Gas Chromatography/Mass Spectrometry Method for the Quantitation of 3-Chloro-*p*-toluidine Hydrochloride in Birds Using a Deuterated Surrogate

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A method was developed for the quantitation of the avicide 3-chloro-*p*-toluidine hydrochloride (CPTH) in pigeon breast muscle and gastrointestinal tract. Accuracy and precision were greatly improved by the use of CPTH-d_6 as a surrogate. CPTH-d_6 was synthesized by the nitration of toluene-d_8 followed by chlorination and reduction. The synthetic product provided mass fragments such that CPTH and the surrogate were differentiated by gas chromatography/mass spectrometry (GC/MS). A tissue extraction process used solid-phase extraction to concentrate the free base forms of CPTH and the surrogate from hexane extracts. These compounds were recovered from the SPE columns with *n*-butyl acetate and quantified by GC/MS. Validation data indicated (1) a linear relation between CPTH/CPTH-d_6 area ratios and CPTH concentration; (2) a method limit of detection of 30 ppb for both tissue matrices; (3) CPTH recoveries from fortified tissues ranging from 56.4 to 92.4%, whereas surrogate corrected recoveries ranged from 95.0 to 101%; and (4) identical extraction efficiencies of both compounds from each matrix.

Keywords: *3-Chloro-p-toluidine hydrochloride; gas chromatography; quantitative analysis; tissue extraction; NMR spectroscopy*

INTRODUCTION

As mankind heads into the 21st century, confrontations between man and nature are becoming more frequent. Airplanes colliding with birds or mammals, crop predation by pest birds, and telephone cable damage by subterranean animals are examples of these ongoing struggles. The mission of the Wildlife Services (WS) program is to balance "...the needs of humans and wildlife in many different situations" (USDA, 1997). In fulfilling this mission, the National Wildlife Research Center (NWRC) "... is the research arm of WS...providing scientific information on conflicts between humans and wildlife. It is the only research facility in the world devoted exclusively to the study of wildlife damage control" (USDA, 1997).

To satisfy federal and state pesticide registration data requirements, the NWRC conducts application and efficacy studies on a number of chemical compounds including the avicide 3-chloro-*p*-toluidine hydrochloride (CPTH). Analysis of test subjects for the presence of the pesticide assists in confirming the cause of death and assesses the possibility of secondary hazards to predator and/or scavenger species that could potentially consume target animals exposed to the avicide. Gas chromatography and mass spectrometry (GC/MS) were used in one such study that evaluated the effectiveness of CPTH corn baits for reducing pest pigeon populations (Cummings et al., 1994). Breast muscle was assayed for CPTH content because this tissue would likely be consumed first by a scavenger species. The gastrointestinal (GI) tract was analyzed for incurred CPTH residues because this matrix may contain the greatest concentration of the avicide shortly after exposure. Previous extraction methods using external standards produced low and variable CPTH recovery data from fortified tissues. These methods were also laborious and time-consuming. To permit the correction of variable recoveries, a residue method was developed that included the use of a surrogate material, a deuterated form of CPTH. This paper discusses the synthesis and purification of deuterated CPTH (Figure 1) and its use as a surrogate in a tissue extraction method for quantifying CPTH residues in the breast muscle and GI tract of pigeons using GC/MS.

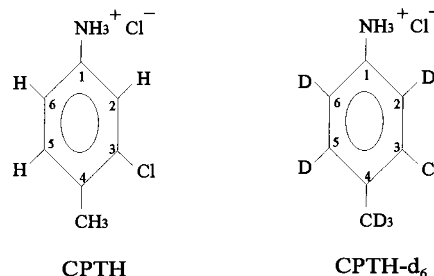

Figure 1. Chemical structures of CPTH and CPTH-d_6.

Properties. CPTH, also known as DRC-1339 or Starlicide, is the hydrochloride salt of 3-chloro-*p*-toluidine (CPT). CPTH is soluble in water and alcohol and insoluble in hydrocarbon, aromatic, ether, and ketone solvents. Due to its photosensitivity, CPTH should be stored in containers that minimize light penetration.

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The pK_a of this compound has been reported to be 3.7 (Kimball and Mishalanie, 1994).

