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Simultaneous determination of fluoroquinolones and tetracyclines in chicken muscle using HPLC with fluorescence detection☆

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Abstract

A multiresidue method has been developed which allows for the simultaneous determination of both fluoroquinolones and tetracyclines in chicken muscle. Samples were extracted with a mix of acetonitrile and 0.1 M citrate, 150 mM $MgCl_2$, pH 5.0. After centrifugation and evaporation, the extracts could be analyzed by liquid chromatography with fluorescence detection. Good recoveries (63–95%) were obtained from samples fortified with a mix of five fluoroquinolones and three tetracyclines, with satisfactory relative standard deviations. Limits of detection were 0.5 ng/g (danofloxacin), 1 ng/g (oxytetracycline, ciprofloxacin, enrofloxacin), 1.5 ng/g (tetracycline), 2 ng/g (difloxacin) and 5 ng/g (sarafloxacin, chlortetracycline). Enrofloxacin and its metabolite ciprofloxacin, as well as oxytetracycline were determined in enrofloxacin and oxytetracycline incurred chicken muscle using this method.

Keywords: Fluoroquinolone; tetracycline; antibiotics

1. Introduction

Veterinary drugs, when used in food animals, have the potential to generate drug residues in the animals and animal products. The U.S. FDA and other regulatory agencies around the world set tolerances or maximum residue levels to ensure residues are not present in excess of the set tolerance levels and that no unapproved drugs are used. Efficient methods are needed for determining these residue levels in food. A large number of available methods have been developed for single drug residues. Multiresidue methods have recently been developed for a number of residue classes in animal tissues, such as the fluoroquinolones [1,2], tetracyclines [3,4], β-lactams [5,6], β-agonists [7,8] and macrolides [9,10], among others, with this approach representing a considerable improvement in efficiency of analysis. Ultimately, methods which allow for the simultaneous determination of more than one class of drug residues will be needed as well. To date, this approach has primarily been demonstrated with microbial or immunochemical screening assays [11,12], or with mass spectrometry [13,14]. Simple screening assays generally do not allow for differentiation between members of a class due to cross reactivity, and mass spectrometry involves the use of complex, expensive instrumentation. The goal of this work was to develop a method which would allow determination of two classes of residues simultaneously using the relatively simple and inexpensive approach of liquid chromatography with fluorescence detection. Application of this method in regulatory monitoring in the U.S., for example, would then only require the use of an additional confirmatory method (e.g. mass spectrometry) in the relatively rare event that violative levels of an analyte appeared to be present.

The tetracyclines (TCs) and fluoroquinolones (FQs) were the two classes chosen for this study. U.S. tolerances and E.U. maximum residue levels for these classes in poultry muscle are listed in Table 1. Members of both these classes are either approved [tetracycline (TC), oxytetracycline (OTC) and chlortetracycline (CTC)] or had been approved [sarafloxacin (SAR) and enrofloxacin (ENRO)] by the U.S. FDA for use in chicken. SAR has now been withdrawn from the market and ENRO has been recently disapproved for use in chicken due to concerns about

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Table 1
Tolerances and maximum residue levels for selected tetracyclines and fluoroquinolones in poultry muscle

Class	Analyte	U.S. tolerance[a] (μg/g)	E.U. maximum residue level[b] (ng/g)
Tetracyclines	Tetracycline Oxytetracycline Chlortetracycline	2 (sum of TC, OTC and CTC)	100 (parent + 4-epimer)
Fluoroquinolones	Danofloxacin	None	200
	Difloxacin		300
	Enrofloxacin		100
	Sarafloxacin		10 (fat) 100 (liver)

[a] http://www.access.gpo.gov/nara/cfr/waisidx_05/21cfr556_05.html.
[b] http://pharmacos.eudra.org/F2/mrl/conspdf/MRL%20consol%202003-07-22%20EN.pdf.

microbial resistance [15], however it is still important to monitor for the possible presence of these and related residues. Both TCs and FQs display fluorescence properties under appropriate conditions. Fluorescence has been used routinely for the detection of FQs [16,17], and occasionally for detection of TCs, generally after post-column treatment [18,19]. In this work, a method is developed which takes advantage of liquid chromatography and the sensitivity of fluorescence for effective detection of three TCs and five FQs simultaneously in chicken muscle.

