Evaluation of antibiotic residues in the liver of broiler by four-plate method in Kazerun city

Habibi GH.[1*]

1Department Of Clinical Scienses, Faculty Of Veterinary Medicine, Kazerun Branch, Islamic Azad University, Kazerun, Iran

***Corresponding author:** H. Habibi, Department Of Clinical Scienses, Faculty Of Veterinary Medicine, Kazerun Branch, Islamic Azad University, Kazerun, Iran, *email:habibigh42@yahoo.com*

(Received: July 1, 2018; Accepted: August 29, 2018)

Abstract

Drugs and antibiotics are widely used in birds for the treatment, prevention, and stimulation of growth. In addition to the desired therapeutic effects or even adverse side effects, the use of these drugs can indirectly affect public health.In this study, 100 packets of livers supplied to the market over a period of three months were collected from protein stores at the city level. First, the susceptible and standard strain of Escherichia coli prepared from the Pasteur Institute of Iran was cultivated and antibiogram was used to determine the sensitivity of the strain. Then, suspension 0.5 McFarland was prepared and Müller-Hinton media with acidity of 7.2 were prepared and autoclaved. After surface and uniform culturing of Müller-Hinton plates with cotton swab with Escherichia coli strains, one fragment of each liver was cut and transferred to surface of four media with sterilizing forceps and by keeping the appropriate distance from the edges. Then, it was placed at 37 ℃ for 24 hours. Finally, the samples were examined in terms of the presence or absence of antibiotics. Out of 100 samples examined, 69 samples with negative result (non-antibiotic) and 31 samples with positive result (containing antibiotic) were observed. The highest frequency was related to non-antibiotic samples. Since the drug residue disrupts public health, it is important to contribute to the health of the community by observing the withdrawal time before slaughter and forcing the producers to measure drug residue at the end of the breeding period.

Keywords: *Drug residue, Liver, Broiler, Four-plate method*

Introduction

In recent years, industrial poultry breeding has been grown significantly. Although control of the diseases has been improved by observing the principles of prevention, the use of drug and antibiotics is still common. The use of these drugs in the veterinary industries is used to control diseases and improve their growth. The use of drugs increases the likelihood of drug residue in tissues. Observing the withdrawal time has a major impact on the health of poultry products and the health of consumer. Determining the minimum drug residue is in fact the maximum amount of drug residue which can be found in the product without leaving adverse effect on the consumer and it is considered as an indicator of the quality control of livestock production (Vahedi *et al.*,2011;Ghasem *et al.*,2014).

The drug residue was measured in dairy products simultaneously with the introduction and use of the drugs. This monitoring was initially performed in the milk industry in order to control

[DOR: 20.1001.1.2717378.2018.2.5.5.6]

fermentation barriers, followed by regular and frequent monitoring of the slaughter products since the early 1970s. The early studies were carried out in the Netherlands and Germany. Then, standard method of four-plate method was introduced in European countries with the help of Bacillus subtilis and Micrococcus luteus strains in three media with PH of 6, 7.2, and 8 (Pikkemaat Mariël,2009). Evaluation of the drug residue through microbial growth prohibition was the first diagnostic method and it is now used commonly (Pikkemaat Mariël,2009). The objective of this study was to evaluate the drug residue in the broiler livers supplied to market by using four-plate method.

Materials and methods

Sampling method

In this research, the required samples were prepared from the protein stores of the city. For this purpose, 100 liver packets supplied to market were purchased from different parts of the city within three months. Then, the relevant containers were transferred to the veterinary laboratory under cool conditions.

Preparation of culture medium

First, a strain of Escherichia coli (with code of MCI No. 1101 and ATCC: 25922), which was sensitive to the antibiotics of enrofloxacin, flumequine, fosbac and sultremia, was prepared from the Pasteur Institute of Iran and transferred to the laboratory. This lyophilized bacterium was dissolved with sterile physiological serum and then cultivated at Brain Heart Infusion medium for 24 hours at 37 ° C. After the growth of the bacterium, a suspension at concentration of 0.5 McFarland was prepared. To ensure the sensitivity of the strain of E. coli used, antibiogram discs of the mentioned antibiotics were used as positive samples in each plate.