Uses. CPTH is used in the United States to control pest bird populations including starlings, crows, ravens, pigeons, blackbirds, and grackles (USEPA, 1995). Bait formulations include water solutions, coatings, and mixtures in margarine or watermelon pulp. Cattle, poultry, and swine producers use CPTH baits in and around animal feedlots to reduce feed consumption and/or contamination by pest birds. Terrestrial and aquatic farmers use the avicide to deter bird predation. Animal refuges use CPTH to control the population of pest birds that threaten the existence of endangered or indigenous bird species.

Symptoms and Mode of Action. Starlings dosed with CPTH at levels slightly higher than their acute LD_{50} level (3.8 mg/kg) appeared to be normal 20 to 30 h after dosing (DeCino et al., 1966). However, water consumption by these birds doubled and then sharply decreased, while food consumption remained constant. Four hours prior to death, the dosed birds refused food and water, became listless, perched themselves with feathers fluffed, and appeared to doze. Near death, the birds became comatose and eventually died without convulsions or spasms.

Internally, Decino found CPTH caused circulatory impairment in the liver, kidneys, and, to some extent, brain. Necrosis of the gizzard lining and kidney tubules caused hemorrhaging and reduction in the excretion of toxic material. "Death apparently results from uremic poisoning and congestion of the major organs" (Decino et al., 1966). A white fatlike material was also observed within the body cavity, particularly in the pericardial region. Preliminary tests indicated this material to be uric acid (Decino et al., 1966). Subsequent testing done in our laboratory showed that uric acid accounted for 2% of this white material. DeCino also reported that symptoms exhibited by other CPTH-dosed birds were similar to those seen for starlings.

Selectivity and Toxicity. Although other avicides are available (Schafer, 1984), CPTH uniquely possesses a high degree of selectivity between birds and mammals (Savarie and Schafer, 1987). This selectivity was also observed within bird species; starlings, red-winged blackbirds, and crows were susceptible to CPTH, whereas other bird species including ducks, sparrows, and hawks were more resistant to the avicide (DeCino et al., 1966).

Toxicological data indicated that CPTH is highly toxic to most pest bird species (oral LD_{50} of 1.0−10 mg/kg) and less toxic to most mammals and predatory birds (oral LD_{50} of 250−1000 mg/kg) (Schafer, 1984). CPTH has a moderately acute toxicity to cold- and warm-water fish and is acutely toxic to aquatic invertebrates (USEPA, 1995). CPTH was also found to be a chronic rather than an acute toxicant to sensitive birds (Schafer et al., 1977). This chronic toxicity appears to be related to irreversible kidney damage (Schafer, 1991).

MATERIALS AND METHODS

Reagents. *Deuterated Materials.* The starting material for the synthesis, toluene-d_8 (99+ atom % D), was purchased from the Aldrich Chemical Co. (Milwaukee, WI). Other deuterated chemicals used included D_2SO_4, 99.5+ atom % D; DNO_3, 99+ atom % D; D_2O, 99+ atom % D; DCl, 99.5+ atom % D.

Solvents used included the following: hexane, J. T. Baker, HPLC grade; CH_2Cl_2, Fisher, Optima; methanol (MeOH), Fisher, HPLC grade; isopropyl alcohol (IPA), Mallinckrodt, analytical reagent; petroleum ether, Fisher, pesticide grade; tetrahydrofuran (THF), J. T. Baker, HPLC grade; hydrochloric acid (HCl), Fisher, ACS Certified Plus; diethyl ether, Aldrich, ACS reagent; ethyl acetate, Fisher, HPLC grade; acetonitrile (ACN), Fisher, Optima; *n*-butyl acetate, B&J, high purity; H_2O, Milli-Q system.

Chemicals used include the following: Na_2CO_3, Aldrich; $SbCl_3$, Fisher, ACS Certified; 10% palladium-on-carbon, Aldrich; $NaBH_4$, Aldrich, 99%; *p*-toluidine, Aldrich, 99%; NaCl, Fisher, USP/FCC granular; CPTH, Purina Mills, 97%; 2-chloro-*p*-nitrotoluene, Chem Services; *p*-nitrotoluene, Chem Services; Cl_2(g), Aldrich, 99.5%+; NaOH, Fisher, 50% w/w in H_2O.