2. Experimental

2.1. Materials

Ciprofloxacin (CIP) and enrofloxacin (ENRO, 99.9%) were obtained from Bayer (Kansas City, MO, USA), danofloxacin (DANO) was obtained from Pfizer (Groton, CT, USA), sarafloxacin hydrochloride (SAR·HCl, 88.5%) and difloxacin hydrochloride (DIF·HCl, 89.0%) were obtained from Abbott (North Chicago, IL, USA), and tetracycline (TC, 95%), oxytetracycline hydrochloride (OTC·HCl, 95%) and chlortetracycline hydrochloride (CTC·HCl, 83%) were obtained from Sigma (St. Louis, MO, USA). Citric acid monohydrate was from Mallinckrodt (Paris, KY, USA), ammonium hydroxide (redistilled) was from GFS Chemicals (Columbus, OH, USA), and malonic acid and magnesium chloride hexahydrate (99.0%) were from Sigma. Methanol was obtained from J.T. Baker (Phillipsburg, NJ, USA) and acetonitrile was obtained from Burdick & Jackson (Muskegon, MI, USA). Deionized water prepared with a Barnstead (Dubuque, IA, USA) E-pure system was used to prepare all aqueous solutions. All solutions prepared for liquid chromatography were filtered through a 0.45 μm filter before use. Control (antibiotic free) chicken breast muscle was obtained from Bell and Evans (Fredericksburg, PA, USA), cut into small pieces, and ground to homogeneity using a Robot coupe (Ridgeland MS, USA) food processor. This material was then kept at −80 °C until use.

2.2. Standard solutions

Stock solutions (100 μg/mL) in 0.03 M sodium hydroxide were prepared for each of the five fluoroquinolones. These solutions were stored at 4 °C and prepared fresh every 6 months. Stock solutions (200 μg/mL) of OTC and CTC were prepared in methanol and TC was prepared in acetonitrile. These solutions were stored at 4 °C and prepared fresh monthly. A fortification solution containing each of the five FQs (2 μg/mL) was prepared in 0.1 M phosphate buffer, pH 9. This solution was stored at 4 °C and prepared fresh monthly. A fortification solution containing each of the three TCs (2 μg/mL) in 0.1 M malonate, 50 mM $MgCl_2$, pH 6.5, was prepared fresh daily.

2.3. Sample preparation

Homogenized chicken muscle samples (1.0 g) were placed in 50 mL centrifuge tubes and either a portion of TC and FQ fortification solutions (fortified samples) or 0.1 M malonate, 50 mM $MgCl_2$, pH 6.5 (control or incurred samples) was added. The samples were then homogenized (Ultra-Turrax T-25, Janke and Kunkel, Cincinnati, OH, USA) with acetonitrile (1.5 mL) and 0.1 M citrate, 150 mM $MgCl_2$, pH 5.0 (1.5 mL) and kept on ice. The samples were centrifuged (5 min, 2791 × *g*) and the supernatants decanted to a 20 mm × 150 mm glass culture tube. The pellets were re-extracted as above and the supernatants combined and evaporated under nitrogen at 40 °C with a TurboVap LV evaporator (Zymark, Hopkinton, MA, USA). The residues were resuspended in 0.1 M malonate, 50 mM $MgCl_2$ pH 6.5 (2.0 mL), vortex mixed, and filtered through a 30 mm, 0.2 μm nylon syringe filter into an autosampler vial for analysis.

2.4. Liquid chromatography-fluorescence

Liquid chromatography was accomplished using a Hewlett–Packard (Wilmington, DE, USA) 1100 Series quaternary LC pump, with on-line degasser, autosampler and column heater. Chemstation software controlled the LC components and processed fluorescence data from a Jasco (Easton, MD, USA) FP-1520 fluorescence detector via a 35900E interface.

