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Quantitation of neonicotinoid insecticides, plus qualitative screening for other xenobiotics, in small-mass avian tissue samples using UHPLC high-resolution mass spectrometry

Michael S. Filigenzi,[1] Emily E. Graves, Lisa A. Tell, Karen A. Jelks, Robert H. Poppenga

Abstract. We developed and validated a liquid chromatography–high-resolution mass spectrometry (LC-HRMS) analytical method for quantitatively measuring pesticide concentrations in small-body avian tissue samples using homogenized 1–2-d-old chicken carcasses as the test matrix. We quantified the following key insecticides: sulfoxaflor (sulfoximine class) and the neonicotinoids dinotefuran, nitenpyram, thiamethoxam, acetamiprid, thiacloprid, clothianidin, and imidacloprid. We used fortified chick carcass samples to validate method accuracy (80–125% recoveries), precision (<20% relative standard deviation), and sensitivity (≤1.2 ppb) for these targeted analytes. This method also uses full-scan, high-resolution MS to screen for the presence of a wide variety of other xenobiotics in bird carcasses. The utility of our screening process was demonstrated by the detection of carbaryl in some samples. This sensitive LC-HRMS analytical method for insecticide detection in a matrix of homogenized carcass is ideal for evaluating small birds for insecticide exposure. This novel whole-carcass method may allow for research studies of small-bodied, free-ranging avian species, and could provide insight regarding their exposure to multiple classes of environmental contaminants.

Key words: electrospray mass spectrometry; hummingbirds; insecticides; pesticide residues; pollinators; small-bodied birds.

Introduction

The health of pollinators is of concern given the potential negative impacts of environmental contaminants combined with other stressors.[10,22] Some studies suggest that declines in biodiversity, as a result of habitat loss or conversion to agriculture, are exacerbated by insecticide use.[9] Mortality from pesticide exposure has been reported in multiple species of bats[5,18] and birds.[8] Neonicotinoid insecticides have been heavily studied for their effects on pollinating insects, specifically the European honey bee (*Apis mellifera*), which is a critical pollinator for many agricultural crops.[17] Because pollination is such an important ecosystem service, there has been concern over the connection between neonicotinoid use and an overall decline of bee populations.[6,12] Many species of bats and some species of birds also perform pollination services, but there is relatively little known about rates of pesticide exposure in these species.

Exposure assessment is a necessary first step in evaluating how pesticides impact wildlife species, but methods for doing so are often lacking.[1] As new pesticides approved by the U.S. Environmental Protection Agency (EPA) become available, there is a growing need for expanded sensitive analytical methods for these compounds. Indirect exposure measures exist for some chemical classes for which direct exposure methods may be limited. For example, organophosphorus and carbamate insecticide exposure in birds can be indirectly evaluated through the measurement of cholinesterase activity,[13] but this method is not effective for evaluating general exposure to other pesticide classes such as neonicotinoids. Thus, expanded sensitive analytical methods are needed to assess exposure to newer pesticide classes. On a broad scale, application rates of neonicotinoids have been shown to be correlated with population declines of insectivorous birds,[11] but direct links between neonicotinoid exposure and reduced bird survival or reproduction have not been demonstrated. Sensitive and broad screening analytical methods for pesticide detection should allow for future

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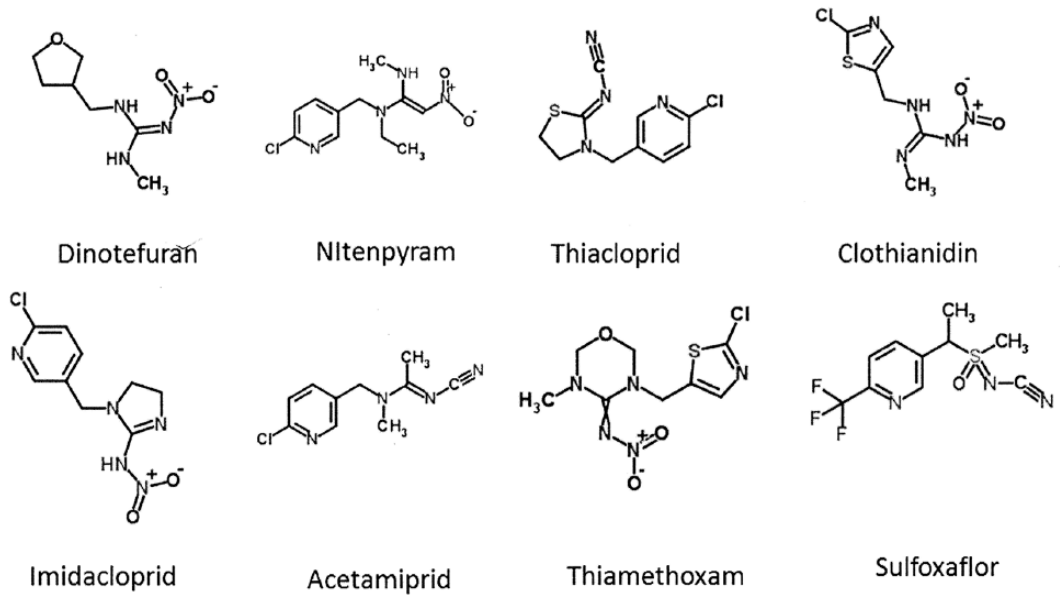

Figure 1. Structures of targeted insecticides.

research on the negative impacts of these chemicals on wildlife species of small-mass birds.