Apparatus. *High-Performance Liquid Chromatography (HPLC).* To avoid byproducts in the final synthesis material, intermediate products were isolated after each reaction using HPLC. These separations were done using a Hewlett-Packard (HP) 1090 liquid chromatograph equipped with an ultraviolet (UV) diode array detector (DAD) and a Phenomenex preparative column (25 cm × 21.2 mm i.d., 5 μm particle size). Instrument parameters included mobile phase flow rate of 5.0 mL/min, ambient column temperature, and DAD wavelength settings of 272 and 300 nm. Each purification step discussed under Synthesis and Purification used a distinctive mobile phase composition and injection volume during the isolation of intermediate products. Liquid fractions were collected using an Eldex Universal fraction collector (Eldex Laboratories, Inc.).

GC/MS. The GC/MS system consisted of an HP 5890 gas chromatograph and an HP 5970 mass selective detector. A DB-1 capillary column (J&W Scientific), 30 m × 0.25 mm i.d. with a 0.25 μm film thickness, was used with a 4 mm Cyclosplitter (Restek Corp.) glass injection port liner. The temperatures of the injection port and transfer line were 280 and 300 °C, respectively. The flow rates of the helium carrier gas through the split and purge vents were 60 and 1 mL/min, respectively, and the column head pressure was 15 psi.

Qualitative evaluations of reaction products were performed using the following GC/MS parameters: oven program, 70 °C, held for 1 min, then increased to 190 °C at 15 °C/min, then increased to 265 °C at 30 °C/min; solvent delay, 4.5 min; purge time, 1.0 min; MS acquisition mode, SCAN, *m/z* 30−200; run time, 16.50 min.

Aliquots of the *n*-butyl acetate solutions, including standards and tissue extracts, were analyzed using the following GC/MS parameters: oven program, 90 °C for 0.25 min, then increased to 300 °C at 35 °C/min; purge time, 1.0 min; solvent delay, 2.25 min; MS acquisition mode, single ion monitoring (SIM), *m/z* 106, 140, 141 for CPTH and 112, 147, 149 for CPTH-d_6; run time, 14.25 min. A macro was developed to sum the abundance data from each set of SIM ions into one chromatogram. Therefore, each GC/MS analysis generated two chromatograms, one each for CPTH (Figure 2A) and CPTH-d_6 (Figure 2B).

Synthesis and Purification. The synthesis of CPTH-d_6 (Figure 3) used three chemical reactions: (A) nitration of toluene-d_8 to produce *p*-NO_2-toluene-d_7, (B) chlorination of the product from reaction A to produce 2-chloro-*p*-NO_2-toluene-d_6, and (C) reduction of the product from reaction B to produce CPT-d_6. A 0.05 N HCl in ethyl acetate solution was used to form the HCl salt, CPTH-d_6. All synthesis reactions were done in air hoods using appropriate personal protective equipment including gloves, lab coat, and safety glasses.

Nitration Reaction. The first step in the synthesis was the nitration of toluene-d_8, based on the method by Dannley and Crum (1968). A 0.10 mol sample of toluene-d_8 was placed in a 50-mL round-bottom flask, which was submerged in an ice bath. A mixture of D_2SO_4(con) (8 mL) and DNO_3(con) (7 mL) cooled to 5 °C was carefully added and vigorously mixed. The two-phase solution was warmed to ambient and mixing continued for another 115 min. The top organic layer was removed and washed with three 20-mL aliquots of D_2O, 10 mL of a 5% Na_2CO_3 in D_2O solution, and 10 mL of D_2O. The organic layer, yellow in color, was evaporated at room temperature to a constant volume to remove any residual toluene-d_8 material. The products from this reaction, determined by GC/MS, included *o*-, *m*-, and *p*- isomers of NO_2-toluene-d_7, along with some $(NO_2)_2$-toluene-d_6 compounds.