A ZORBAX Eclipse XDB-Phenyl chromatography column (3.0 mm × 150 mm, 3.5 μ, Agilent, Palo Alto, CA, USA) was used, with an in-line 2-μm filter and a Phenomenex (Torrence, CA, USA) Security Guard column (C_{18}, 2.0 mm i.d. cartridge). Solvent A (0.1 M malonate, 50 mM Mg^{+2}, adjusted to pH 6.5 with concentrated NH_4OH) and solvent B (methanol) were combined in a gradient as follows: 16% B (6 min), 16–40% B (4 min), 40% B (8 min), 40–80% B (3 min), 80% B (2 min), 80–16% B (3 min), 16%B (4 min). The flow rate was 0.5 mL/min, and the

column heater was set at 30 °C. Fluorescence of TCs used λ_{ex} 375 nm and λ_{em} 535 nm, while FQs used λ_{ex} 275 nm and λ_{ex} 425 nm. A program on the fluorescence detector changed wavelengths between the TC and FQ conditions as analytes eluted from the column. Analyte retention times were checked daily and the fluorescence detector program modified, if needed, for any small shifts in analyte retention, so that optimum wavelength switching could be maintained.

The liquid chromatograhy column was washed after each day's run and stored in 50/50 acetonitrile/water. The solvent A channel for the pump and degasser was flushed daily with water.

2.5. Quantitation

Normal quantitation was achieved by measuring fluorescence peak heights and comparing with calibration curves prepared with standards in 0.1 M malonate, 50 mM $MgCl_2$, pH 6.5. Peak heights were monitored, rather than peak areas, due to occasional abrupt baseline changes accompanying wavelength shifts by the fluorescence detector. For quantitation using matrix matching, calibration curve standards were prepared in control chicken muscle extract and fluorescence peak heights were measured for all analytes except DIF with matrix matching, for which peak area was monitored.

2.6. Incurred chicken tissue

Broiler chickens were obtained from a local hatchery and housed at the University of Arkansas Poultry Farm. All birds had *ad libitum* access to a standard nonmedicated broiler diet and water. The chickens were randomly divided into two treatment groups and starting at week 4 of age, were dosed for 3 days in the drinking water, with either 50 μg/mL enrofloxacin (Baytril® Bayer) or 800 mg/gal (211 mg/L) oxytetracycline dihydrate (Sigma–Aldrich). Medicated water was prepared fresh daily. Breast samples were collected from two to three birds from each group at each sample point during the 3 day dosing period and during the 3 days post-drug withdrawal period. Due to mortality, only 1 bird was collected for ENRO for the withdrawal day 3 sample. All samples were stored at −80 °C until shipped overnight on dry ice to the USDA facilities in Pennsylvania. These samples were then homogenized in the same way as the control tissue and maintained at −80 °C. An initial extraction and analysis was performed for each incurred sample to determine the approximate ENRO or OTC concentrations. The incurred samples were then diluted, as needed, by homogenizing with control chicken muscle in a food processor, in order to produce a sample within the desired concentration range for analysis (5–150 ng/g). These diluted samples were then stored at −80 °C prior to analysis.

3. Results and discussion

3.1. Chromatography-fluorescence

Chromatographic conditions were required which would allow both FQs and TCs to fluoresce well, preferably without need for a post-column treatment. TCs do not fluoresce significantly at pH 3–4, but they do fluoresce in base, particularly in the presence of divalent ions. Unfortunately, FQs do not fluoresce strongly at pH 10, as they do at pH 3–4. We chose to begin with the general approach of Iwaki et al. [20], who had developed a method for TCs using pH 6.5 acetate in the presence of calcium chloride and EDTA. In our study, malonate (0.1 M) and citrate (0.1 M) were tested as potentially more effective buffers at this pH than acetate. Malonate appeared to provide better TC fluorescence than citrate and was selected for further experiments. The presence of a divalent cation in the mobile phase was determined to be a requirement for significant TC fluorescence. In fluorescence experiments, magnesium ions were found more effective than calcium ions and EDTA was found to decrease TC fluorescence response. Increasing magnesium ion concentration above 50 mM to either 75 or 100 mM did not dramatically improve fluorescence, so the optimum aqueous portion of the mobile phase was selected as 0.1 M malonate, 50 mM magnesium chloride, pH 6.5.

