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Determination of 20, 25-diazacholesterol in avian matrices by high performance liquid chromatography/tandem mass spectrometry

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ABSTRACT

Wildlife contraceptives are an emerging tool for minimizing human-wildlife conflicts. One promising avian contraceptive compound, 20,25-diazacholesterol (DAC), reduces fertility by inhibiting cholesterol synthesis. A reliable analytical method for DAC was required in support of its registration for use as a reproductive control agent in pest bird species. A liquid chromatographic method employing tandem mass spectrometry (LC–MS/MS) was developed for the analysis of tissue extracts following solid phase extraction clean-up. Tissues analyzed were whole body samples from crows, monk parakeets, and quails and liver samples from crows and quails. Excellent sensitivity and selectivity was afforded by tandem mass spectrometry. The method accuracy of DAC from various tissue samples fortified at parts-per-million (ppm) and parts-per-billion (ppb) concentrations was high (> 90%) with excellent precision (< 10% relative standard deviation). Lower limits of detection were excellent in all tissues types, ranging from 1 to 11 ppb in whole body matrices and 9.9–34 ppb in liver matrices.

1. Introduction

Bird damage to agriculture, human health, and physical structures can result in substantial monetary loss and potential loss of life. For example, blackbirds cause approximately $5.4 million worth of damage to sunflower crops alone every year [1] . Birds may also spread zoonotic diseases and pathogens such as salmonella [2], influenza [3], and histoplasmosis [4,5]. The influx of invasive species such as the Monk parakeet (*Myiopsitta monachus*) has caused extensive problems. Monk parakeets nest on power lines, causing blackouts [6], bird strikes are an ongoing concern for the aviation industry [7,8], and pigeon excrement erodes historical monuments and damages roofs and statues [9,10].

Many approaches have been used to reduce damage caused by birds, including reduction of nuisance bird populations. However, lethal control is often met with public scrutiny and outcry. Furthermore, lethal control may be ineffective in some bird populations. New populations of birds simply immigrate into areas where previous populations were reduced and the problem continues [11]. Similarly, egg removal often increases the number of eggs laid and negatively impacts the health of laying birds [12]. Thus, more effective and socially acceptable methods must be developed. Wildlife contraceptives are an emerging tool for minimizing human-wildlife conflicts. One promising compound, 20,25-diazacholesterol (DAC), reduces fertility by inhibiting cholesterol synthesis. Animals exposed to DAC are unable to produce testosterone and progesterone necessary for sexual reproduction [13].

DAC was originally developed by the G.D. Searle Co. for use as a cholesterol-reducing agent in humans and registered as a feral pigeon contraceptive under the trade name Ornitrol™ in the early 1970′s [14,15]. Continuing throughout the 1970′s and into the 1980′s, several species of wild birds were studied to determine if reproduction could be altered with Ornitrol™. The results showed varying degrees of success [16,17]. Recently, scientists at the USDA APHIS WS National Wildlife Research Center (NWRC) evaluated DAC as a fertility control agent in quail [18] (Yoder, Bynum, & Miller, Development of Diazacon™ as an avian contraceptive, 2005). Results of this study demonstrated that egg production decreased by 85%, fertility of laid eggs by 70%, and hatchability of eggs that were laid decreased by 100% upon 12–14 days of treated feed consumption. Quail fertility remained suppressed for up to three months or longer. In a similar study with monk parakeets, fertility was reduced by 68% in that species [19].

Research with DAC has advanced toward registration of the compound with the US Environmental Protection Agency for use as a reproductive control agent for pest bird species. Evaluation of primary and secondary hazards (both mammalian and avian) is an important registration requirement. A precise analytical method for quantifying DAC in species exposed to it was required to evaluate these potential hazards. However, as a relatively low-use compound, a reliably sensitive method for quantifying DAC in bird tissues was not available. Modifying previous methods, we extracted DAC from avian tissues by saponification followed by liquid–liquid extraction with an organic

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solvent, then an acidified water:methanol solution and an SPE clean-up [20]. An HPLC method was developed previously for the determination of DAC in quail serum [21]. The NWRC had earlier developed an unpublished method for DAC in homogenized whole quail employing high performance liquid chromatography (HPLC) with ultraviolet detection [Goldade, personal communication]. The lower limit of detection (LLOD) of 1.1 μg/g proved to be insufficiently sensitive, causing this method to be of limited utility. Here, we describe a liquid chromatographic method using tandem mass spectrometry (LC–MS/MS) with a significantly lower detection limit. Furthermore, the sensitivity is greatly improved owing to the increased signal to noise afforded by tandem mass spectrometry.