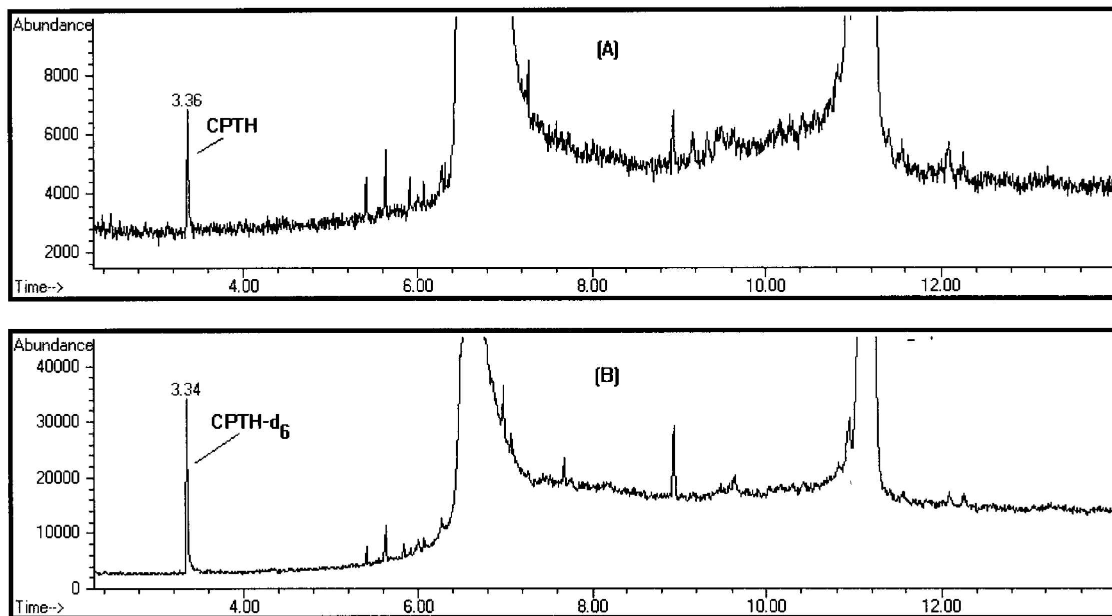

Figure 2. CPTH and CPTH-d_6 chromatograms from fortified breast tissue extract.

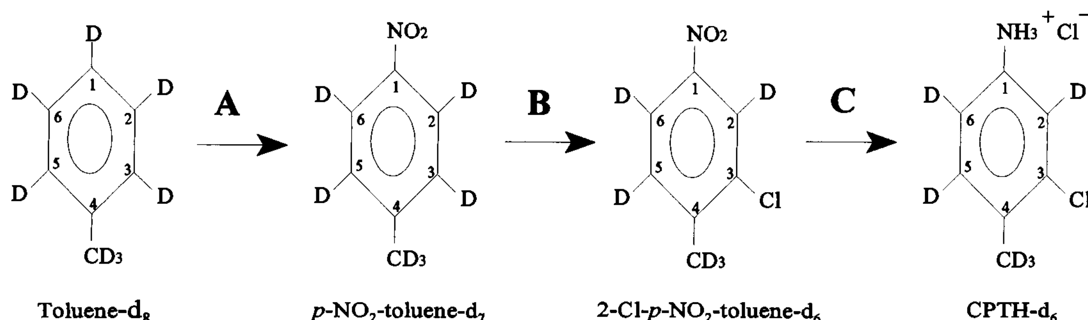

Figure 3. CPTH-d_6 synthetic reactions.

The target product, p-NO_2-toluene-d_7, was isolated using a two-step HPLC separation process. To separate the mono- and dinitro compounds, 75-μL aliquots from the nitration product solution were chromatographed with a hexane/CH_2Cl_2 (55:45) mobile phase. Fractions containing the mononitro compounds were collected during the 24−35-min retention time window. These fractions were combined and evaporated at room temperature to a constant volume. An equal volume of MeOH was added and mixed, and 100-μL aliquots were separated using a hexane/CH_2Cl_2/IPA (75.0:24.5:0.5) mobile phase. Fractions containing p-NO_2-toluene-d_7 were collected during the 45−60-min retention time window and analyzed by GC/MS. Fractions that were 99+% in the target product, based on relative area response, were combined and saved. Fractions having relative area responses <99% p-NO_2-toluene-d_7 were combined, evaporated, dissolved in MeOH, and repurified by HPLC. All LC fractions of 99+% purity were combined and evaporated, producing a pale yellow crystalline material.

Chlorination Reaction. A molar ratio of 1:38, $SbCl_3$(s)/p-NO_2-toluene-d_7 was placed in a 50-mL graduated glass centrifuge tube. This reaction vessel was placed in a 55 °C water bath, liquefying both components (Davies, 1921). A small stir bar was placed in the tube to mix the reaction solution, and Cl_2(g) was carefully bubbled into the mixture for 6 h. The reaction vessel was removed from the warm water bath, and 0.300 mL of a 0.5 N DCl in D_2O was added, forming a white precipitate. The reaction solution was extracted with petroleum ether, and the combined extracts were removed and evaporated to dryness. The residue was dissolved in a minimal amount of CH_2Cl_2, and 100-μL aliquots were purified by HPLC using a hexane/CH_2Cl_2 (85:15) mobile phase. Fractions containing the 2-chloro-p-NO_2-toluene-d_6 target product were collected during the 50−62-min retention time window and confirmed by GC/MS. These fractions were combined, and the mobile phase was evaporated, leaving a white precipitate.

