.80	0.80	–	–
Sulfapyridine	V	Not approved	–	0.68[d]	0.40	0.40	–	–

[a] A – acaricide, B – bactericide, AH – anthelminthic, V – veterinary and human pharmaceuticals, F – fungicide, H – herbicide, I – insecticide, R – plant growth regulator, WP – wood preservative, PHP – post-harvest preservative, M – Molluscicide, Met - metabolite.

[b] For pesticides and rodenticides the legal status reflecting the EU Pesticide Database was considered (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN), which is valid for the entire EU. For veterinary drugs, the marketing status in Spain is specified, as shown in the Cima vet search engine of the Spanish agency for drugs and health products (https://cimavet.aemps.es/cimavet/publico/home.html).

[c] Pesticide considered in the coordinated multi-annual plan of the EU for the investigation of residues in food of vegetable or animal origin during the years 2020, 2021 and 2022 (Regulation CE/2019/533).

[d] These compounds were detected in only one individual each.

two other very striking events, such as the fact that six of the seven rodenticides detected in this series are not authorized for agricultural or environmental use in the EU, and also that one of them - brodifacoum - has been the most frequently detected in this series of birds of prey (11 individuals).

Finally, although detection frequencies were low in almost all cases, up to 11 different pharmaceuticals were detected in this series of birds of prey blood samples (Table 3). As in the previous cases, the fact that the sample analyzed is blood implies that the exposure to these contaminants has been recent (and probably regular), possibly only a few hours before sampling. More in-depth studies are needed to assess the toxicological significance of these findings. Still, it is worth noting some data such as the high frequency of detection with which levamisole has appeared in this series (12 individuals with levels > LOQ). To the best of our knowledge, this is the first study that shows data about levamisole in wild raptors. However, some other authors have indicated that this is a prevalent environmental pollutant, and have reported its presence in non-target wild organisms, such as marine mollusks and fish (Moreno-Gonzalez et al., 2016), with frequencies even higher than those reported in this series.

4. Conclusions

The current method, with a one-step miniaturized QuEChERS sample preparation, followed by both LC–MS/MS and GC–MS/MS analyses, allows the simultaneous determination of 360 toxic or potentially toxic environmental pollutants in small amounts of whole blood (250 μl). The analytical scope of this optimized and fully validated method includes a vast number of chemicals of environmental concern for wildlife, and also for humans. Thus, it includes: i) the most relevant POPs (organochlorine pesticides, polychlorinated biphenyls, polybrominated diphenyl ethers, and polycyclic aromatic hydrocarbons); ii) almost 90% of the active substances of the plant protection products included in the coordinated multi-annual plan of the EU for the investigation of residues in food of vegetable or animal origin; iii) the most commonly employed chemicals that are involved in deliberate poisoning of wildlife; iv) the most widely used anticoagulant rodenticides; v) pharmaceuticals, including many of those of major use in veterinary practice; vi) and a suite of metabolites that can be used as biomarkers of exposure. The application of the method to actual raptors samples allows to verify its suitability for biomonitoring studies and to glimpse its potential for obtaining valuable exposure data in ecotoxicological studies.

CRediT authorship contribution statement

Cristian Rial-Berriel: Conceptualization, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Andrea Acosta-Dacal:** Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Manuel Zumbado:** Investigation, Writing - original draft, Writing - review & editing. **Octavio P. Luzardo:** Conceptualization, Investigation, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare no conflict of interest. This is an independent research. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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