2. Material and methods

2.1. Reagents and standards

HPLC grade methanol, hexane, chloroform, acetonitrile, ammonium formate, and hydrochloric acid were obtained from Fisher Chemical (Pittsburgh, PA). Formic acid (88%), (3-dimethylamino)-1-propylamine, and potassium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO). Ammonium hydroxide (50%) was obtained from Ricca Chemical (Arlington, TX). Water was obtained from a Milli-Q UV plus water purification system manufactured by Millipore Corporation, (Burlington, MA).

A deuterated form of DAC was synthesized for use as an internal standard (IS). 7B-([3-(Dimethylamino) propyl]-methylamino) androst-5-en-3B-ol (DAC) was purchased from Asis Chemical (Waltham, MA). 5-Androsten-3β-ol-17-one-16, 16-d_2 was purchased from Steraloids (Newport, RI) for preparation of deuterated DAC dihydrochloride (DAC-d_2) which was synthesized in-house by reductive amination of the ketone in 5-Androsten-3β-ol-17-one-16, 16-d_2 with (3-dimethylamino)-1-propylamine with simultaneous formylation of the primary amine in (3-dimethylamino)-1-propylamine. The aldehyde was reduced with sodium borohydride and the dihydrochloride salt was produced by treatment with hydrochloric acid.

Concentrated DAC (1000 μg/mL) and DAC-d_2 (1000 μg/mL) standard solutions were prepared in 0.1% formic acid in 1:1 methanol:-water and used to prepare a series of calibration standard solutions by dilution. Calibration standard solutions were prepared from the concentrated DAC standard solution at 7.50, 10.0, 25.0, 100, 250, and 500 ng DAC/mL in 0.1% formic acid and each solution contained 100 ng/mL DAC-d_2. Fortification solutions (1.00 μg DAC-d_2/mL and 1.00 and 10.0 μg DAC/mL) were prepared in 0.1% formic acid in 1:1 methanol:water and used to fortify control tissues.

For DAC screening with UV detection, a working standard was prepared in 0.1% formic acid in 1:1 methanol:water at 5 μg/mL without addition of the DAC-d_2 internal standard.

2.2. Instrumentation

A Univex MG8912 meat grinder (Salem, NH) was used for tissue preparation and a microwave digestion unit was used for extraction of tissue samples (MARS, CEM Inc, Matthews, NC). An Eberbach mechanical shaker with a 2–3/8" stroke length was used for liquid/liquid extraction of digests (Eberbach Corp., Ann Arbor, MI). SPE was performed on a Zymark Rapid Trace SPE Workstation (Zymark Corp., Hopkinton, MA).

Samples were prescreened to avoid injecting a high concentration of DAC into the mass spectrometer. For screening an Agilent Technologies HPLC series 1260 (Agilent Technologies, Wilmington, DE) equipped with vacuum de-gas, binary pump, temperature-controlled column compartment, wellplate sampler, and diode array detector was used.

Sample analysis was performed on an Agilent Technologies series 1200 HPLC connected to an Agilent 6410A–2 K triple quadrapole mass spectrometer (QQQ-MS). MassHunter® software (Agilent Technologies, Wilmington, DE) was used for quantitation analysis. Chromatographic separations for both instruments were achieved with a Waters XBridge C-18 column (2.5 μm, 50 × 2.1 mm, Milford, MA).

Table 1
HPLC and MS/MS Parameters.