Non-lethal assessment of pesticide exposure is desirable for population monitoring of animals. Organic pollutants such as dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs) can be detected in feather samples from common magpies (*Pica pica*) using gas chromatography.[14] Imidacloprid (a neonicotinoid) was successfully detected in 500-µL blood samples from Eurasian eagle owl (*Bubo bubo*) nestlings[21] using a quick, easy, cheap, effective, reliable, and safe (QuEChERS) extraction method and high-performance liquid chromatography combined with time-of-flight mass spectrometry (HPLC/TOF-MS). This was the first study to measure neonicotinoids in wild birds using non-lethal sampling, to our knowledge. A 2018 study utilized cloacal fluid and fecal pellets from hummingbirds for the detection of neonicotinoid and organophosphate pesticides.[4] An alternative to sampling from live birds is postmortem testing of tissue samples. Hummingbirds and other small-bodied birds are often taken to rehabilitation facilities when they are found injured and, in some cases, these birds do not survive. An analytical method to determine the presence of neonicotinoid pesticides in the carcasses of such birds would be useful in assessing their exposure to these chemicals.

Postmortem analysis for pesticide residues in animals typically involves analysis of tissue samples taken from target organs. However, for animal species with small body masses, testing is often constrained by the tissue mass required for many existing analytical methods. One gram or more of a single tissue type (e.g., liver) is commonly used for analysis,[2] which makes it difficult to use established methods for evaluating pesticide exposure of small-bodied individual animals with internal organ weights that do not meet this threshold without pooling samples from multiple individuals. In one report, whole liver samples from hummingbirds weighed ~100 mg[20]; in another report, hummingbird livers were found to weigh 27–120 mg.[19] Therefore, the standard approach of analyzing a specific tissue type for pesticides is not practical in such small-bodied birds.

The objective of our study was to develop and validate an analytical method for assessing common insecticide exposure of birds with limited available sample amounts so that this method could be used in future studies to evaluate insecticide exposure of small free-ranging birds. Target compounds included sulfoxaflor (sulfoximine class) and 7 neonicotinoids: dinotefuran, nitenpyram, thiamethoxam, acetamiprid, thiacloprid, clothianidin, and imidacloprid (Fig. 1). A secondary objective was to use the method to screen for a broad range of other potential contaminants of concern. Using a homogenized carcass matrix and liquid chromatography–high-resolution mass spectrometry (LC-HRMS), we established and validated an analytical method for quantitatively measuring insecticide concentrations in samples from hummingbirds and established the method's utility in detecting other contaminants.

Materials and methods

Matrix source

Individual 1–2-d-old chicken (*Gallus gallus domesticus*) carcasses supplied by the Department of Animal Science Avian Facility (ASAF) at the University of California–Davis (UC Davis) were used as the test matrix for method validation. Birds were euthanized as part of a standard

management program for the poultry species housed at the UC Davis ASAF. All aspects related to the management of birds were approved by the UC Davis Institutional Animal Care and Use Committee. Three chick carcasses were ground, homogenized, and pooled to be used as the control matrix for method development, validation, and sample analysis.

Reagents and reference standards

Reagents included HPLC-grade water, acetonitrile, formic acid, and methanol (Thermo Fisher Scientific, Waltham, MA). QuEChERS extraction tubes containing magnesium sulfate and sodium acetate as well as loose magnesium sulfate and primary-secondary amine were used for sample extraction (United Chemical Technologies, Bristol, PA). Sulfoxaflor (98% purity) was purchased from Toronto Research Chemicals (Toronto, ON). All other chemical standards including the isotopically labeled compounds (minimum 98% purity) were purchased from Millipore Sigma (St. Louis, MO). Isotopically labeled internal standard compounds included d3-thiamethoxam, d3-clothianidin, d4-imidacloprid, d3-acetamiprid, and d4-thiacloprid.

Preparation of standard solutions

For each neat standard material, ~10 ± 0.5 mg was weighed out and diluted to 10 mL in methanol to produce stock solutions. Appropriate volumes of these stock solutions were combined and diluted to produce working solutions for the targeted analytes at a concentration of 10 µg/mL each. A similar procedure was used to produce a mixed solution of the 5 labeled internal standards at 10 µg/mL each. The mixed neonicotinoid solution was further diluted to concentrations of 1.0 and 0.10 ng/mL to use for spiking and for analytical standard preparation, respectively. Analytical standards in solution containing each of the target analytes at 6 concentrations (0.50–100 ng/mL) were used to construct calibration curves. Either linear or quadratic curve fits were used depending on which model best fit the data for each analyte. A weighting of 1/X was used for all samples.

