UHPLC Analysis of OTC Residue Present In Meat of Poultry Treated With Therapeutic Dose of The Antibiotic Orally And Intramuscularly – A Comparison

Manu George C.[1], M.V. Chinnu[2], Susmitha Rajan[3]
[1, 2] Dept of Veterinary Biochemistry
[3]Central Instruments Laboratory
[1, 2, 3] College of Veterinary & Animal Sciences,Mannuthy, Kerala - 680651

Abstract- *Antibiotics are widely used as therapeutic, prophylactic and growth promoting agents. The indiscriminate uses of antibiotics will results 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 to comparethe amount of OTC residue in meat of poultry treated with therapeutic dose of the antibiotic orally and intramuscularly by ultra high performance/pressure liquid chromatography. The assay was performed on eighteen numbers of broiler chicks which were maintained on a standard diet for one month. After attaining one month old, the chicks were randomly divided into three groups; one control (C) group and two treatment groups (T1 andT2) each consists of six birds. Long acting OTC was injected @10mg/kg bodyweight intramuscularly (three times at three day interval) for the treatment group I (6 birds),given OTC orally for a period of 15 days @ 500mg/2.5kg to the treatment group II (6 birds)and rest of the birds were kept as control (6 birds). Muscle samples were collected on the first day after the last treatment. Samples were processed and Ultra High Performance Liquid Chromatography (UHPLC) was performed with mobile phase consisting of 0.03M oxalic acid, methanol, acetonitrile (60:20:20, v/v/v) by isocratic elution, with a flow-rate of 1.0 ml/min, and UV detection at 360 nm. A noticeable amount of OTC residue was detected in the muscle samples of T1compared to T2. LOD, LOQ & Linearity of the method was found to be 0.0181 ppm, 0.0549 ppm & 0.999 respectively.*

Keywords- OTC, UHPLC, antibioticresidue,muscle

I. INTRODUCTION

Antibiotics are therapeutic agentsused in the treatment and prevention of bacterial infections. They act either by blocking vital processes in bacteria, killing the bacteria, or halting their multiplication. The uncontrolled use of these drugs for treatment or prophylaxis (to prevent the infection) and as growth enhancers,will result in accumulation of these antibiotics or their metabolites in various tissues like muscle, liver and kidney.Poultry industry is a rapidly growing sector of animal husbandry. For economic farming, the stakeholders of poultry industry often use different types of antibiotics as therapeutic, prophylactic and growth promoting agents.Oxytetracycline (OTC) is the most widely used antibiotic in veterinary medicine under tetracycline group. Unless proper withdrawal period is given, the meat having antibiotic residue enters in the food chain leading to various health hazards like allergic reactions, different kinds of toxicity and antibiotic resistance in the consumers.Considering the public health and food safety aspects, it is necessary to identify and quantify the antibiotic residues in different animal derived food products and to make public awareness about the harmful effects of these residues.

As per the recommendation of joint Food and Agriculture Organization(FAO) / World Health Organization(WHO) Expert Committee on Food Additives (2002) the acceptable maximum residue limit (MRL)for OTC in cattle, pig, sheep and poultry is 0.2 ppm, 0.6 ppm and 1.2 ppm in muscle, liver and kidneys respectively. The European Union (EU) recommended MRL is 0.1 ppm and 0.3 ppm in muscle and liver respectively in poultry and the recommendation by US food and drug administration (USFDA- 2011) for poultry is 2 ppm in muscles, 6 ppm in liver and 12 ppm in kidneys.

The present study was to comparethe amount of OTC residue present in meat of poultry treated with therapeutic dose of the antibiotic orally and intramuscularly by ultra high performance/pressure liquid chromatography.

II. MATERIALS AND METHODS

The assay was performed on eighteen broiler chicks which were maintained on a standard diet for one month. On 31st day, long acting OTC was injected @10mg/kg bodyweight intramuscularly (three times at three day interval) for the treatment group I(6 birds),given OTC orally for a period of 15 days @ 500mg/2.5kg to the treatment group II (6 birds)and rest of the birds were kept as control (6 birds). At the end of trial, chicken were sacrificed by electrical stunning followed by neck-cutting. The muscle samples were collected immediately and stored at -20^0 C.

III. CHEMICALS AND REAGENT

Standardoxytetracyclinedihydrate obtained from Himedia. HPLC grade methanol,acetonitrile and Oxalic acid, obtained from Merck. High purity Milli-Q water was used.Oxytrtracycline dehydrate (injection long acting) obtained from Zydus AH.