Reduction Reaction. A 1.38 mmol sample of 2-chloro-p-NO_2-toluene-d_6 was combined with 5.5 mL of THF and 55.1 mg of a 10% palladium-on-carbon catalyst in a 50-mL round-bottom flask (Petrini et al., 1987). The reaction solution was placed in an ice bath and mixed for 5 min. $NaBH_4$(s), 3.47 mmol, was added to the flask in three portions over 10 min. The ice bath was removed and mixture warmed to ambient; mixing continued for another 15 min. The flask was returned to the ice bath, and 2.3 mL of a 2 N HCl(aq) solution was slowly added to the mixture to decompose any residual $NaBH_4$ present. Diethyl ether, 9.7 mL, was added and mixing continued for another 5 min. The two liquid layers were separated, and the pH of the aqueous layer was adjusted to 8 with 2 N NaOH(aq) and extracted with hexane. The diethyl ether and hexane layers were combined and evaporated to constant volume at room temperature. The residue was dissolved in and chromatographed with a hexane/CH_2Cl_2/IPA (8.5:90.0:1.5) mobile phase. Fractions collected during the 34−60-min retention time window and analyzed by GC/MS contained only the free base product, CPT-d_6.

HCl Salt Formation. All of the CPT-d_6-containing LC fractions were combined, and the mobile phase was evaporated to a volume of 35 mL. The concentrated solution was transferred to a 50-mL tube and evaporated at room temperature to a constant volume. The residual material was dissolved in 5 mL of hexane, and three 7-mL aliquots of a 0.05 N HCl in ethyl acetate solution were added, forming the white precipitate, CPTH-d_6. After each addition, the solution was vortex mixed and centrifuged for 2 min. The final organic

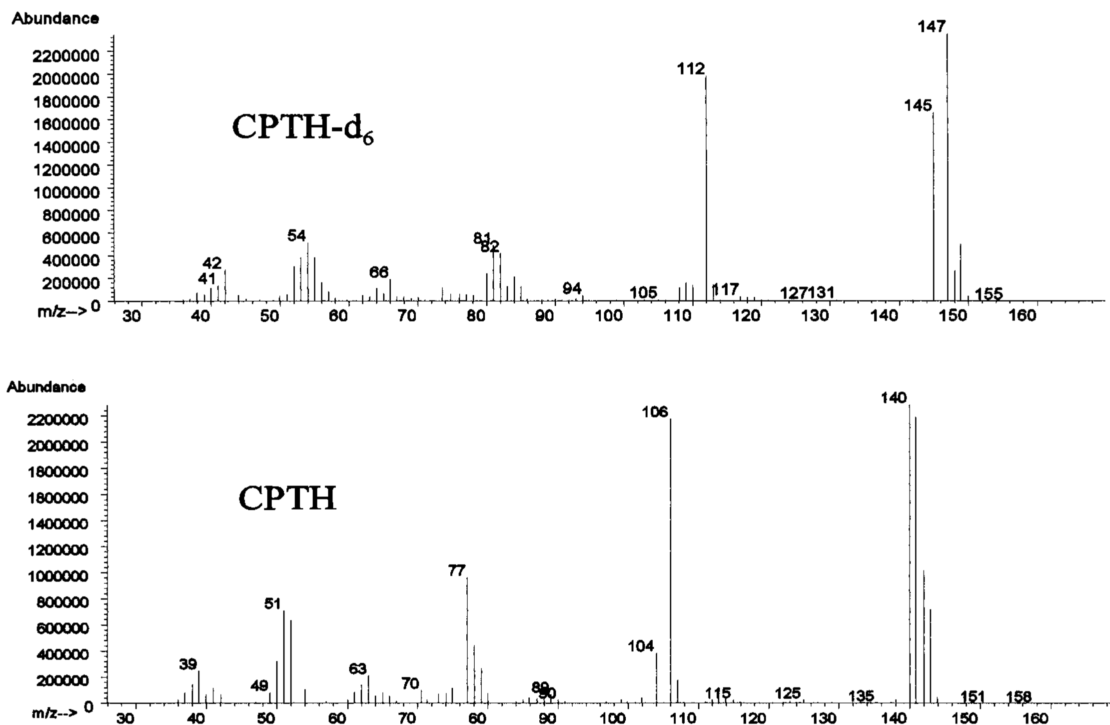

Figure 4. Mass spectra for CPTH-d_6 and CPTH.