In preparation of the culture media in the four-plate test, Müller-Hinton Agar plate was used. After setting the medium PH at 7.2, they were autoclaved. After performing the bacteria sensitivity test, surface and uniform culture of Müller-Hinton plates with sterile cotton swab and Escherichia coli strains and suspension 0.5 McFarland was performed. Then, a liver was randomly selected from each packet and cut at dimensions of 8*2 mm and an approximate weight of 0.5 g. Then, one fragment of each liver was cut and transferred to four media with sterilizing forceps and by keeping the appropriate distance from the edges. Then, it was

incubated for 24 hours at 37 ℃ (Ghasem *et al*., 2014; Vahedi *et al*., 2011; Tajik *et al*., 2010; Pikkemaat Mariël, 2009).

Finally, culture media were checked, and if the diameter of the transparent medium around each sample is greater than 2 milliliters, it was considered positive; otherwise, it was considered negative (Ghasem, *et al*., 2014) (Figure 1).

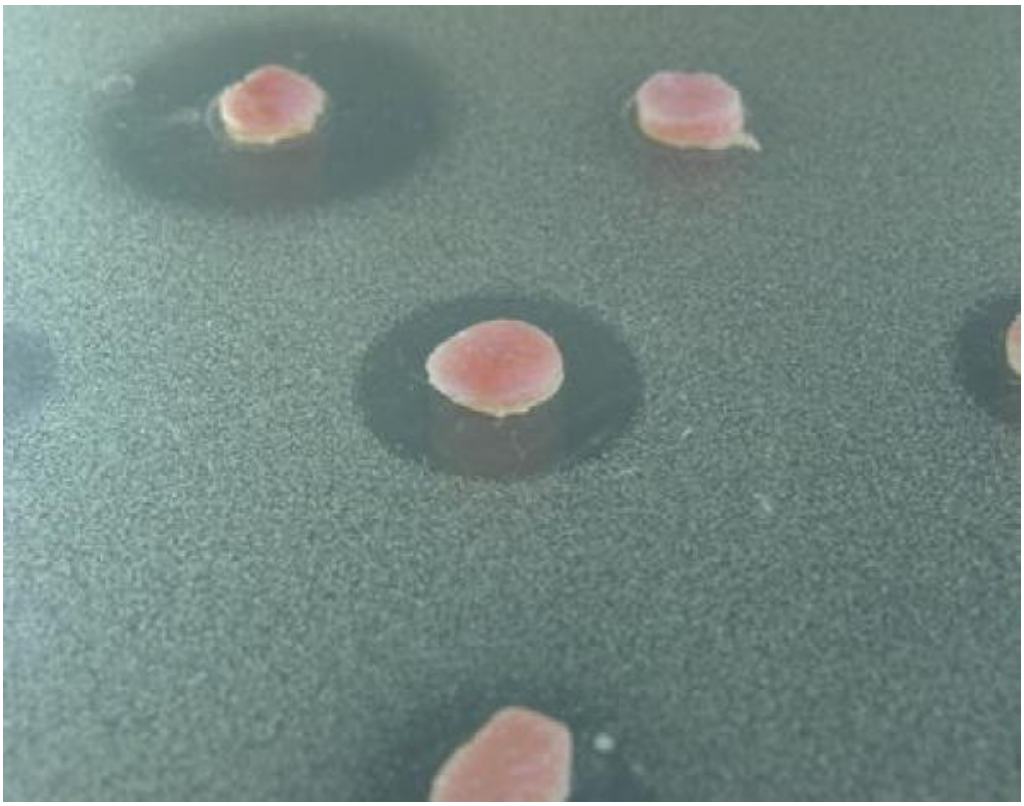

Figure 1.Microbial inhibition zones (Pikkemaat Mariël, 2009)

Results

Out of the 100 samples examined, 69 samples with negative result (non-antibiotic) and 31 samples with positive result (containing antibiotic) were seen. The highest frequency was related to non-antibiotic samples (Figure 2). No significant difference was seen in the results by examining the results of different geographic regions and sampling periods and there was a uniform dispersion in all samples.

