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OXYTETRACYCLINE RESIDUE ANALYSIS IN POULTRY SERUM BY UHPLC

Chinnu, M.V.[1*], Manu George, C.[2] and Ramnath, V.[3]

[1]Department of Veterinary Biochemistry, [2,3]Central Instruments Laboratory, College of Veterinary & Animal Sciences, Mannuthy, Kerala - 680651
**Corresponding author: chinnu@kvasu.ac.in*

ABSTRACT

Antibiotics are widely used as therapeutic, prophylactic and as growth promoting agents. The indiscriminate use of antibiotics will result in accumulation of these antibiotics/their metabolites in various tissues like muscle, liver and kidney. Consumption of meat of treated birds during withdrawal period would result in health hazards like antibiotic resistance in the consumers. The present study was carried out to develop a simple and sensitive method for the determination of oxytetracycline (OTC) residue in serum of poultry. The assay was performed on twelve broiler chicks which were maintained on a standard diet for one month. On 31st day, long acting OTC was injected @10mg/kg body weight intramuscularly (three times at three day interval) for the treatment group (6 birds) and rest of the birds were kept as control. Blood samples were collected on the first day and 7th day after the last treatment. Serum was separated and Ultra High Performance Liquid Chromatography (UHPLC) was performed by isocratic elution, with a flow-rate of 1.0 ml/min, and UV detection at 360 nm. A noticeable amount of OTC residue could be detected in all the samples on first day while on 7th day only two samples out of six showed antibiotic residue. The method is simple, rapid and very sensitive for the determination of OTC residue in serum samples.

Keywords: Antibiotics, OTC, UHPLC, Serum, Poultry

INTRODUCTION

Antibiotics are therapeutic agents used in the treatment and prevention of bacterial infections (Black, 1977). They act by blocking vital processes in bacteria, thereby killing the bacteria or halting their multiplication. Tetracycline group of antibiotics are commonly used in veterinary medicine to treat various diseases, among which oxytetracycline (OTC) is the most

commonly used. Uncontrolled use of these drugs not only for treatment but also for prophylaxis, results in accumulation of these antibiotics/their metabolites in various tissues like muscle, liver and kidney (Alhendi *et al.,* 2000 and Abdel-Mohsein *et al.,* 2015). Consumption of meat of treated animals during withdrawal period would result in various health hazards like antibiotic resistance, allergic reactions and toxicity in the consumers (Czeizel and Rockenbauer, 2000; Salama *et al*., 2011). The acceptable Maximum Residue Limit (MRL) for OTC as recommended by the joint FAO/WHO Expert Committee on Food Additives (2002) is 0.2 mg/kg in muscle (cattle, pig, sheep and poultry), 0.6 mg/kg in liver (cattle, pig, sheep and poultry), 1.2 mg/kg in kidney (cattle, pig, sheep and poultry) and 0.4 mg/kg in poultry egg (Walker and Ayres, 1958).

Poultry industry is a rapidly growing sector of animal husbandry. For economic farming, different types of antibiotics are often used as therapeutic, prophylactic and growth promoting agents which could remain in various tissues of birds if slaughtered before withdrawal period ends. Different analytical techniques like microbiological evaluation, mass spectrometry (MS) and high performance liquid chromatography (HPLC) (Black, 1977; De Ruyck *et al.,* 1999) are routinely applied for the determination of antibiotic residues. Separation of oxytetracycline residues isolated from biological matrices has been performed on a variety of HPLC columns, especially reversed- phase column type C18, using different mobile phases by isocratic and gradient elution.

The objective of the present study was to develop a simple and sensitive method for the determination of OTC residue in the serum of poultry.

MATERIALS AND METHODS

The assay was performed on twelve broiler chicks which were maintained on a standard diet for one month. On 31st day, long acting OTC was injected as per the instruction on the labeled bottle, @10mg/kg bodyweight intramuscularly (three times at three day interval) for the treatment group (6 birds) and rest of the birds were kept as control (6 birds). Blood samples were collected on the first day and 7th day after the last treatment. Serum was separated, filtered through 0.45µm syringe filter and used for the analysis.

Chemicals and reagent

Standard oxytetracycline dihydrate was obtained from Himedia. HPLC grade methanol, acetonitrile and oxalic acid were obtained from Merck. Oxytetracycline

dehydrate (injection long acting) obtained from Zydus AH. High purity Milli-Q water generated in the laboratory was used for the study. Standard solution was prepared by dissolving 1mg of OTC standard powder in 10ml of mobile phase (100ppm). Stock standard solutions were filtered and kept in amber coloured glass bottles to prevent the photo-degradation and stored at 4ºC and were stable for 2-3 days. Standards (50 ppm) and (20 ppm) were prepared from this stock solution.