layer was removed, and the white precipitate was further washed with two 5-mL volumes of hexane. The CPTH-d_6 precipitate was analyzed by GC/MS and nuclear magnetic resonance (NMR) to confirm the identity and isotopic purity of the final product. A 13.3-mg sample of the CPTH-d_6 material was dissolved in 10 mL of MeOH. A 100-μL aliquot was subsequently diluted to 10 mL with H_2O. A CPTH solution with a known concentration was prepared in the same manner. Aliquots of 10 μL from both solutions were chromatographed with an acetonitrile/H_2O (80:20) mobile phase flowing through a C-8 column (Keystone, 25 cm $\times$ 4.6 mm i.d., 5 μm particle size) at a rate of 1.0 mL/min. UV absorbance was monitored at 241 nm. On the basis of the CPTH area responses and the molecular weight difference between the two compounds, the purity of the CPTH-d_6 material was found to be 97.1%.

Tissue Extractions. To quantify CPTH residues in ground breast muscle and GI tract from pigeon, an extraction procedure was developed using this deuterated material as a surrogate. Appropriate protective equipment was used including gloves, lab coat, and safety glasses.

Glassware and SPE Conditioning. To minimize any binding of CPTH to glassware, glass centrifuge tubes were rinsed with a 7 μg/mL *p*-toluidine in *n*-butyl acetate solution prior to use. Silica (1 g) solid-phase extraction (SPE) columns (International Sorbent Technology) were preconditioned by eluting 2 mL of *n*-butyl acetate followed 2 mL of the *p*-toluidine solution and 2 mL of *n*-butyl acetate through the sorbent bed. The columns were vacuum-dried and rinsed with three 5-mL volumes of hexane. The columns were stored in 2 mL of hexane prior to use.

Standard Preparation. To optimize reproducibility of the GC/MS responses, the analytes were injected into the GC/MS in their free base forms of CPT and CPT-d_6. Intermediate solutions of each compound were prepared by combining an aliquot from a concentrated aqueous solution with an equal volume of 2 N NaOH(aq). The free base analyte was partitioned into and diluted with *n*-butyl acetate. The final CPTH and CPTH-d_6 concentrations of these intermediate solutions were 100 and 50 μg/mL, respectively. Aliquots from these *n*-butyl acetate solutions were used in preparing calibration standards.

Breast Tissue Extraction. A 5-g sample of CPTH-fortified ground breast tissue was placed in a 35-mL Teflon tube equipped with a screw cap. The CPTH-d_6 surrogate material, 5 μg, was added to the tissue and was vortex mixed. Both analytes were allowed to absorb into the tissue for 30 min. A 5-mL aliquot of NaCl(s)-saturated H_2O was added, and the sample was vortex mixed and then sonicated for 5 min. A 10-mL volume of a NaCl(s)-saturated 2 N NaOH(aq) solution was mixed with the sample. After 5 min, 15 mL of hexane was added and the sample was mixed and mechanically shaken (horizontally) for 10 min. After shaking, 200 μL of IPA was added and the sample shaken by hand. The tube was centrifuged for 2 min at 835*g*. The hexane layer was removed and eluted through a preconditioned SPE column by gravity flow. The sample extraction and centrifugation were repeated two additional times using 10 mL of hexane and 100 μL of IPA. After the elution of the last hexane extract, the SPE column was vacuum-dried. Analytes were recovered from the column by eluting 2 mL of *n*-butyl acetate through the SPE cartridge into a prerinsed centrifuge tube. Vacuum was applied to the column to recover any residual *n*-butyl acetate present. The resulting extract in the centrifuge tube was diluted to a final volume of 5.00 mL with *n*-butyl acetate, which was then analyzed for both the surrogate, CPTH-d_6, and CPTH by GC/MS.