Mobile Phase:	(A) 25 mM ammonium formate (pH 8.5)		
	(B) 25 mM ammonium formate in 1:1 methanol:acetonitrile		
Gradient:	Time (min)	%A	%B
	0.0	80	20
	1.0	80	20
	1.1	70	30
	5.0	30	70
	5.1	0	100
	6.5	0	100

Transitions and Collision Energies

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (v)
DAC	389.4	344.3	29
	389.4	316.2	29
DAC-d_2	391.4	346.3	29
	391.4	318.3	33

The mobile phase consisted of 25 mM ammonium formate (pH 8.5) in a gradient of 25 mM ammonium formate in 1:1 methanol:acetonitrile (Table 1) with a flow rate of 0.5 mL/min. The column temperature was 80 °C and the run time was 6.5 min. A wavelength of 206 nm was used for UV detection. The mass spectrometer employed electrospray ionization with nitrogen nebulizer gas (35 psi; 12 L/min; 350 °C) and fragmentor voltages of 126 V for DAC and 148 V for DAC-d_2. The collision energies were optimized for two transitions for both DAC and DAC-d_2 (Table 1).

2.3. Tissue preparation

Whole body and liver tissue were analyzed for three different species: Coturnix quail (*Coturnix coturnix*), American crow (*Corvus brachyrhynchos*) and Monk parakeet (*Myiopsitta monarchus*). Livers were removed from each specimen and reserved for analysis. Whole-body samples were prepared by removing and disposing of wings, legs, and skin, and homogenizing the remaining carcass using the meat grinder. Liver samples were prepared by grinding the frozen tissue in liquid nitrogen using a mortar and pestle.

2.4. Extraction procedure

One gram of whole-body tissue or 0.5 g of liver tissue was placed in a 55-mL Teflon microwave digestion tube. Samples were fortified with 0.100 mL of the DAC-d_2 internal standard solution (yielding 100 ng/g) and digested with 4 mL of 10% KOH solution via microwave (432 kJ) to saponify the fatty acids in the sample. The digestion temperature began at room temperature and increased over 8 min to 80 °C and held for 10 min. After cooling, digests were transferred to screw-cap culture tubes. They were then extracted with 10 mL of 2:1 hexane:chloroform (one gram of sodium chloride was added to reduce emulsification) and mixed on a mechanical shaker for 10 min. This extracted DAC from the aqueous phase and left behind any water-soluble contaminants. The samples were centrifuged at approximately 450 x g for 5 min and the organic layer removed by Pasteur pipette to a clean culture tube. This extraction was performed a total of 3 times with the organic layer combined in the same tube. The organic layer was extracted with 4.0 mL 0.1 M hydrochloric acid in 2:1 methanol:water and mixed on a vortex mixer for 30 s. This protonated the DAC and allowed it to be extracted into the aqueous phase and separate out organic-soluble

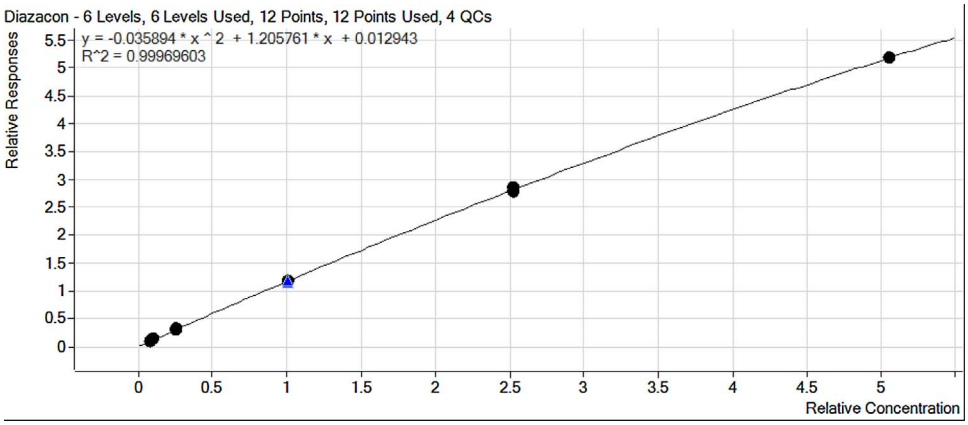

Fig. 1. Representative Calibration Curve for Diazacon: The calibration curve uses six points: 7.65 ng/mL, 10.2 ng/mL, 25.5 ng/mL, 102 ng/mL, 255 ng/mL, and 510 ng/mL. The concentration of the DAC-d_2 internal standard is 101 ng/mL.

Table 2
DAC recoveries for tissue samples fortified at multiple concentrations.