Homogenized tissue sample preparation

Carcasses were frozen in liquid nitrogen. In our early work, carcasses were immersed in liquid nitrogen and ground in a commercial tissue homogenizer (Stein M-2 sample mill; Hoffman Manufacturing, Jefferson, OR). This homogenization method resulted in a frozen powdered tissue matrix with most feathers intact, and was used for analysis of the majority of the hummingbirds. A freezer mill (model 6875; SPEX SamplePrep, Metuchen, NJ) was used for later samples, and this provided complete grinding of all parts of the carcass, including feathers. This grinding process was used for method validation. One gram of the ground carcass material was weighed in a 50-mL polypropylene centrifuge tube (Corning, Corning, NY). This aliquot was then fortified with 5 µL of the labeled internal standard mix, providing a concentration of 50 ng/g for each labeled compound. Targeted analytes and the labeled internal standards were fortified as appropriate, and the sample was allowed to stand for 5–10 min. Extraction and dispersive solid-phase cleanup procedures were by a modified QuEChERS method developed for HRMS analysis of a variety of matrices.[7] Five mL of water was added to the samples, and the tubes placed on a commercial shaker (Geno/Grinder; SPEX SamplePrep) for 5 min at 750 rpm. Fifteen mL of 0.1% formic acid in acetonitrile was added, and samples were shaken again for 5 min at 750 rpm. Samples were then centrifuged for 5 min at 1,300 × *g* (Avanti J-E centrifuge; Beckman Coulter, Brea, CA). The supernatant was decanted into a second 50-mL tube containing 6 g of magnesium sulfate and 1.5 g of sodium acetate. This tube was immediately hand shaken until the reagents were well mixed and then centrifuged for 5 min at 2,500 × *g*. The acetonitrile (upper) layer of the supernatant was then transferred to a 15-mL tube containing 150 mg of potassium sulfate and 50 mg of primary-secondary amine. These tubes were placed in a tube rotator for 15 min and then centrifuged for 5 min at 2,500 × *g*. The supernatant was transferred to a 15-mL glass tube and evaporated to dryness under nitrogen. The dried extract was reconstituted by adding 40 µL of methanol, vortex mixing, adding 160 µL of water, and vortex mixing again. The resulting solution was filtered through a 0.22-µm polyethersulfone syringe filter (Millipore Sigma) into a 2-mL autosampler vial equipped with a 250-µL glass insert.

HPLC-HRMS parameters

All samples were analyzed using a Q Exactive Quadruple-Orbitrap mass spectrometer interfaced with a Dionex UltiMate 3000 ultra HPLC (UHPLC; Thermo Fisher Scientific, San Jose, CA). The UHPLC system was equipped with a 100 × 2 mm, 1.8-µm Eclipse Plus C18 column (Agilent Technologies, Santa Clara, CA). Mobile phase A was 0.1% formic acid in water, and mobile phase B was 0.1% formic acid in acetonitrile; column flow was 0.35 mL/min, and injection volume was 20 µL The mobile phase gradient started with 1% solvent B, which was held for 1.5 min after injection. The concentration of solvent B was ramped in a linear gradient to 98% at 9.5 min and held until 13.5 min, at which time it was dropped back to 1%. It was held at 1% for 4 min in order to re-equilibrate the column.

The mass spectrometer was equipped with a heated electrospray ionization probe and run in positive ion mode. Two acquisition functions were used for each sample. The first function utilized time-segmented "parallel reaction monitoring" (PRM), in which precursor ions were selected by a quadrupole mass filter, fragmented in the instrument's higher-energy collisional dissociation (HCD) cell, and then analyzed in the Orbitrap mass analyzer. This function provided

Table 1. Precursor/product ions, retention times, and retention time windows for targeted pesticides.

Compound	Precursor ion (*m/z*)	Quantitation ion (*m/z*)	Qualifier ion (*m/z*)	Retention time (min)	Start time (min)	End time (min)
Dinotefuran	203.114	129.0890	87.0789	3.9	3.4	4.5
Nitenpyram	271.096	99.0911	56.0495	4.2	4.0	4.4
Thiamethoxam	292.027	131.9663	181.0535	4.8	4.5	5.0
Clothianidin	250.016	169.0504	131.9663	5.0	4.8	5.2
Imidacloprid	256.060	175.0970	209.0583	5.1	4.9	5.4
Acetamiprid	223.175	126.0099	56.0495	5.3	5.0	5.8
Sulfoxaflor	174.052	172.0564	154.0459	5.76	5.5	6.3
Thiacloprid	253.031	126.0099	171.0869	5.8	5.5	6.1
Carbaryl	202.086	145.0648	117.0700	6.5	6.2	7.5

Start and end times refer to the time window during which tandem mass spectrometer data was acquired for each compound.

Table 2. Internal standards, quantitation ions, and native analytes referenced to each internal standard.