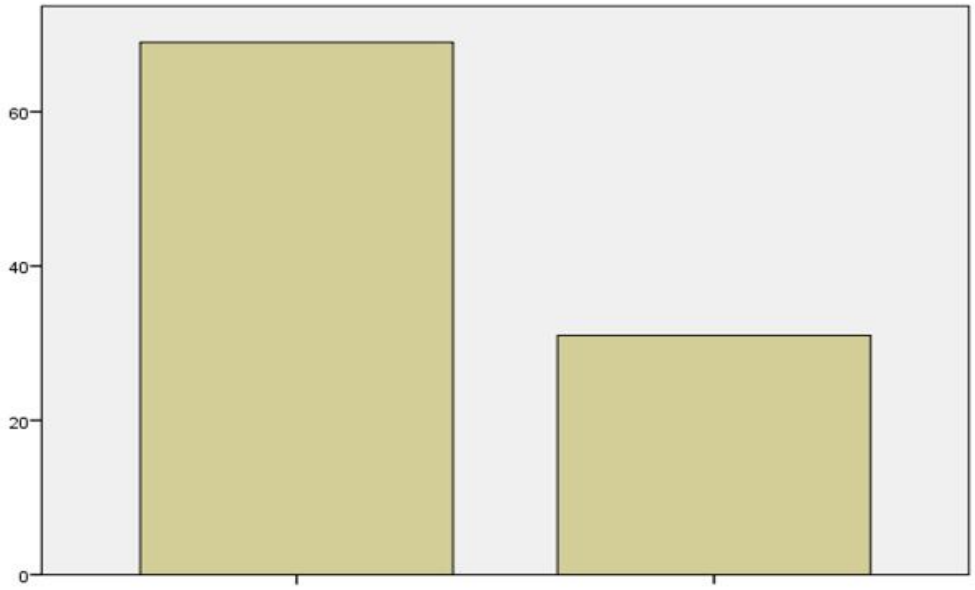

Figure 2 .The percentage of frequency of negative and positive results

Discussion

Drugs are used mainly in poultry breeding as a means of prevention and treatment and stimulation of growth. The presence of these drugs in products causes disorders such as allergies, intestinal microbial flora imbalance, cellular mutation, creation of drug residue, and finally, drug resistance in the consumer (Muaz *et al*., 2018; Munda *et al*., 2017; Ghasem *et al*., 2014; Vahedi *et al*., 2011; Tajick *et al*., 2006). Drug residue searching and determining their values have been investigated by numerous researchers. For this purpose, various tools and methods such as ELISA, microbiology, immunochemistry, chromatography, and Spectrometry have been used. Each of these methods has many advantages and disadvantages (Ghasem *et al*.,2014; Tajik *et al*., 2010). The quality evaluation of the drug residue is mainly performed using the microbiology method. Simplicity, low cost, and measuring multiple samples with single test are advantages of this method (Vahedi *et al*.,2011; Pikkemaat Mariël,2009). However, the type of bacterium and degree of their sensitivity, the medium and their PH and the type of tissue selected for testing change the results. Accordingly, the presence of the drug residue in the samples of this study is predicted to be higher due to the limited sensitivity spectrum of the used bacteria, the limited

[DOR: 20.1001.1.2717378.2018.2.5.5.6]

acidity of the culture medium and its effect, and the extent of tissue diversity and laboratory methods (Pikkemaat Mariël, 2009; Okerman *et al.*,2007; Shim *et al*.,2003). Other diagnostic methods are used to search the quantitative values of drug residue (Ha *et al*., 2016; Chusri *et al*.,2013; Gao *et al*.,2013; Yibar *et al*., 2011; Le *et al*., 2011; San Martin *et al*., 2010; Tajic *et al*.,2010; Reyes-Herrera *et al*., 2008; Reyes-Herrera *et al*., 2005; Zhang *et al*., 2006; Shim *et al*., 2003). The muscles of the chest and thigh, liver and kidney tissues have been commonly used to determine the antibiotic values. However, other uncommon methods such as sampling from blood and feather have been also used (Haag *et al*.,2016; Reyes-Herrera *et al*., 2011).

Ghasemi *et al*. (2014) examined antibiotic residues of 160 muscle and liver samples in broiler in Mashhad using four-plate method and reported that 18.7% of the cases were positive, so that Sulfamide antibiotic values were 22% in muscles and 11.7% in the liver. Tajik *et al*. (2010) examined 160 samples of heart, liver and kidney of broiler chickens using the four-plate method and they observed drug residues in 17.5% of the samples. Al-Mustafa *et al*. (2000) used four-plate method for screening 32 broiler chickens in Saudi Arabia, and they reported that liver and muscle samples of 22 farms were positive. Pikmat mariel (2009) also emphasized the role of PH in the culture medium and the diversity of bacterial strains used in this test and their effect on improving the results. Although this method requires more work and effort, it reduced the costs and accelerated the diagnosis performance due to increasing the accuracy and extent of diagnosis spectrum (Vahedi *et al*.,2011; Pikkemaat Mariël,2009).