The next step was to optimize the chromatographic separation of the FQs and TCs. Several different columns were tried, including a Luna C-8, Discovery RP amide C-16, Zorbax Bonus RP, Kromasil 100-5Phenyl, Pursuit Diphenyl, and Eclipse XDB-phenyl, with either acetonitrile or methanol used in a gradient elution with the malonate–magnesium buffer. The best separation for the five FQs and three TCs tested was achieved with the Eclipse XDB-phenyl column. Fig. 1 shows chromatograms for control chicken, fortified chicken, ENRO-incurred chicken and OTC-incurred chicken muscle extracts.

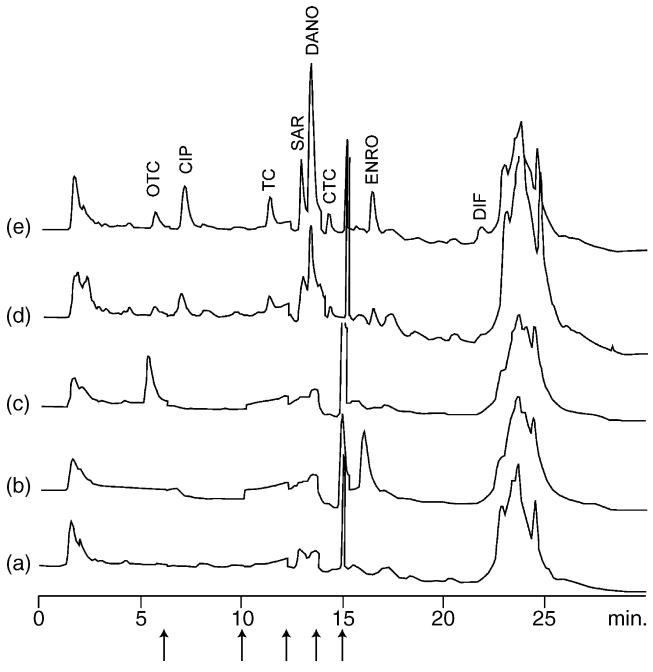

Fig. 1. Liquid chromatograms of (a) control chicken muscle extract, (b) ENRO-incurred chicken muscle extract, dosing day 1, (c) OTC-incurred chicken muscle extract, dosing day 1, (d) 5 ng/g matrix matched calibration curve standard of CIP, SAR, DANO, ENRO, DIF, OTC, TC and CTC (×2), (e) 50 ng/g CIP, SAR, DANO, ENRO, DIF, OTC, TC, CTC-fortified chicken muscle extract. Arrows indicate times of wavelength changes in fluorescence detector program.

3.2. Sample extraction

Extraction of chicken muscle samples with an aqueous solution, such as 0.1 M malonate, 50 mM $MgCl_2$, pH 6.5, produced very turbid extracts, presumably due to slow but continual protein precipitation. In an effort to eliminate this difficulty, samples were kept chilled in ice and extracted with a mixture of buffer and either methanol or acetonitrile. This approach produced clear extracts. The ratio of aqueous:organic solvent for the extraction was varied between 2:1, 1:1 and 1:5, and a 1:1 ratio was found to produce the best recoveries, with acetonitrile giving the higher recovery for the organic component.

For the buffer component of the extraction medium, 0.1 M malonate, 50 mM $MgCl_2$, pH 6.5, 0.1 M citrate, 50 mM $MgCl_2$, pH 5.0 and McIlvaine buffer were tested with either TCs, FQs, or both. Citrate produced higher recoveries from the chicken muscle. Buffer pH was varied between 4.0, 4.5, and 5.0 (citrate buffer) and 5.0, 5.75, and 6.5 (malonate). The pH 5.0 citrate buffer produced the best recoveries. Finally, the magnesium ion concentration was varied between 50 and 200 mM. This showed little effect on recovery of TCs, but a significant effect on FQs, with improvement becoming somewhat less after 150 mM. Thus, the optimum buffer for the extraction was chosen as 0.1 M citrate, 150 mM $MgCl_2$, pH 5.0.