Compound	Quantitation ion (*m/z*)	Targeted analyte(s)
d3-dinotefuran	206.1325	Dinotefuran
d_3-thiamethoxam	295.0454	Thiamethoxam, nitenpyram
d_3-clothianidin	252.0348	Clothianidin, sulfoxaflor
d_4-imidacloprid	260.0847	Imidacloprid
d_3-acetamiprid	226.0933	Acetamiprid
d_4-thiacloprid	257.0560	Thiacloprid

targeted high-resolution, full-scan tandem MS (MS/MS) data acquisition for each of the neonicotinoids. (Normalized collision energy was 35 for all compounds.) The second acquisition function utilized full-scan analysis to provide non-targeted HRMS data acquisition that allowed for the detection of other xenobiotics potentially present in small bird carcasses and for detection of the labeled internal standards. Analysis of standard solutions was used to determine the retention times, precursor, and product ions for the targeted analytes (Table 1) and to determine retention times and protonated molecular ions for the labeled internal standards (Table 2). Mass spectrometer instrument settings were as follow: AGC target: 5 E5; max injection time: 50 msec; sheath gas setting: 35; aux gas setting: 15; sweep gas setting: 1; spray voltage: 4.5; capillary temperature: 320°C; aux gas heater temperature: 280°C; resolution ($M/\Delta M$ at m/z 200): 70,000 (full scan), 17,500 (PRM).

Confirmatory analysis of non-targeted compounds was performed (Velos Pro linear ion trap mass spectrometer, interfaced to a Dionex Ultimate 3000 UHPLC; Thermo Fisher Scientific). The UHPLC was equipped with a 150 × 2.1 mm, 1.8-µm SB-C18 column (Agilent). Mobile phase A was 0.1% formic acid in water, and B was 0.1% formic acid in acetonitrile. Column flow was 0.20 mL/min, and injection volume was 20 µL. The mobile phase gradient started with 5% solvent B, which was ramped upon injection to 35% B at 15 min and then ramped to 95% B at 20 min. It was held at 95% until 25 min and then immediately dropped back to 5% B and held for 7 min to re-equilibrate the column. The mass spectrometer was run in full scan MS/MS mode. The precursor ion for each compound was the one utilized in the ToxFinder database (Thermo Fisher Scientific), with product ions and collision energy determined by analysis of the appropriate standard solution. Mass spectrometer instrument settings were as follows: isolation width: 2.5; sheath gas setting: 35; aux gas setting: 15; sweep gas setting: 1; spray voltage: 4.5; capillary temperature: 320°C; aux gas heater temperature: 280°C.

Quality control samples

Hummingbird samples were batched in groups of 10 or fewer. Each batch included a negative control chick carcass sample, fortified only with the labeled internal standards. These samples were monitored for the absence of targeted analytes or interfering compounds. Each batch also included 2 chick carcass samples fortified prior to extraction: 1 fortified at 1 ppb, and 1 at 10 ppb. The 1-ppb spike was used to demonstrate detectability of all target analytes at this level, with the exception of nitenpyram, given that the limit of detection (LOD) for nitenpyram was slightly >1.0 ppb. The 10-ppb spike was used to monitor target analyte recoveries for each batch.

Analytical sequences

Each analytical sequence began and ended with a 6-point calibration curve containing all target insecticides and their associated internal standards in a range of 0.5–100 ng/mL. An additional standard solution containing most the compounds in the ToxFinder database was also analyzed (Supplementary Table 1).

Data analysis

Quantitation software (Xcalibur; Thermo Fisher Scientific) was used for quantitation of the targeted neonicotinoids.

Quantitation ions were chosen from full-scan, high-resolution MS/MS scans. The mass tolerance for fragment ion detection was set at 10 ppm. Excel (Microsoft, Redmond, WA) was used to calculate mean, percent relative standard deviation (%RSD), LOD, and limit of quantification (LOQ) values for method validation.

ToxFinder software was used for screening for other xenobiotics. ToxFinder is a program that identifies chemicals using a database containing the accurate masses of their ions and their retention times. The ToxFinder database (Supplementary Table 1) included 154 compounds: organophosphorus and carbamate insecticides, veterinary drugs, plant alkaloids, drugs of abuse, and others. This database was developed in our laboratory using the same chromatographic conditions used for the hummingbird work. The mass tolerance was set at 5 ppm, and the retention time window was set to ± 1 min.