Weighed 5g of muscle sample in a 50ml capacity polypropylene tube, 3ml of citrate buffer was added and blended in a high speed tissue blender for 1000 rpm for 10-15 minutes. The mixture was vortexed at high speed for 5 minute, and then centrifuged at 3500 rpm for 10 minutes in a cooling centrifuge. The supernatant was transferred to another tube and the extraction was repeated by adding 2ml of citrate buffer. The final supernatant was taken for the clean-up procedure. For clean-up the supernatant was filtered through 0.45µm nylon filter. The 0 final filtrates were used for analysis. The filtrate can be stored at -50 c.

IV. APPARATUS

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. The chromatographic column was a reversed-phased C18column[7](Thermo Scientific, C18, 3µm, 2.1 × 250mm).

Standard solution

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 colouredglassbottles to prevent the photo-degradation and stored at 4^0C and were stable for 2-3 days. 50ppm and 20ppm standards were prepared from this stock solution.

Chromatographic condition

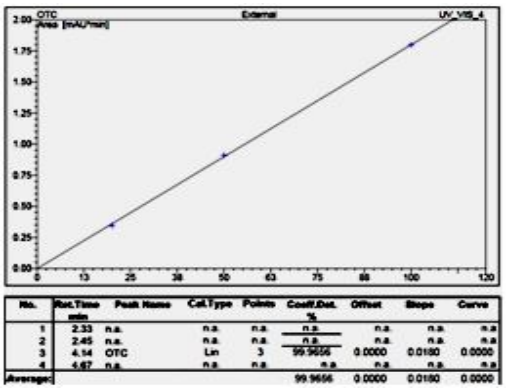
The mobile phase used was 0.03M oxalic acid, methanol, acetonitrile (60:20:20, v/v/v) by isocratic elution. The flow-rate was 1.0 ml/min, and the UV detector was set at 360 nm. The sample volume injected on column was 1µl and the run time was 10 minutes[6-10].

Detection was done using UV detector at 360nm and quantification was integrated by HPLC software interfaced to the computer.

V. RESULT

Analysis was carried out by injection of the extracted sample with the mobile phase [0.03M oxalic acid, methanol, acetonitrile (60:20:20, v/v/v)][18]. A satisfactory separation was obtained with this mobile phase.

For quantification of OTC residue in samples, calibration curve with the standards 20ppm,50ppm and 100ppmwas plotted as shown in (Fig.1).The chromatogram of the control (Fig.2) has only one peak which will not interfere with the sample peak or standard peak.LOD, LOQ & Linearity of the method was found to be 0.0181 ppm, 0.0549 ppm & 0.999 respectively.



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

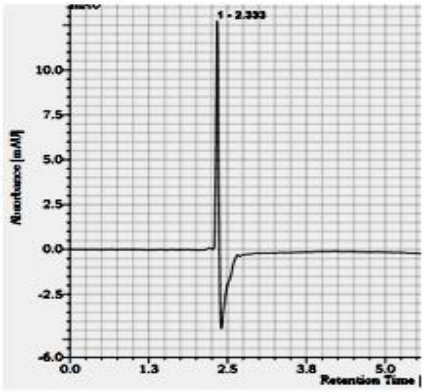


Fig.2

Fig.1 shows calibration curve using standards, Fig.2 chromatogram of control serum

The representative chromatogram of muscle samples from six birds on the first day after injection of OTC are shown in the Fig.3. An average RT of OTC was found to be 4.30 mints. An average peak area of OTC residues present in the sample was 0.290mAU and average peak height was 2.83cm. The average amount of OTC residues present in the sample was found to be 27.69 ppm (Table 1).