Vahedi *et al*. (2011) examined 815 industrial poultry carcasses in Mazandaran slaughterhouses and reported 65.4% of antibiotic residues in the muscles, liver and kidney, either alone or simultaneously. In this study, kidneys had the most positive cases (52.2%), followed by liver (51%) and then muscle (44.5%) . Tajik *et al*. (2006) reported the drug residue was found 50% of fifty poultry carcasses in Mazandaran using chromatography method. Sarker *et al*. (2018) examined 160 carcasses of broiler by using chromatography in Bangladesh and showed that the highest concentration of drug residue, especially ciprofloxacin, was seen in the liver, followed by thigh and chest muscles . Ramatella *et al*. (2017) examined drug residues in 150 poultry carcasses by using Eliza and two chromatographic methods. They reported that ciprofloxacin and streptomycin levels were higher than the normal level. In addition, 3% of the samples showed multiple drugs residuals simultaneously. Slama *et al.* (2011) examined the tetracycline compounds residues in meat and liver of broiler chickens using chromatography method and

[DOR: 20.1001.1.2717378.2018.2.5.5.6]

they reported that 44% of the samples were positive. The highest residue level was observed in the liver, followed by chest and thigh. Moreover, 7% to 13% of the samples had non-allowed residue titer.

Tavakoli *et al*. (2015) examined the drug residue in chicken meats in Tehran with using chromatography. They reported that the level of chloramphenicol was high, but 99% reported the tetracycline and enrofloxacin residues at the allowed level. By examining 250 samples of liver, muscle, heart, gizzard and skin of chickens in Tabriz by using chromatography, Rezaee Moghadam *et al*. (2018) reported enrofloxacin level 52% in liver and 2% in skin. They also reported ciprofloxacin level 30% in the liver and 6 % in skin . Ha *et al*. (2016) examined the enrofloxacin level in chicken meat by Antibody Fluorescent Kit method and reported as 43.4-62.3% .

Nasim *et al*. (2016) tested the enrofloxacin level in liver and muscle of 150 broiler carcasses by using chromatography method. A total of 126 samples contained the drug residue and 84 had a concentration higher than the allowed level . Panzhenhagen *et al*. (2016) evaluated the level of enrofloxacin residues in 200 chicken livers and muscles. The drug residue was reported 72% using ELISA method and 22% using chromatography method . Ebrahimzadeh *et al*. (2014) reported the residues of two drugs of enrofloxacin and chloramphenicol in Tabriz 91% and 31%, respectively, although their values were lower than the allowed level . Olusola *et al.* (2012) reported that the level of tetracycline residue in frozen meat supplied to the market in Nigeria was higher than the allowed level .

Although the use of certain drugs, such as chloramphenicol and nitrofuran, has been prohibited over the recent years in many countries due to their carcinogenicity, their drug residues have been reported in poultry products. Studies have shown that plants have been able to absorb some drugs such as tetracycline from the soil. The production of chloramphenicol in the soil by Streptomyces Venezuela or Actinomyces bacteria in soil causes the drug to be absorbed by the forage and the bushes and the use of the forage and bushes by birds leads to drug residue and its transmission to consumer.

After collecting plant samples from different countries such as Thailand, Mongolia and the Netherlands, they were analyzed by using chromatography method and high levels of drug residues were confirmed in some plants and geographical areas. Weather conditions such as temperature, rainfall and humidity affect the activity of bacteria in the soil (Gao *et al*.,2013;

[DOR: 20.1001.1.2717378.2018.2.5.5.6]

Berendsen *et al.*,2010). The feather powder, which is used as a protein in the poultry and livestock chain, contains significant amount of drug residues, which can be transmitted to other consumers, resulting in increased drug resistance (Haag *et al*.,2016). The addition of the birds' fertilizers to soil in order to enrich it results in the transmission of drugs to plants and consumers and, leading to drug resistance of microorganisms (Sarmah *et al*.,2006).

Conclusion

Generally, the administration of drugs in feeding of the poultry and its hazards to human through the food chain has led to many problems. Providing training for producers on the prohibition of using growth stimulation drugs and mutagen drugs, observing the withdrawal time before slaughter and forcing to measure the drug residue at the end of breeding period are among the effective methods used to reduce these problems.

Acknowledgements

We thereby appreciate Dr. Saeed Salami for his cooperation and diligence in this study.