Method validation

The extraction method was tested to determine the feasibility of detecting the targeted compounds at low ng/g concentrations in whole bird samples. Peak heights and areas obtained by analysis of whole chick tissue fortified with the target analytes at 1 ng/g were sufficient to warrant method validation. The method was validated by analyzing whole control chicks that were ground, fortified with the target analytes, and then extracted and analyzed. The method detection limit for each compound was determined by following the EPA's procedure outlined in 40 CFR Appendix B (U.S. EPA. 40 CFR Appendix B to Part 136—definition and procedure for the determination of the method detection limit–revision 1.11, 2011. Available from: https://www.gpo.gov/fdsys/granule/CFR-2011-title40-vol23/CFR-2011-title40-vol23-part136-appB/content-detail.html) with 7 replicate control samples fortified at 1 ng/g prior to extraction. Precision and accuracy were measured by analyzing 3 additional replicates of samples at concentrations of 5.0, 20, and 50 ng/g. To assess method selectivity, unfortified chick carcasses were analyzed prior to method validation and with each batch of samples analyzed. The results were checked for the absence of interfering peaks at the retention times of each of the targeted analytes. Linearity in matrix was assessed by analysis of a calibration curve consisting of extracts of the control matrix fortified at 5 concentration levels ranging from 1.0 to 100 ppm. Note that, although no specific published method validation protocol was followed, the procedures used for this validation are consistent with guidelines published by the FDA, SANCO, and other such groups (USFDA Office of Foods and Veterinary Medicine. Guidelines for the validation of chemical methods for the FDA FVM Program. 2nd ed. 2015. Available from: https://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM273418.pdf).

Results

The established conditions provided adequate separation for the use of multiple PRM time segments (Figs. 2, 3). After adding the PRM segments to the acquisition method, standards were analyzed to determine the accurate mass MS/MS spectra and to establish quantitation and qualifier ions for each compound. Under these chromatographic conditions, 2 chromatographic peaks with identical MS/MS spectra were detected for sulfoxaflor (Fig. 3). For quantitative purposes, the 2 peaks were integrated, and the summed peak areas were used for calculations.

The r^2 values for the calibration curves were consistently >0.99 for all compounds. The calculated residuals from the calibration curves (the difference between the concentration in the standard and the calculated concentration) were <30%, except for nitenpyram, which showed residuals up to 40%. Results were similar for the extracted matrix calibration curve, with r^2 values >0.99 for all compounds, and residuals <25% for all compounds at all levels with the exception of nitenpyram (residuals up to 40%).

LODs were determined by analysis of 7 replicates fortified at 1 ng/g each. The mean, SD, and %RSD of the concentrations were calculated for each analyte. The SD was multiplied by 3.14 (Student t value at the 99% confidence level with 6 degrees of freedom). These LODs are consistent with those determined by this method in other matrices, including bovine liver tissue[7] (Table 3).

Analyses of reagent blanks and unfortified chick control matrices were consistently free of any signals matching those of the targeted analytes (Figs. 4, 5). The lack of any detectible background signal for many ions (Figs. 4, 5) is characteristic of HRMS.

Average recoveries were within 80–120% for all analytes at all concentrations except for sulfoxaflor at the 50 ng/g concentration, which averaged 125% (Table 3). The RSD was <20% for all analytes at all concentrations.

We have applied this method to the analysis of carcasses of hummingbirds that did not survive rehabilitation, and both targeted and non-targeted compounds were detected in wild bird samples (Graves et al., 2019; in review, Environmental Science and Pollution Research). In general, carbaryl, which was not originally one of the target analytes, was detected using the ToxFinder program in several of the first carcasses analyzed. Targeted MS/MS conditions for carbaryl analysis were added to the LC-MS method in order to provide definitive qualitative identification in subsequent samples without further confirmatory analysis.

Discussion

Our results support the assumption that neonicotinoids can be quantified in fortified chick carcass homogenates using a sensitive LC-MS method while simultaneously screening