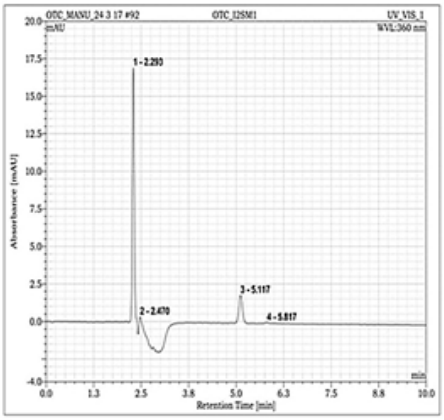


Fig. 3

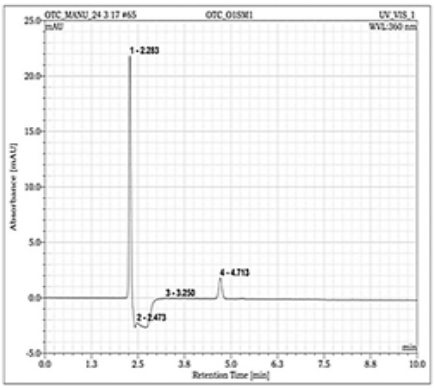


Fig. 4

Fig. 4 shows the representative chromatograms of the muscle samples spiked along with 20ppm of standard OTC day one after oral administration. The average RT was found to be 4.73 mints. The average peak area and peak height was found to be 0.200 mAU and 1.97cm respectively. The average amount of OTC residues present in the muscle samples 0.167 ppm (Table 2).

Table 1

Sl.No.	RT (min)	Height (mAU)	Area (min * mAU)	Amount (ppm)
1	4.28	0.24	0.024	2.32
2	4.29	0.62	0.063	6.04
3	4.30	0.52	0.052	4.94
4	4.31	6.30	0.640	60.92
5	4.32	0.56	0.056	5.36
6	4.33	8.76	0.909	86.59
Avg.	4.30	2.83	0.290	27.69

Table 2

1	2	3	4	5	6	7 (5-6)*
Sl.No.	**RT (min)**	**Height (mAU)**	**Area (min*mAU)**	**Amount of spiked sample (ppm)**	**Amount of 20 ppm std**	**Amount (ppm)**
1	4.71	1.88	0.190	20.66	20.63	0.03
2	4.72	1.99	0.202	20.63	20.63	0
3	4.72	1.96	0.198	20.70	20.63	0.07
4	4.73	1.98	0.201	20.63	20.63	0
5	4.73	2.01	0.204	20.63	20.63	0
6	4.74	2.03	0.206	20.63	20.63	0
Avg.	4.73	1.97	0.200	20.63	20.63	0.0167

VI. DISCUSSION

The current study was carried out to determine OTC residue by Ultra High Performance Liquid Chromatography (UHPLC) in muscle tissue of poultry treated with therapeutic dose of the antibiotic both orally and intramuscularly. Continuous treatment with antibiotics will results in accumulation of residues in different body parts of animals/poultry. If proper withdrawal periods is not given it will enter into the food chain and cause serious health hazards to the consumers1.

Quantitative analysis of OTC residue on day 1 after injection using UHPLC showed residues in all samples of muscle.The mean value of OTC residues presents in the muscle samples were 27.69 ± 14.95 ppm. All this value showed a level above the MRLrecommended by EUand USFDA. Whereas on first day after oral administration, the level of OTC residue present in muscle sample were 0.017 ± 0.012 ppm. All this value showed a level within the MRL recommended by EU and USFDA.

In the present study, first day after injection of the antibiotic OTC, shown that there is residue above the MRL. So if proper withdrawal period is given it will prevent consumers being exposed to antibiotic residues at concentrations greater than the MRLs.If a residue of antibiotic medicine is at a concentration below the MRL1, 20, then it does not pose a threat to consumer health.

VII. ACKNOWLEDGEMENT

The work was carried out at Central Instruments Laboratory and Department of Veterinary biochemistry, College of Veterinary & Animal Sciences, Mannuthy, Kerala. The support and assistance given by all the staff members in the above mentioned departments areduly acknowledged

REFERENCES

[1] Alhendi, A.B., Homeida, A.M. and Gaili, S. 2000.Drug residues in broiler chickens fed with antibiotics in ration. *VeterinarskiArhiv***70(4)**: 199-205

[2] Abdel-Mohsein, H.S., Mahmoud, M.A.M. and Ibrahim, A.H. 2015. Tetracycline residues in intensive broiler farms in Upper Egypt: Hazards and Risks. *J. Wld's. Poult. Res. 5***(3)**:48-58.

[3] Black, W. D. 1977. A study of the pharmacodynamics of oxytetracycline in the chicken. Poult.sci. 56(5): 1430-1434

[4] Czeizel, A. E. and Rockenbauer, M. 2000. A population basrd case control teratologic stud of oral oxytetracycline treatment during pregnancy. European J. Obstetrics &Gynecology and Reproductive Biol. 88: 27-33

[5] De Ruyck, H., De Ridder, H., Renterghem, R.V. and Van Wambeke, F. 1999. Validation of HPLC method of analysis of tetracycline residues in eggs and broiler meat and its application to a feeding trial.*FoodAdditives and Contaminants***. 16(2):** 47-56.