Chromatographic conditions

The HPLC system of UltiMate™ 3000 Standard Dual System (Thermo Scientific™) equipped with auto-sampler, quaternary pump, UV Vis detector and a DAD was used in the study. The chromatographic column was a reversed-phased C18 column (Gajda and Posynlak, 2009) (Thermo Scientific, C18, 3µm, 2.1 × 250 mm). The mobile phase used was 0.03 M oxalic acid, methanol, acetonitrile (60:20:20, v/v/v) by isocratic elution. The flow-rate was 1.0 mL/min, and the UV detector was set at 360 nm. The sample volume injected was 1µL and the run time was 10 min (Furusawa, 1999; Gupta *et al*., 2014; Ibrahim *et al*., 2015 and Meredith *et al.,*1965). Quantification was integrated by HPLC software interfaced to the computer.

Statistical analysis

The statistical analysis of OTC residues in samples was performed using SPSS software.

RESULTS AND DISCUSSION

Analysis was carried out by direct injection of the serum sample with the mobile phase comprising of 0.03M oxalic acid, methanol and acetonitrile in the ratio 60:20:20 (Ueno *et al.,*1992). A satisfactory separation was obtained with this mobile phase. For quantification of OTC residue in samples, calibration curve with the standards 20 ppm, 50 ppm and 100 ppm were plotted as shown in Fig. 1. The chromatogram of the control (Fig. 2) had another one peak which did not interfere with the peak in standard or sample.

Figure 3 is the representative chromatogram of the serum samples collected on the first day after injection. The amount of OTC present in the samples were calculated (Tables 1 and 2) using the software Chromeleon (c) Dionex 1996-2006 Version 6.80 SR14 Build 4527 (238909). A noticeable amount of OTC residue could be detected in all the samples on first day (an average of 3.51 ppm). On seventh day post injection, (Fig. 4) traces of OTC residue could be detected only in two samples out of six (an average of 0.13 ppm).

Statistical analysis of the samples collected on the first and the seventh day post injection showed significant difference between control and treatment groups (paired t test, p value < 0.05). A good linearity of the method was observed with a correlation coefficient of 0.999998, in the concentration range of 20 ppm to 100 ppm. LOD and LOQ of this method were 0.0181 ppm and 0.0549 ppm respectively.

The current study was carried out to determine OTC residue in serum of poultry treated with therapeutic dose of the antibiotic by Ultra High Performance Liquid Chromatography (UHPLC). Continuous treatment with antibiotics will results in accumulation of residues in different body parts of animals/poultry. If the correct withdrawal periods are not followed, the antibiotic residue will enter into the food chain and cause serious health hazards like allergic reactions, different kinds of toxicity and antibiotic resistance in the consumers. Withdrawal period is the time required for drug residues to reach a safe concentration in tissues/secretions for human or animal consumption. Alhendi *et al*. (2000) reported five days withdrawal period for OTC and sulphadimidine after oral administration @ 100 mg/kg body mass. De Ruyck *et al.* (1999) recommended a withdrawal period of 14 days for eggs and 5 days for broiler meat after treatment with TC and OTC.

In the present study, serum of all birds in the treatment group on the first day after last injection of OTC, had an average residue level of 3.51 ± 0.32 ppm which is above the MRL. But after seven days the antibiotic residue could be detected only in two birds out of six and the level detected was below the MRL (0.044 ± 0.028ppm). So following the correct withdrawal period would prevent consumers from being exposed to antibiotic residues at concentrations greater than the MRLs. Consuming of antibiotic residue at a concentration below the MRL, will not pose any health hazards to consumers

Table 1. HPLC retention time (RT), area and amount of OTC residue in serum samples after day first of injection