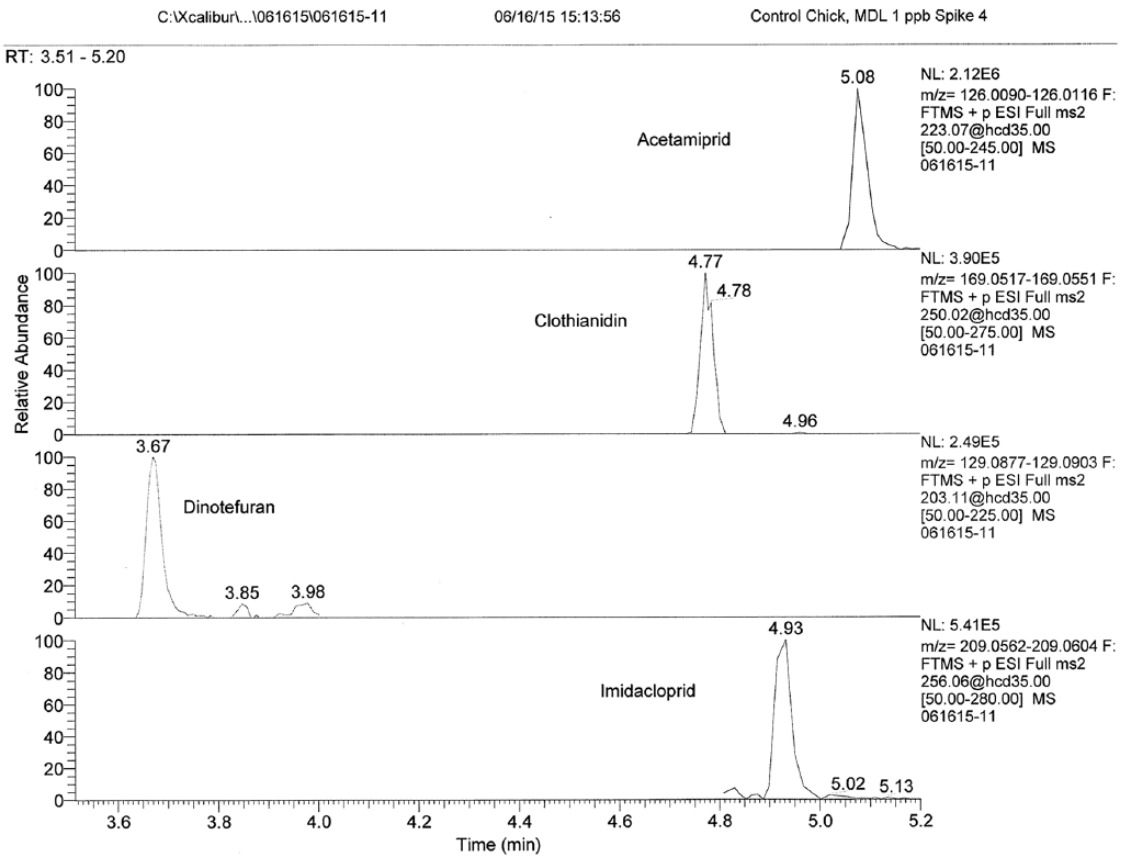

Figure 2. Selected ion chromatograms for acetamiprid, clothianidin, dinotefuran, and imidacloprid from analysis of an extract of control matrix (homogenized chick carcasses) fortified with 1 ng/g of all targeted analytes.

for a broad range of other analytes. Although the Orbitrap mass analyzer scans at a slow rate relative to other types of mass analyzers, the use of multiple PRM segments as well as optimized mass spectrometer settings, such as injection time and mass resolution, provided sufficient data points across the narrow UHPLC chromatographic peaks for accurate quantification.

Our method compared well with other methods developed for the detection of neonicotinoid samples in tissue matrices. One report compared the use of subcritical water extraction to ultrasonic extraction and shaking extraction, all with analysis by LC-MS/MS; LODs of 0.12–0.36 ng/g were achieved using subcritical water extraction, with LODs >0.78 ng/g for the other 2 methods.[24] Another method developed for the detection of neonicotinoid residues in bovine tissue using pressurized solvent extraction and LC-MS/MS achieved LODs of 0.8–1.5 ng/g.[23] A third study of a method developed for analysis of pesticides in bee samples and bee products reported LODs of 0.2–1.3 ng/g for a more limited suite of neonicotinoids.[15]

A modification for quantifying sulfoxaflor for our study was to sum the detected chromatographic peaks. Sulfoxaflor is present as 2 diastereomers and 4 enantiomers,[3] and other investigators report separation of sulfoxaflor into 2 separate chromatographic peaks using typical UHPLC conditions.[16] For our study, summing the 2 areas of the detected chromatographic peaks provided adequate quantitative performance for this compound.

It is important to note that nitenpyram and sulfoxaflor are the 2 analytes for which there were no isotopically labeled analogs available. In general, quantitative performance for compounds that cannot be referenced against their own isotopically labeled analogs is poorer compared to compounds that can be referenced, because of differences in matrix-related response suppression. Matrix-matched calibration is often used to compensate for this issue, but this approach would require one 4–5-g bird carcass for each calibration standard, and it would require birds that are known to have not been exposed to any of the analytes. Another approach for quantitative LC-MS work is the use of the standard additions technique, but this requires multiple extractions for each quantified sample, which is a problem given that a single analysis would consume most, if not all, of a small-bodied bird. Neither of these approaches is practical, and we feel that the quantitative performance obtained for these compounds in our study was acceptable, as reflected in the