	Concentration					
Tissue	**n =**	**25 ng/g**	**n =**	**250 ng/g**	**n =**	**100,000 ng/g**
Monk Parakeet, Whole Body	7	105 ± 9.7	7	103 ± 3.1	7	94.4 ± 6.3
Crow, Whole Body	7	112 ± 9.7	7	106 ± 5.6	7	90.3 ± 7.5
Quail, Whole Body	7	102 ± 3.3	7	105 ± 3.8	7	95.4 ± 14.1
	n =	**30 ng/g**	**n =**	**300 ng/g**	**n =**	**100,000 ng/g**
Monk Parakeet, Liver	7	116 ± 9.2	7	106 ± 5.4	7	95.6 ± 5.4
Crow, Liver		n/a	7	107 ± 2.6	7	92.5 ± 5.2

Table 3
DAC recoveries for fortified quality control samples.

	Concentration					
Tissue	**n =**	**25 ng/g**	**n =**	**250 ng/g**	**n =**	**100,000 ng/g**
Monk Parakeet, Whole Body	4	105 ± 6.6	4	113 ± 5.8	3	95.9 ± 1.3
Crow, Whole Body	8	111 ± 7.7	8	103 ± 2.9	8	97.8 ± 5.4
Quail, Whole Body	6	115 ± 4.7	4	103 ± 1.2	6	89.2 ± 6.6
	n =	**30 ng/g**	**n =**	**300 ng/g**	**n =**	**100,000 µg/g**
Monk Parakeet, Liver	4	105 ± 6.8	4	104 ± 3.1	4	101 ± 1.3
Crow, Liver		n/a	8	104 ± 3.7	8	94.0 ± 3.4

contaminants. The samples were centrifuged at approximately 450 x g for 5 min and the aqueous layer removed by Pasteur pipette to a clean culture tube. This extraction was repeated two more times using 3.0 mL 0.1 M hydrochloric acid in 2:1 methanol:water, combining the aqueous layers in the same tube. Samples were then placed in a water bath at approximately 50 °C under a gentle stream of nitrogen for 15 min to remove any residual organic solvent.

2.5. Extract cleanup

Cleanup was performed by solid phase extraction using Strata-X-C–33 µm polymeric strong cation solid phase extraction cartridges (500 mg/3 mL) (Phenomenex, Torrance, CA). Cartridges were conditioned with 4.0 mL methanol followed by 4.0 mL 0.1 M hydrochloric acid in 2:1 methanol:water prior to loading the acidified extracts and washing with 5.0 mL 0.1 M hydrochloric acid in 2:1 methanol:water, 3.0 mL methanol, and 6.0 mL 1:1 acetonitrile: methanol. Analytes were eluted with 6.0 mL 5% ammonium hydroxide in 10:1 methanol:water. 2.0 mL of 10% formic acid in methanol was added to the eluate to prevent the analyte from sticking to the glassware. The samples were evaporated to dryness in a water bath at 50 °C under a gentle stream of nitrogen. Samples were reconstituted with 1.00 mL 0.1% formic acid in water and sonicated for 10 min before being transferred to autosampler vials for injection (25 µL) into the HPLC-UV for prescreening.

2.6. Preliminary screening

Working standard containing DAC only and extracts were injected (25 µL) into the HPLC equipped with ultraviolet detection; DAC was estimated using a single-point calibration. Those extracts determined to have DAC concentrations exceeding 5 µg/mL were diluted appropriately in a solution containing 100 ng/mL DAC-d_2 in 0.1% formic acid such that the new DAC concentrations fell into the calibrated range of the more sensitive LC–MS/MS.

2.7. Calibration and quantitation

Calibration standards were injected into the HPLC equipped with MS/MS detection at the beginning and end of each run. Peak responses ratios (DAC/DAC-d_2) were calculated and used to generate a calibration curve using quadratic fit and 1/x weighting. (Fig. 1) The MassHunter® (version B.07.00) software was used to automatically calculate DAC concentrations using peak response ratios from the extracts and the daily calibration curve. The 100 ng/mL DAC calibration standard was frequently injected (every 10 samples) to monitor calibration accuracy and drift.