[6] Furusawa, N. 1999. High-performance liquid chromatographic determination/identification of oxytetracycline and sulphadimidine in meat and eggs. *Chromatographia*. ***49*(7):** 369-373.

[7] Gajda, A. and Posynlak, A. 2009. Tetracyclines and their epimers in animal tissues by high-performance liquid chromatography. *Bull Vet InstPulawy*. ***53*(2):** 263-267.

[8] Gupta,V., Ahlawat, S., Patel, B., Shankar, O., Kumar, K., Shukla, S.K., Shukla, S., Navneet, K., Dubey, M. and Veer singh, L. 2014.High-performance liquid chromatography method validation for determination of tetracycline residues in poultry meat. *Chronicles of Young Scientists***5(1)**:72-74

[9] Ibrahim, I.G., Sabiel, Y.A., Thoria, O.O., Khalafalla, A.E. and Safa, O.S. 2015.Microbiological and HP LC assays for detection of tetracycline residues in chicken meat. *Asian J. Sci. and Technol.***6(2)**:1020-1022

[10] Meredith, W.E., Weiser, H.H. and Winter, A.R.1965. Chlortetracycline and oxytetracycline residues in poultry tissues and eggs. *Appl. Microbiol.* **13(1**): 86-88.

[11] Odore, R., De Marco, M., Gasco, L., Rotolo, L., Meucci, V., Palatucci, A.T., Rubino, V., Ruggiero, G., Canello, S., Guidetti, G. and Centenaro, S. 2015. Cytotoxic effects of oxytetracycline residues in the bones of broiler chickens following therapeutic oral administration of a water formulation. *Poult. sci.* **141**.

[12] Salama, N.A., Abou-Raya, S.H., Shalaby, A.R., Emam, W.H. and Mehaya, F.M. 2011. Incidence of tetracycline residues in chicken meat and liver retailed to consumers. *Food Additives and Contaminants: Part B*. ***4*(2):** 88-93.

[13] Senyuva, H., Ozden, T. and Sarica, D.Y. 2000. High-Performance Liquid Chromatographic Determination of Oxytetracycline Residue in Cured Meat Products.Instrumental Analysis Centre. *Scient. and Tech. Res. Council of Turkey* (TUBITAK) **24:**395 -400.

[14] Shalaby, A.R., Salama, N.A., Abou-Raya, S.H., Emam, W.H. and Mehaya, F.M. 2011. Validation of HPLC method for determination of tetracycline residues in chicken meat and liver. *Food Chem.***124(4):**1660-1666.

[15] Shama, S.A., Mahmoud, A.H., Hassan, H.M. and Nassar, S.H. 2016.Validation of modified HPLC method for determination of oxytetracycline, tetracycline and doxycycline in chicken meat and liver.*New York Sci. J.***9(5)**:68-73

[16] Tauber, V., Patruț, E. and Chiurciu, V. 2015. Development and validationof an HPLC method for the determination of oxytetracycline and enrofloxacin in veterinary formulations.*Medicamentul Vet. / Vet. Drug*. **9(2):**65-69

[17] Ueno, R., Uno, K. and Aoki, T., 1992. Determination of oxytetracycline in blood serum by high performance liquid chromatography with direct injection. *J. chromatography***573**:333-335.

[18] Van Wambeke, F. 1999. Validation of HPLC method of analysis of tetracycline residues in eggs and broiler meat and its application to a feeding trial. *Food Additives & Contaminants*. ***16*(2):** 47-56.

[19] Vishnuraj, M. R., Kandeepan, G., Rao, K. H., Chand, S., and Kumbhar, V. 2016. Occurrence, public health hazards and detection methods of antibiotic residues in foods of animal origin: A comprehensive review. Cogent Food & Agric. 2(1): 1235458.

[20] Walker, H.W. and Ayres, J.C. 1958. Antibiotic residuals and microbial resistance in poultry treated with tetracyclines. *J. Food Sci.* **23(5):**525-531

[21] Ziółkowski, H., Grabowski, T., Jasiecka, A., Zuśka-Prot,

M., Barski, D. and Jaroszewski, J. J. 2016. Pharmacokinetics of oxytetracycline in broiler chickens following different routes of administration. *The Vet. J.* **208***:* 96-98.