Introduction of a defatting step by extraction of the preliminary extract with ether, hexane, or ether/hexane (1:1) was tested. Ether alone significantly reduced the recovery of DIF. Hexane or a 1:1 mix of ether:hexane provided acceptable recoveries, but did not dramatically decrease background.

The effect of repetitive extractions of the same sample was studied. Recoveries improved significantly from one to two extractions, but increased less for the 3rd repetition.

3.3. Recovery of FQs and TCs from fortified samples

Samples of control chicken muscle were fortified with a mix of the five FQs and three TCs at levels of 20, 50, and 100 ng/g, and then extracted and analyzed. These experiments were conducted with and without matrix matching (calibration standards prepared in chicken muscle extract), and the resultant data is presented in Table 2. Five replicate samples were used for each level on each day. Each fortification level was carried out on 3 separate days to determine inter-day variation.

Good recoveries were calculated for all analytes at 50 and 100 ng/g fortification levels, as well as for five of the eight analytes at 20 ng/g. SAR and CTC each provided one instance of low calculated recoveries at the 20 ng/g level, which was remedied by matrix matching. DIF consistently provided low calculated recoveries at 20 ng/g. Matrix matching provided improved accuracy, which resulted in improved recovery values (56.1–85.6%), but intra-day variations were still high (11.3–20.8% R.S.D.). As the last analyte to elute from the column, the peak for DIF was