2.8. Bias and repeatability

Control (no known DAC) carcasses of Monk parakeet, quail, and crow were prepared for analyses. Carcass tissue samples (1.0 g) were fortified with either 0.025 or 0.100 mL of the DAC fortification standard, yielding seven tissue samples each with the following DAC concentrations: 25 ng/g, 250 ng/g, and 100,000 ng/g. Similarly, replicates of Monk parakeet and quail control liver tissue (0.5 g) were fortified to yield seven liver samples each with DAC concentration of 30 ng/g, 300 ng/g, and 100,000 ng/g. Crow livers were fortified at 300 ng/g and 100,000 ng/g, but not at 30 ng/g due to high background from the matrix. Tissue samples were analyzed over a two-day period according to the procedures above. The accuracy of the fortified samples was determined by quantifying the fortified samples with the calibration curve with internal standard ratio correction and comparing this value to their calculated theoretical concentration.

2.9. Matrix interference

Seven representative control tissue samples of each type were assayed according to the procedures in this method without fortification with DAC or DAC-d_2.

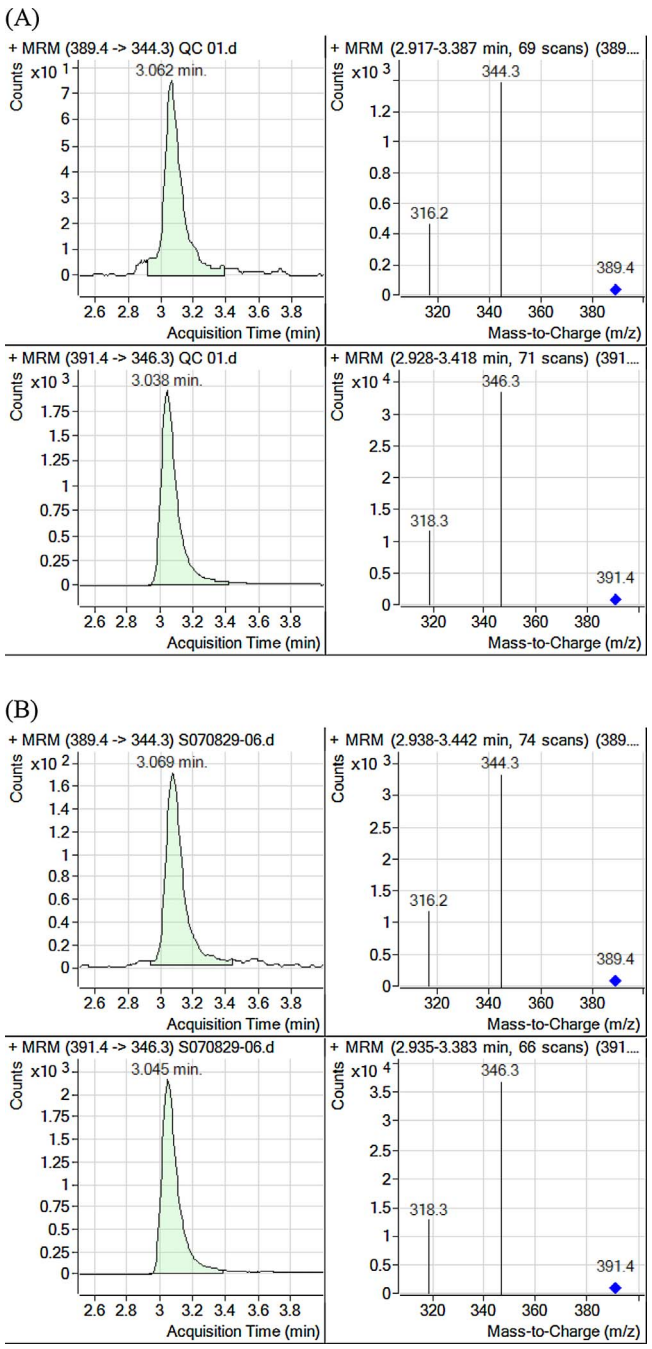

Fig. 2. Representative Chromatograms and MRM for DAC in Coturnix Quail whole body field samples: (A) Control matrix showing a DAC concentration corresponding to 2.3 ng/g (top: DAC; bottom: DAC-d_2) and (B) a field sample showing a DAC concentration of 6.3 ng/g.