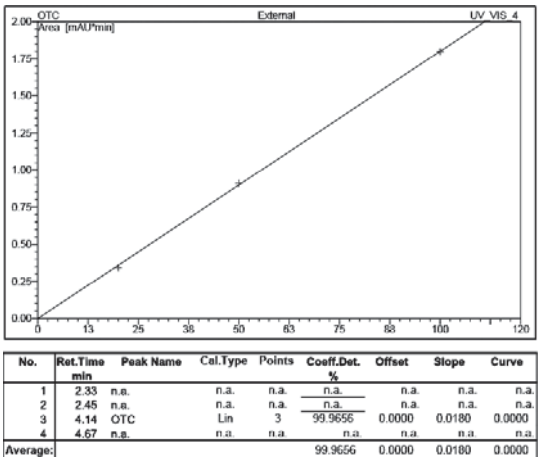
Sl. No.	RT	Area	Amount of OTC (ppm)
1	4.043	0.0833	4.62
2	4.047	0.0574	3.18
3	4.050	0.0495	2.74
4	4.053	0.0579	3.21
5	4.053	0.0790	4.39
6	4.057	0.0527	2.93

Table 2. HPLC retention time (RT), area and amount of OTC residue in serum samples after day first of injection

Sl.No.	RT	Area	Amount of OTC (ppm)
1	4.167	0.0023	0.131
2	4.163	0.0025	0.136
3	4.163	0	0
4	4.163	0	0
5	4.163	0	0
6	4.163	0	0

(Vishnuraj *et al*., 2016). Withdrawal period of different classes of antibiotics in various tissues of different species of animals or poultry is different depending on the dosage, route of administration and the presentation of the drug. Accordingly, withdrawal period varies from a minimum period of 3 days to 28 days and 10 day is considered optimum for most of the drugs. In the case of OTC, the recommended withdrawal period is 7 days for meat and egg (Khatun *et al.,* 2018).

The UHPLC method mentioned in the present study, with the above mentioned column, mobile phase and standard is a rapid and sensitive method for the determination of OTC residue in serum samples.



No.	Ret.Time min	Peak Name	Cal.Type	Points	Coeff.Det. %	Offset	Slope	Curve
1	2.33	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	2.45	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	4.14	OTC	Lin	3	99.9656	0.0000	0.0180	0.0000
4	4.67	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Average:					99.9656	0.0000	0.0180	0.0000

Fig. 1. Calibration curve using standards

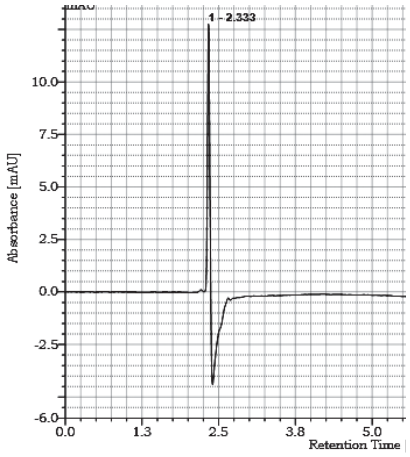


Fig. 2. Chromatogram of control serum

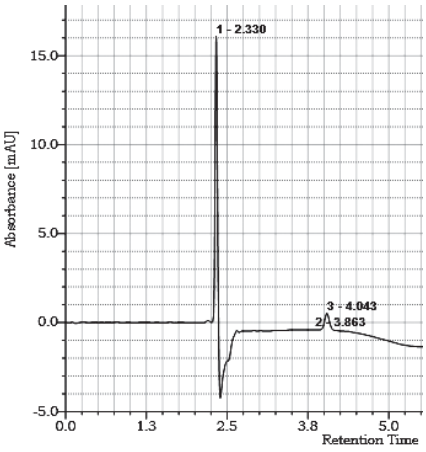


Fig. 3. Chromatogram – Day 1 post injection

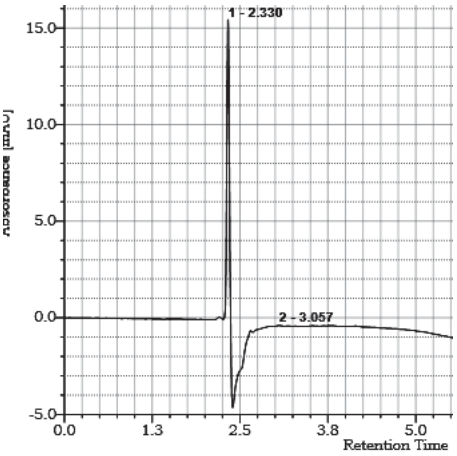


Fig. 4. Chromatogram – Day 7 post injection

SUMMARY

The present study reports the sensitive UHPLC protocol for detection of OTC residues in serum samples. The study also points out the need to adhere to antibiotic withdrawal period to circumvent issues of antibiotic residues in foods of animal origin.

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