Table 2
Recoveries of FQs and TCs from fortified samples

		Recovery (%R.S.D.)							
Fortification level	Day	CIP	SAR	DANO	ENRO	DIF	OTC	TC	CTC
20 ng/g	1[a]	83.3 (2.7)	80.5 (2.6)	85.1 (1.4)	73.0 (2.5)	18.9 (73.7)	71.8 (4.3)	76.4 (1.1)	23.8 (15.2)
	2[a]	90.3 (1.4)	89.3 (1.0)	84.2 (8.2)	77.1 (1.4)	46.6 (32.6)	77.2 (2.0)	81.7 (2.2)	62.5 (2.8)
	3[a]	86.9 (1.8)	46.7 (7.2)	93.3 (2.0)	76.5 (3.1)	41.3 (46.8)	75.0 (2.6)	78.2 (3.0)	61.2 (2.6)
	Ave 1–3[b]	86.8 (3.9)	72.1 (26.5)	87.5 (6.6)	75.5 (3.3)	35.6 (55.0)	74.7 (4.2)	78.8 (3.5)	49.2 (38.1)
20 ng/g matrix matched	1[a]	75.5 (2.6)	74.1 (2.8)	74.0 (1.4)	71.4 (2.2)	68.6 (4.0)	66.9 (4.7)	72.5 (2.8)	73.6 (6.4)
	2[a]	79.2 (1.0)	79.4 (0.8)	78.4 (0.9)	81.0 (0.9)	87.4 (1.8)	74.2 (1.7)	74.9 (1.3)	65.1 (3.5)
	3[a]	79.2 (2.8)	85.7 (3.4)	79.1 (2.8)	80.9 (2.3)	84.1 (1.2)	71.2 (2.6)	77.5 (2.1)	65.3 (1.9)
	Ave 1–3[b]	77.9 (3.1)	79.7 (6.6)	77.2 (3.5)	77.8 (6.3)	80.0 (10.8)	70.8 (5.3)	75.0 (3.4)	68.0 (7.4)
50 ng/g	1[a]	87.4 (1.5)	94.2 (1.4)	94.6 (1.4)	89.4 (1.2)	69.4 (2.8)	78.2 (2.0)	80.8 (1.2)	71.2 (2.9)
	2[a]	82.7 (0.7)	87.1 (0.7)	86.6 (0.8)	79.7 (2.2)	66.8 (1.6)	75.0 (1.6)	77.8 (1.4)	65.8 (2.1)
	3[a]	86.2 (2.1)	87.8 (0.8)	91.1 (0.9)	87.9 (2.0)	77.4 (2.7)	78.8 (2.3)	82.8 (2.1)	71.4 (2.0)
	Ave 1–3[b]	85.4 (2.8)	89.7 (3.8)	90.8 (3.9)	85.7 (5.4)	71.2 (7.0)	77.3 (2.9)	80.5 (3.0)	69.5 (4.5)
50 ng/g matrix matched	1[a]	79.4 (1.8)	80.9 (1.1)	80.5 (1.4)	81.9 (1.8)	85.3 (4.2)	74.2 (2.1)	77.3 (1.6)	69.0 (2.1)
	2[a]	79.5 (0.5)	80.3 (0.8)	80.6 (0.6)	83.3 (0.6)	78.6 (3.1)	72.2 (2.1)	75.7 (2.1)	65.0 (1.3)
	3[a]	81.1 (1.8)	84.4 (1.1)	83.4 (0.9)	86.2 (1.6)	81.1 (2.1)	75.1 (1.4)	79.2 (2.2)	71.1 (1.7)
	Ave 1–3[b]	80.0 (1.7)	81.9 (2.4)	81.5 (2.0)	83.8 (2.5)	81.7 (4.6)	73.8 (2.4)	77.4 (2.7)	68.4 (4.2)
100 ng/g	1[a]	83.4 (1.6)	85.5 (2.0)	85.8 (1.8)	84.4 (2.4)	75.4 (2.6)	75.7 (2.0)	77.2 (2.7)	67.8 (1.6)
	2[a]	84.5 (1.2)	87.4 (2.4)	88.6 (1.9)	85.8 (1.6)	76.7 (3.1)	76.5 (1.2)	79.5 (1.9)	72.9 (1.0)
	3[a]	87.0 (1.2)	87.6 (1.9)	90.5 (1.9)	84.9 (1.7)	66.0 (3.7)	77.8 (2.4)	80.2 (1.5)	62.9 (2.0)
	Ave 1–3[b]	85.0 (2.2)	86.8 (2.2)	88.3 (2.8)	85.0 (1.9)	72.7 (7.4)	76.6 (2.1)	79.0 (2.6)	67.9 (6.4)
100 ng/g matrix matched	1[a]	81.5 (1.6)	82.8 (2.1)	81.4 (1.9)	84.4 (1.9)	84.2 (5.5)	73.6 (2.1)	77.1 (2.6)	66.5 (2.8)
	2[a]	81.4 (1.6)	84.7 (2.2)	83.4 (1.7)	85.1 (1.1)	81.4 (1.4)	74.4 (1.1)	79.1 (1.4)	71.6 (1.0)
	3[a]	77.9 (1.4)	79.3 (2.4)	79.9 (2.2)	82.8 (2.3)	88.8 (4.0)	73.4 (1.4)	76.4 (1.6)	65.7 (2.8)
	Ave 1–3[b]	80.3 (2.6)	82.3 (3.5)	81.5(2.5)	84.1 (2.1)	84.8 (5.3)	73.8 (1.6)	77.5 (2.4)	67.9 (4.5)

[a] $n = 5$.
[b] $n = 15$.

Table 3
FQ and TC levels from incurred chicken tissue[a]

Analyte incurred	Day	Measured ENRO (ng/g) (R.S.D.)	Measured CIP (ng/g) (R.S.D.)	Measured OTC (ng/g) (R.S.D.)	Dilution required	Corrected ENRO (ng/g)	Corrected CIP (ng/g)	Corrected OTC (ng/g)
ENRO	Dose day 1	99.7 (0.8)	8.0 (4.2)	–	1:20	1990	160	–
	Dose day 3	128 (5.4)	4.3 (8.8)	–	1:20	2560	86.2	–
	Withdrawal day 1	106 (2.8)	det[b]	–	1:4	423	det[b]	–
	Withdrawal day 3	44.5 (1.6)	3.7 (10.0)	–	–	44.5	3.7	–
OTC	Dose day 1	–	–	135 (3.3)	1:4	–	–	539
	Dose day 3	–	–	141 (3.0)	1:4	–	–	563
	Withdrawal day 1	–	–	100 (4.5)	–	–	–	100
	Withdrawal day 2	–	–	108 (3.3)	–	–	–	108

[a] $n = 5$.
[b] det = >LOD, <LOQ.

broad and on an upslope. Measurement of peak area for DIF, along with matrix matching, gave the improved results shown in Table 2. Matrix matched data for DIF at 50 and 100 ng/g fortification levels, generated in order to determine its necessity, also used peak area to provide consistency.