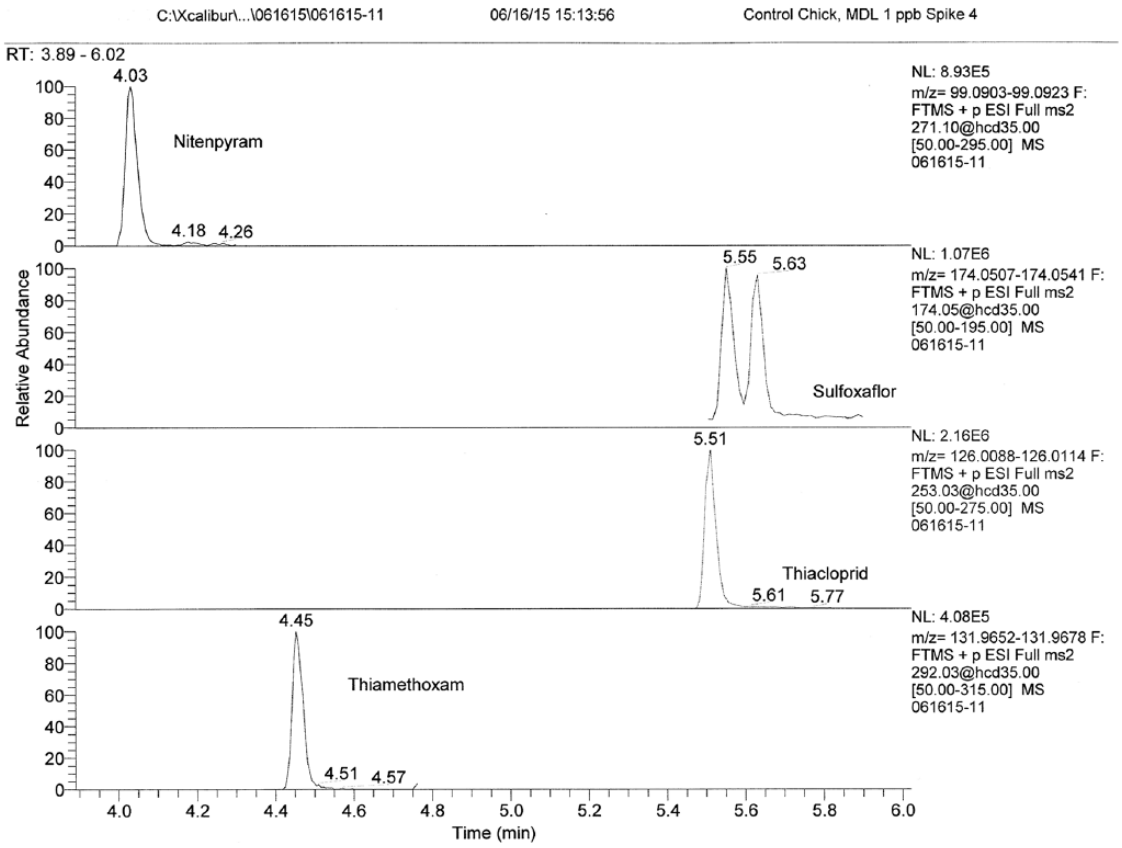

Figure 3. Selected ion chromatograms for nitenpyram, sulfoxaflor, thiacloprid, and thiamethoxam from analysis of an extract of control matrix (homogenized chick carcasses) fortified with 1 ng/g of all targeted analytes.

Table 3. Method validation parameters for insecticides detected via high-resolution mass spectrometry. Parameters include percent relative standard deviation (%RSD; replicate control samples fortified with 1 ng/g of each analyte; $n = 7$), limit of detection and quantification (LOD and LOQ, respectively), and recovery/%RSD evaluation of analytes at 3 spiking concentrations in control chick matrix.

Compound	Measured mean (ng/g)	%RSD	LOD (ng/g)	LOQ (ng/g)	% Recovery/%RSD spike concentrations (ng/g)		
					5	20	50
Dinotefuran	1.2	14	0.52	1.6	116/14	99/7.4	119/14
Nitenpyram	1.58	25	1.2	3.6	NV	NV	NV
Thiamethoxam	1.44	16	0.74	2.2	85/11	111/10	108/10
Clothianidin	1.17	7.8	0.29	0.87	101/2.1	95/16	91/16
Imidacloprid	1.3	12	0.47	1.41	109/6.8	116/16	89/15
Acetamiprid	1.2	6.6	0.25	0.75	105/3.7	104/15	99/18
Thiacloprid	1.1	9.9	0.36	1.1	109/0.18	104/16	99/19
Sulfoxaflor	0.89	23	0.63	1.9	112/3.0	82/6.3	125/17

NV = no value because percent recovery was not evaluated for this analyte.

accuracy and precision statistics, and would be useful for exposure monitoring.

To our knowledge, no other method has been published that combines the ability to quantify low levels of neonicotinoids in avian tissue matrices while also allowing for broad-range screening for many other types of pesticides as well as retrospective data analysis for emerging contaminants of concern. The utility of this approach was demonstrated by the early detection of carbaryl residues in a number of birds that allowed modification of the method for subsequent sample analysis.