GI Tract Extraction. The same extraction process used for ground breast muscle was used for ground GI tract with the following modifications: (1) 2 g of ground tissue was extracted; (2) 2 μg of CPTH-d_6 was combined with the tissue sample; (3) 5 mL of a NaCl(s)-saturated 2 N NaOH(aq) solution was used; and (4) the final volume of the *n*-butyl acetate solution was 2.00 mL.

RESULTS AND DISCUSSION

Spectral Data. Figure 4 contains the mass spectra of CPTH and CPTH-d_6. The analyte concentration for each solution was 100 μg/mL. The spectral patterns indicate the two analytes can be distinguished from each other without the concern of mass fragment overlap between the two compounds. Extracted ion chromatograms for CPTH were generated by summing abundance data for the three most abundant mass fragments (*m*/*z* 106, 140, and 141). CPTH-d_6 chromatograms were

produced by summing abundance data for mass fragments (m/z) 112, 147, and 149.

The structures of both compounds (Figure 1) were determined by combining proton, carbon-13, and deuterium NMR spectroscopy using a Bruker spectrometer.

CPTH: ^1H (300 MHz, D_2O, 4.70 ppm) δ 2.24 (s, 3, CH_3), 7.11 (dd, 1, J = 2.3, 8.2 Hz, H-6), 7.30 (d, 1, J = 8.2 Hz, H-5), 7.33 (d, 1, J = 2.3 Hz, H-2); ^{13}C (75 MHz, D_2O) δ 21.6 (CH_3), 124.1 (C-6), 126.2 (C-2), 134.9 (C-5), 131.3, 137.6, 140.4 (C-1, C-3, C-4). Absolute ^{13}C assignments were made by HETCOR.

CPTH-d_6: ^{2}H (76.75 MHz, H_2O, 4.80 ppm) δ 2.28 (s, 3, CD_3), 7.21 (s, 1, D-6), 7.41 (bs, 2, D-2, D-5).

The synthetic scheme produced CPTH-d_6 with sufficient chemical and isotopic purity to be used as a surrogate for the quantification of incurred CPTH residues in pigeon GI tract and breast muscle.

Validation of Extraction Method. The extraction method was validated by evaluating (1) area ratios (CPTH/CPTH-d_6) from the MS detector response for CPTH concentrations from 0.025 to 2.00 μg/mL and CPTH-d_6 at 1.00 μg/mL versus CPTH concentration, (2) matrix interferences, (3) method limit of detection (MLOD) using tissue samples fortified with 0.050 μg/g CPTH and 1.00 μg/g CPTH-d_6 and baseline responses from the extraction of nonfortified tissues, (4) recovery and repeatability at three different CPTH fortification levels of 2.00, 0.500, 0.100 μg/g. The CPTH-d_6 concentration in these tissue samples was 1.00 μg/g.

Composite samples of each tissue matrix were prepared by blending dissected tissues from 10 pigeons not exposed to CPTH. All statistical analyses, including linear regressions and t tests, were done using SAS (version 6.11, Cary, NC).

Response Linearity. Prior to assessment of analyte and surrogate-containing calibration solutions, a set of CPTH-d_6 solutions ranging from 0.100 to 2.00 μg/mL were injected and evaluated. Regression analysis indicated a linear relation (r^2 = 0.995, p < 0.0001) between analyte response and CPTH-d_6 concentration. Furthermore, the y-intercept was not found to be significantly different from zero ($p_{[\text{intercept}=0]}$ = 0.844), indicating a directly proportional relation between analyte response and CPTH-d_6 concentration. Evaluation of a 2.00 μg/mL CPTH-d_6 solution did not produce any chromatographic response in the corresponding CPTH chromatogram. These data indicate that the GC/MS response of the CPTH-d_6 did not contribute to the response of CPTH, and surrogate responses were found to be linear for recoveries as low as 10%. Therefore, using a 1 μg/mL CPTH-d_6 concentration in standard and extract solutions was acceptable.

After the CPTH-d_6 evaluation, calibration solutions containing 1.00 μg/mL of the surrogate along with CPTH concentrations ranging from 0.025 to 2.00 μg/mL were prepared and analyzed. Regression analysis indicated a linear relation (r^2 = 0.999, p < 0.0001) between area ratio (CPTH/CPTH-d_6) and CPTH concentration. In addition, the y-intercept was not found to be significantly different from zero ($p_{[\text{intercept}=0]}$ = 0.234), indicating a directly proportional relation between area ratio and CPTH concentration. Injection of a 2.00 μg/mL CPTH solution did not produce any response in the corresponding CPTH-d_6 chromatogram.