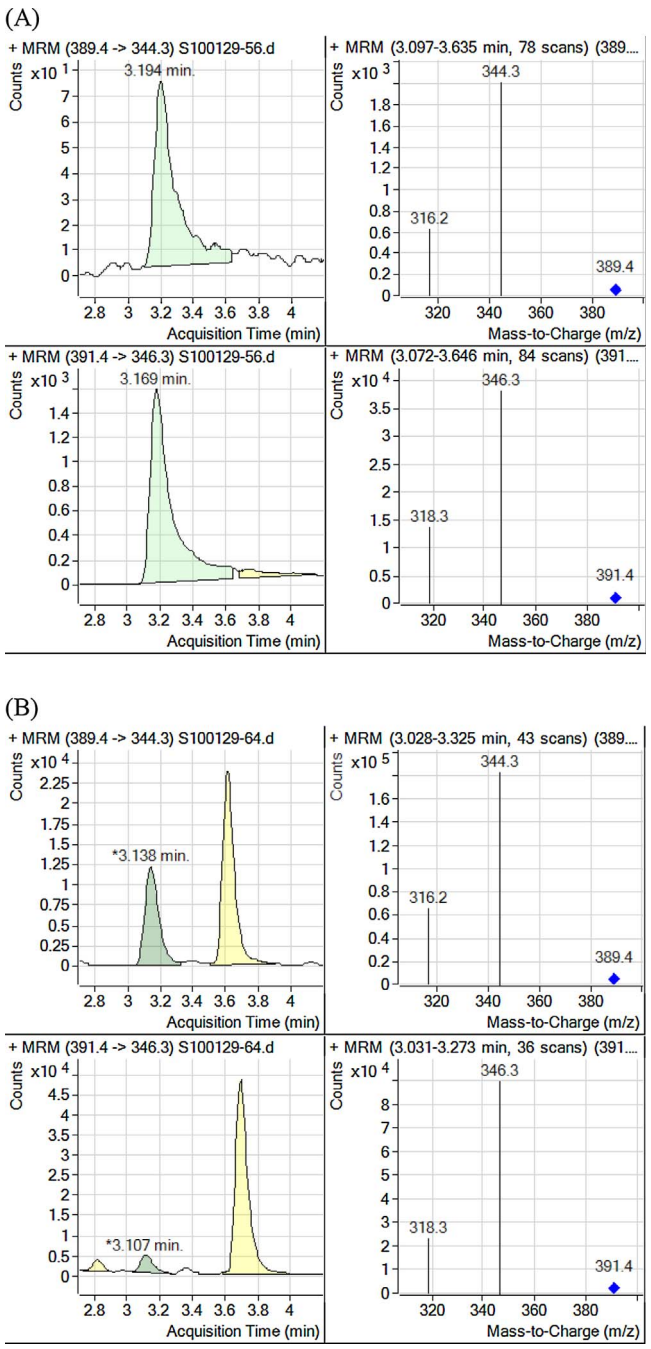

Fig. 3. Representative Chromatograms and MRM for DAC in American Crow liver field samples: (A) Control matrix showing a DAC concentration corresponding to 5.4 ng/g (top: DAC; bottom: DAC-d_2) and (B) a field sample showing a DAC concentration of 528 ng/g.

2.10. Lower limits of detection

Chromatographic peak responses at the retention time of DAC from bias and repeatability samples and matrix interference samples were used to estimate detection and quantitation limits. The lower limit of detection (LLOD) was defined as the concentration of DAC in the sample required to generate a signal equal to the mean response chromatographic peak response observed at the DAC retention time in replicate control tissues plus three times the standard deviation. The lower limit of quantitation (LLOQ) was defined as the concentration of DAC in the sample to generate a signal equal to the mean response chromatographic peak response observed at the DAC retention time in

Table 4
DAC Lower Limit of Detection/Quantitation (LLOD/LLOQ).

Tissue	LLOD (ng/g)	LLOQ (ng/g)
Monk Parakeet, Whole Body	11	28
Crow, Whole Body	4.0	7.9
Quail, Whole Body	1.0	2.5
Monk Parakeet, Liver	9.9	26
Crow, Liver	34	80

replicate control tissues plus ten times the standard deviation. Fortified samples at levels near the LLOQ showed accuracy and precision values similar to samples fortified at higher levels (Tables 2 and 4).