As can be seen from Table 2, matrix matching was not necessary for the 50 and 100 ng/g fortification levels as it provided no dramatic improvement in the determined recoveries. As matrix matching requires more analyst time and effort, based on our results, it would not be recommended for quantitation of any of the analytes at levels >20 ng/g, for quantitation of CIP, DANO, ENRO, OTC, and TC at 20 ng/g, or for screening of any of the analytes at concentrations ≥20 ng/g. It would be recommended to use matrix matching if low level quantitation of SAR, CTC, or DIF was required. With exception of the three cases discussed above (SAR, CTC and DIF at 20 ng/g, without matrix matching), calculated recoveries of the analytes in this work were quite satisfactory, ranging from 63 to 95%.

3.4. Linearity and limits of detection

Linearity of the analysis was demonstrated over the concentration range of 5–150 ng/mL. This is equivalent to 10–300 ng/g muscle, as muscle extracts (from 1.0 g tissue) were taken up in 2.0 mL prior to analysis. Five to six point calibration curves were run daily from 5 to 100 ng/mL for fortified samples, and 5 to 150 ng/mL for incurred samples, and R^2 values were ≥0.999 for calibration curve samples prepared in buffer as well as those prepared in control chicken muscle extract (matrix matched).

The limits of detection (LODs) were obtained for all analytes by taking three times the standard deviation (over 7 days) of the peak height (or peak area for DIF) of the lowest matrix matched calibration standard for each analyte (5 ng/g), dividing by the slope of the calibration curve, and multiplying by 2 to correct for dilution of muscle extracts. The following LODs were obtained: 0.5 ng/g (DANO), 1 ng/g (OTC, CIP, ENRO), 1.5 ng/g (TC), 2 ng/g (DIF), 5 ng/g (SAR, CTC). The lower limit of quantitation (LLOQ) is 5 ng/g for DANO, OTC, CIP, ENRO and TC, and 10 ng/g for DIF, SAR and CTC. The ability of the method to accurately quantitate at low levels is reflected in the low R.S.D.s associated with inter-day values ($n = 6$) for calibration standards prepared in matrix or in buffer (<20% R.S.D. for all analytes except DIF at 5 ng/g, and <20% for DIF at 10 ng/g).

3.5. Inter- and Intra-day variation

Relative standard deviation values (R.S.D.s) for inter- and intra-day variation are provided in Table 2. With the exception of DIF and one instance each for SAR and CTC at 20 ng/g without matrix matching, all values are quite good. Considering matrix matched data for 20 ng/g and both matrix matched and non-matrix matched data at 50 and 100 ng/g, intra-day R.S.D.s range from 0.5 to 6.4%, and inter-day R.S.D.s range from 0.7 to 12.5%.

3.6. Determination of ENRO and OTC in incurred samples

The method was used to determine ENRO and OTC in incurred chicken muscle samples. The analysis was performed with and without matrix matching, although the latter was not required, as per the above discussion regarding fortified samples. The results from the two approaches were comparable, and the non-matrix matched data are shown in Table 3. Some of the samples required dilution prior to analysis and the "corrected" values in the last three columns represent the actual analyte concentration found in the samples, after correction for dilution. The results for ENRO displayed a very similar pattern to what had been obtained previously using another method [21]. The ENRO metabolite CIP was detected as well. For the OTC samples, the levels were found to be moderate during dosing and decreased to a lower level after withdrawal of the drug, as would be expected.

4. Conclusions

This method allows for an efficient, simultaneous, multiresidue determination of both FQs and TCs in chicken muscle samples and takes advantage of the fluorescence properties of both analyte classes. Good recoveries were obtained from fortified samples, with low R.S.D.s and low limits of detection. ENRO- and OTC-incurred chicken muscle samples were also successfully analyzed using this method.

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