Matrix Interference and MLOD Determination. The MLOD calculations were based on the mass of CPTH that would generate a chromatographic response equal to 3 times the peak to peak baseline noise at the analyte retention time in the control chromatograms. Five ground tissue samples from each matrix were extracted to evaluate potential chromatographic interferences. An additional five samples from each matrix were fortified with 0.050 μg of CPTH/g and 1.00 μg of CPTH-d_6/g and extracted.

The extraction of nonfortified tissue samples indicated no chromatographic response with the same retention time as CPTH. Chromatograms of nonfortified tissues and the low-level fortified tissues were used to determine MLODs. For each tissue matrix, the MLOD was calculated to be 0.030 μg/g.

Recovery and Repeatability. Five ground samples from each matrix were fortified at each of three CPTH levels; 2.00, 0.500, and 0.100 μg/g. The CPTH-d_6 content in all of these samples was 1.00 μg/g. After fortification, the samples were vortex mixed. After 30 min, the samples were extracted and analyzed as previously described.

Table 1. Recovery and Repeatability Results for the Determination of CPTH Residues in Fortified Breast Muscle and GI Tract

CPTH level (μg/g)	compd	breast mean	breast s	breast RSD	GI tract mean	GI tract s	GI tract RSD
(A) Recovery Data (%) Using Surrogate							
2.00		101	1.6	1.7	99.3	2.0	2.0
0.500		98.3	3.6	3.7	99.4	3.7	3.7
0.100		95.0	5.6	5.9	97.8	5.2	5.3
(B) Absolute Recovery Data (%)							
2.00	CPTH	79.9	3.8	4.7	65.1	2.6	3.9
	CPTH-d_6	79.8	4.6	5.8	66.3	2.6	4.0
0.500	CPTH	64.8	4.2	6.5	56.4	1.9	3.3
	CPTH-d_6	65.0	3.2	4.9	56.0	2.6	4.6
0.100	CPTH	68.2	10	15	92.4	9.1	9.9
	CPTH-d_6	68.7	7.9	12	90.9	8.2	9.0

Using calibration data that included CPTH-d_6 responses, the surrogate-corrected CPTH recoveries ranged from 95.0 to 101% (Table 1A). Using calibration data that excluded the use of the surrogate data, the mean CPTH recovery values ranged from 56.4 to 92.4% (Table 1B). In addition, the recovery variability using the surrogate-corrected values ranged from 1.7 to 5.9%, whereas the nonsurrogate data exhibited variabilities ranging from 3.3 to 15%.

Further evaluation of the absolute recovery data in Table 1B showed no statistical difference between the mean recovery values for both CPTH and CPTH-d_6 from fortified breast and GI samples. For example, a t test performed on the recovery data from tissues samples fortified at 0.500 μg of CPTH/g of tissue and 1.00 μg of CPTH-d_6/g of tissue levels resulted in p values of 0.9152 and 0.7834 for breast tissue and GI tract, respectively. This provides additional evidence that the behavior of the surrogate mimics that of the analyte in both matrices.

The synthetic reactions produced a CPTH-d_6 material that was well suited as a surrogate in the development of an extraction method for the analysis of CPTH residues in pigeon tissues. Regression analysis indicated a linear and directly proportional relation between instrument response (CPTH area/CPTH-d_6 area) and CPTH concentration. The MLOD of 30 ppb for both tissue matrices offered acceptable sensitivity for residue determination. Observed recoveries based on external standard solution responses indicated similar extraction efficiencies for CPTH and CPTH-d_6 from both the breast

muscle and GI tract samples. As surrogate-corrected recoveries accurately reflected CPTH fortification levels, this approach would generate highly precise and accurate quantification data for incurred CPTH residues in pigeons. Furthermore, this method permitted the analysis of 24 tissue samples in an 8-h day. The methodology is well suited for the support of registration studies or monitoring wildlife damage management control efforts. This analytical method should be applicable to the quantitation of CPTH in other relevant matrices such as soil, plants, and other animal tissues. CPTH-d_6 may also be a suitable surrogate for analysis of CPTH residues by HPLC/MS.

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