3. Results and discussion

During method development, an interfering peak with identical m/z as one of the product ions (m/z = 391) as the internal standard was observed in many tissue extracts, particularly crow livers. This interfering peak was resolved from DAC chromatographically. Changing the mobile phase components from 0.1% formic acid in water and 0.1% formic acid in acetonitrile to 25 mM ammonium formate and 25 mM ammonium formate in 1:1 acetonitrile:methanol yielded significant resolution of the peaks. Under the conditions specified in this method, the retention times of DAC and DAC-d_2 were approximately 3.2 min (Table 2). Day to day variability of the peak ratio determined from repeated injection of the 100 ng/mL calibration standard was good, with a relative standard deviation of 18% across 15 runs.

Injection of high DAC concentrations into the LC–MS/MS system during initial method development resulted in significant persistence of the analyte in the atmospheric ionization source. Because carryover of a DAC chromatographic signal would have a detrimental impact on LLOD (requiring it to be sufficiently high enough to preclude false identification of DAC in actual samples), sample extracts were first screened by UV under similar LC conditions to identify which samples had levels above the range of the calibration curve. These samples were then diluted into the linear range.

The same properties leading to DAC persistence in the mass spectrometer may also lead to its persistence in glassware used during extraction. In fact, significant loss of DAC was observed during SPE cleanup when the eluate, which was at a high pH, was not acidified. Addition of 10% formic acid in methanol to the ammonium hydroxide SPE eluate facilitated DAC solvation and efficient transfer.

A quadratic model was made necessary because of isotopic interference between DAC and the deuterated internal standard which share m/z = 391 (representing $M + H^+$ for DAC-d_2 and $M + 2 + H^+$ for DAC). Although DAC M + 2 abundance is relatively small (approximately 4%), its contribution to the internal standard peak response makes a simple linear regression invalid. Minimizing isotopic interference through synthesis of a DAC-d_6 internal standard proved to be unfeasible. However, correction for isotopic interferences is possible using a nonlinear function [22]. The quadratic relationship between the DAC/DAC-d_2 chromatographic peak ratio and DAC concentration consistently yielded coefficients of determination (R-square) between 0.9970 and 0.9999. Excellent results for calibration curves as evidenced by R-square and method accuracy were obtained by employing the nonlinear function.

Accuracy of DAC from fortified tissue samples were high with excellent repeatability (Table 2). When bias and repeatability evaluation was performed during analysis of actual samples, accuracy data from these fortified quality control samples were nearly identical (Table 3). Likely owing to the high sensitivity of tandem mass spectrometry, a small chromatographic interference was observed in most control extracts (Fig. 2). The highest interference was observed in crow liver, corresponding to 15 ng/g (Fig. 3). As a result of this matrix interference, LLOD was defined as the DAC signal equal to the mean response of the interference plus three times its standard deviation. Excellent LLODs were evident in all tissues representing an improvement of several orders of magnitude over the previous LLOD of 1.1 μg/g obtained by HPLC-UV analysis (Table 4).

Selection of matrices for analyses was carefully considered. Whole body tissue residues provide an estimation of the dose a predator would receive if it consumed a treated animal. Because the liver is particularly metabolically active, it tends to have a higher concentration of active ingredient; determining the concentration of active ingredient in the liver provides data as to the highest dose a non-target species might receive in normal field conditions. This method was applied by our group for the analyses of field samples quail whole body tissues, crow whole body and liver tissues, and monk parakeet whole body and liver tissues. Quail livers proved to be too small to feasibly process for individual sampling, ergo only whole body quail tissues were analyzed.

This method represents a highly accurate procedure for quantifying DAC residues in the carcasses of target bird species that have ingested DAC treated feeds. Accuracy of fortified quality control samples were excellent with a high degree of repeatability. These data are essential for evaluating the potential impacts on predators or scavengers that consume target animals that have ingested the treated feed and have facilitated the use of DAC as a wildlife control technique.