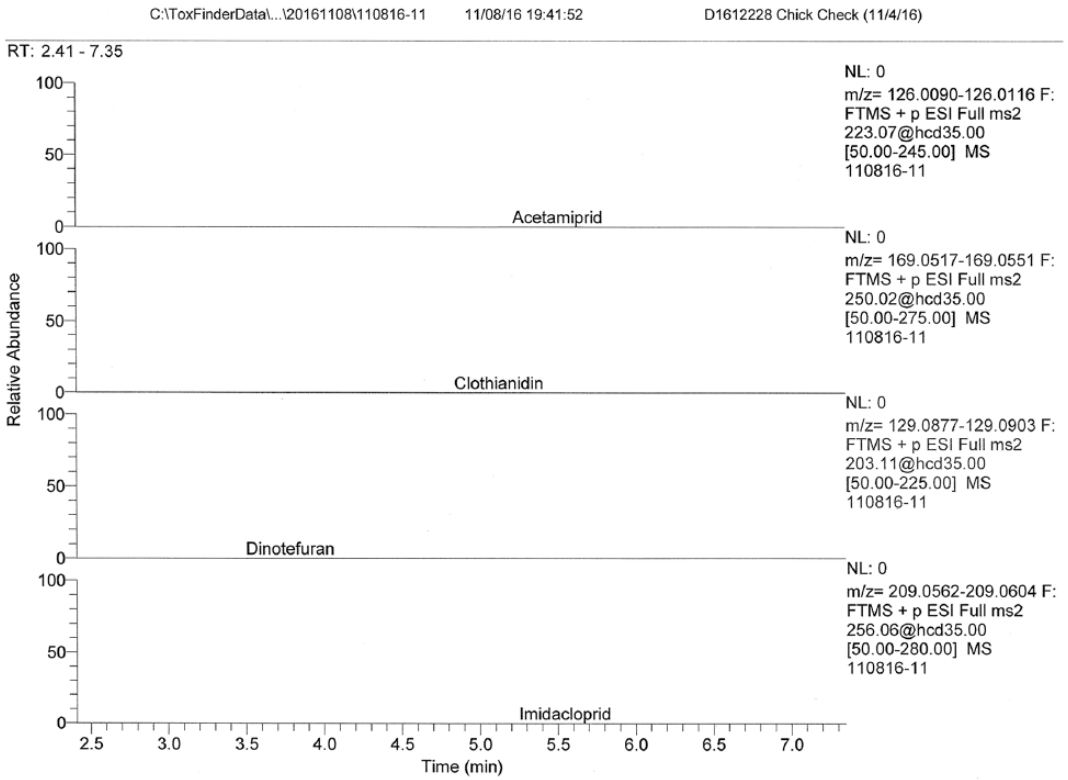

Figure 4. Selected ion chromatograms for acetamiprid, clothianidin, dinotefuran, and imidacloprid from analysis of an extract of negative control matrix (homogenized chick carcasses) demonstrating freedom from signals matching those of the targeted analytes.

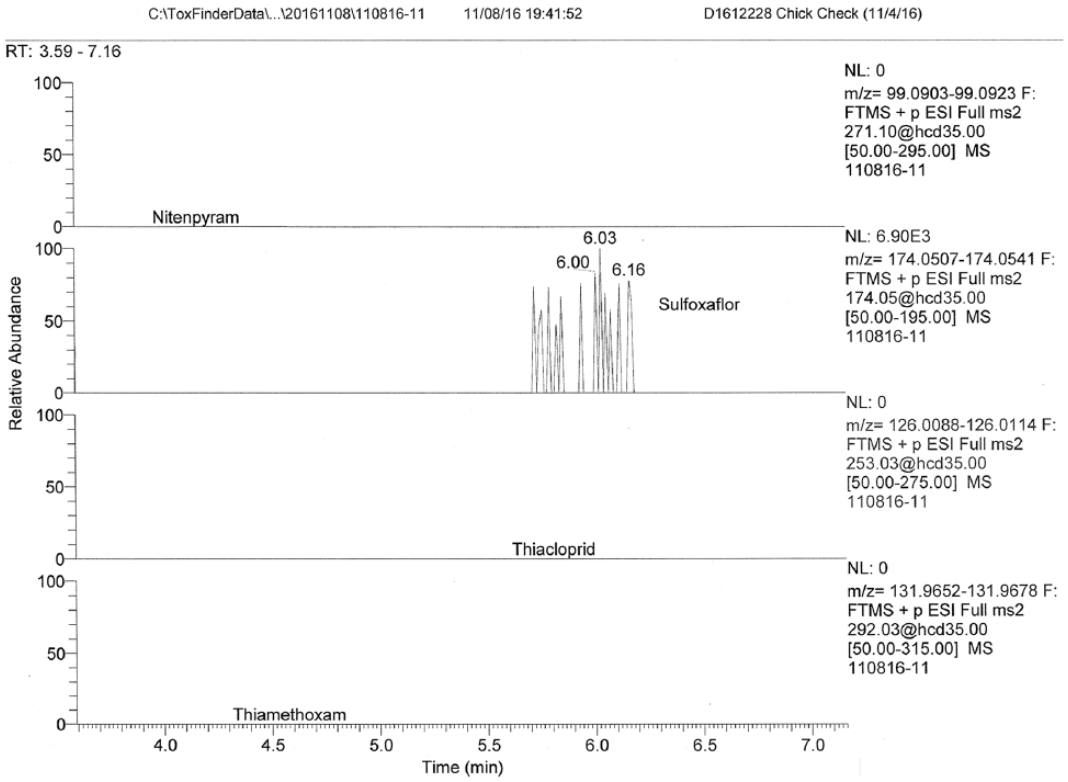

Figure 5. Selected ion chromatograms for nitenpyram, sulfoxaflor, thiacloprid, and thiamethoxam from analysis of an extract of negative control matrix (homogenized chick carcasses).

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplementary material

Supplementary material for this article is available online.

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