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References

[1] B. Peer, H.J. Homan, G.M. Linz, W.J. Bleir, Impact of Blackbird damage to sunflower: bioenergetic and economic models, Ecol. Appl. 13 (2003) 248–256.
[2] I. Tizard, Salmonellosis in wild birds, Semin. Avian Exotic Pet Med. 13 (2004) 50–66.
[3] T. DeLiberto, S. Swafford, D. Nolte, K. Pedersen, M. Lutman, B. Schmit, J. Baroch, D. Kohler, A. Franklin, Surveillance for highly pathogenic avian influenza in wild birds in the USA, Integr. Zool. 4 (2009) 426–439.
[4] A.R.J. Stickley, J.R. Pruitt, C.E. Hume, T.I. Pass, C.H. Gayle, Decontamination of a Histoplasma capsultum-infested blackbird roost: use of a sprinkler system to apply formalin, Proc. East. Damage Control Conf. 3 (1987) 171–176.
[5] J. Fischer, Human health concerns in the practice of wildlife damage management, Twelfth Great Plains Wildlife Damage Control Workshop, Noble Foundation, Ardmore, Okla, 1995, pp. 21–26.
[6] M.L. Avery, J.R. Lindsay, J.R. Newman, S. Pruett-Jones, E.A. Tillman, Reducing monk parakeet impacts to electric utility facilities in south Florida, Advances in Vertebrate Pest Management vol. IV, Flanger Verlag, Furth, 2006, pp. 125–136.
[7] R.A. Dolbeer, S.E. Wright, E.C. Cleary, Ranking the hazard level of wildlife species to aviation, Wildl. Soc. Bull. 28 (2000) 372–378.
[8] N. Sodhi, Competition in the air: birds versus aircraft, Auk 119 (2002) 587–595.
[9] D. Channon, Feral pigeon excrement on heritage stonework, Inte. Pest Control 46 (2004) 24–27.
[10] E. Bernardi, D. Bowden, P. Brimblecombe, H. Kenneally, L. Morselli, The effect of uric acid on outdoor copper and bronze, Sci. Total Environ. 407 (2009) 2383–2389.
[11] D. Sol, J. Senar, Urban pigeon populations: stability, home range, and the effect of removing individuals, Can. J. Zool. 73 (1995) 1154–1160.
[12] L. Jacquin, B. Cazelles, A. Julliard, G. Leboucher, J. Gasparini, Reproduction management affects breeding ecology and reproduction costs in urban feral pigeons Columba livia, Can. J. Zool. 88 (2010) 781–787.
[13] C. Yoder, W. Andelt, L. Miller, J. Johnston, M. Goodall, Effectiness of twenty, twenty-five diazacholesterol, avian gonadotropin-releasing hormone, and chicken riboflavin carrier protein for inhibiting reproduction in Coturnix quail, Poult. Sci. 83 (2004) 234–244.
[14] J. Schortemyer, S. Beckwith, Chemical control of pigeon reproduction, Trans. North Am. Wildl. Nat. Resour. Conf. 35 (1970) 47–55.
[15] J. Sturtevant, Pigeon control by chemosterilization: population model from laboratory results, Science 170 (1970) 322–324.
[16] C. Mitchell, R. Hayes, T.J. Hughes, Effects of the chemosterilant ornitrol on house sparrow reproduction, Am. Midland Nat. 101 (1979) 443–446.
[17] D. Lacombe, A. Cyr, J. Bergeron, Effects of the chemosterilant ornitrol on the nesting success of red winged blackbirds, J. Appl. Ecol. 23 (1986) 773–779.
[18] C. Yoder, K. Bynum, L. Miller, Development of Diazacon™ as an avian contraceptive, Proceedings of the 11th Wildlife Damage Management Conference, Traverse City, Michigan, 2005.
[19] M. Avery, C. Yoder, E. Tillman, Diazacon inhibits reproduction in invasive monk parakeet populations, J. Wildl. Manage. 72 (2008) 1449–1452.
[20] M. Fenton, Chromatographic separation of cholesterol in foods, J. Chromatogr. 624 (1992) 369–388.
[21] J. Johnston, M. Goodall, J. Hurley, C. Yoder, L. Miller, Determination of diazcon in quail feed and quail serum by ion pair reversed-phase chromatography, J. AOAC Int. 84 (2001) 634–639.
[22] G. Rule, Z. Clark, B. Yue, A. Rockwood, Correction for isotopic interferences between analyte and internal standard in quantitative mass spectrometry by a nonlinear calibration function, Anal. Chem. 85 (2013) 3879–3885.