References

Al-Mustafa ZH. and Al-Ghamdi MS. Use of norfloxacin in poultry production in the eastern province of Saudi Arabia and its possible impact on public health. International Journal of Environmental Healthy Research, 2000;10(4):291-299.

Berendsen B., Stolker L., De Jong G., Nielen M., Tserendor E., Sodnomdarjaa R., Cannavan A., and Elliott C. Evidence of natural occurrence of the banned antibiotic chloramphenicol in herbs and grass. Analytical and Bioanalytic Chemistry, 2010; 397(5):1955–1963.

Chusri M., Wongphanit P., Palaga T., Puthong S., Sooksai S. and Komolpis K. Production and characterization of a monoclonal antibody against enrofloxacin. Journal of Microbiology and Biotechnology, 2013;23(1):69-75.

Ebrahimzadeh Attari V., Mesgari Abbasi M., Abedimanesh N., Ostadrahimi A. and Gorbani A. Investigation of enrofloxacin and chloramphenicol residues in broiler chickens carcasses collected from local markets of tabriz, northwestern iran. Health Promotion Perspectives, 2014 30;4(2):151-157.

Gao F., Zhang QD., Zhang ZH., Yan XD., Zhang HC. and Wang JP. Residue depletion of nifuroxazide in broiler chicken. Journal of Sciences Food and Agriculture, 2013;93(9):2172-2178.

Ghasem F., Fathi B. and Jamshidi A. Detection of antibiotic residues in poultry carcasses in Mashhad poultry abattoir . Iranian Journal of Veterinary Science and Technology, 2014; 6(1):30-36.

Haag G., Marin G., and Errecalde G. Quantification of residual enrofloxacin and ciprofloxacin in feathers of broiler chickens by high-performance liquid chromatography-fluorescence after oral

administration of the drugs . Journal of Advances Pharmacology and Technology Research, 2016; 7(1): 2–5.

Ha MS., Chung MS. and Bae DH. Simple detection of residual enrofloxacin in meat products using microparticles and biochips. Food Additives Contaminants Part A Chemistry, Analysis, Control Expo Risk Assess, 2016;33(5):817-823.

Le T., Yi SH., Zhao ZW. and Wei W. Rapid and sensitive enzyme-linked immunosorbent assay and immunochromatographic assay for the detection of chlortetracycline residues in edible animal tissues. Food Additives Contaminants Part A Chemistry, Analysis, Control Expo Risk Assess, 2011;28(11):1516-1523.

Muaz K., Riaz M., Akhtar S., Park S., and Ismail A. Antibiotic Residues in Chicken Meat: Global Prevalence, Threats, and Decontamination Strategies: A Review Journal of Food Protection, 2018;81(4):619–627

Munda M., Khanb U., Tahir U., Mustafab B. and Fayyazd A. Antimicrobial drug residues in poultry products and implications on public health: A review International Journal Of Food Properties, 2017;20(7)1433–1446

Nasim A., Aslam B., Javed I., Ali A., Muhammad F., Raza A. and Sindhu ZU. Determination of florfenicol residues in broiler meat and liver samples using RP-HPLC with UV-visible detection. Journal of Science Food and Agriculture, 2016;96(4):1284-1288.

Okerman L., Noppe H., Cornet V. and De Zutter L. Microbiological detection of 10 quinolone antibiotic residues and its application to artificially contaminated poultry samples. Food Additives and Contaminants ,2007;24(3):252-257.

Olusola AV., Diana BE. and Ayoade OI. Assessment of tetracycline, lead and cadmium residues in frozen chicken vended in Lagos and Ibadan, Nigeria. Pakistan Journal of Biology Sciences, 2012;15(17):839-844.

Panzenhagen PH., Aguiar WS., Gouvêa R., de Oliveira AM., Barreto F., Pereira VL. and Aquino MH. Investigation of enrofloxacin residues in broiler tissues using ELISA and LC-MS/MS. Food Additives Contaminants Part A Chemistry, Analysis, Control Expo Risk Assess, 2016;33(4):639-643.

Pikkemaat Mariël G. Microbial screening methods for detection of antibiotic residues in slaughter animals . Analytical and Bioanalytical Chemistry, 2009; 395(4): 893–905.

Ramatla T., Ngoma L., Adetunj M.i, and Mwanza M. Evaluation of Antibiotic Residues in Raw Meat Using Different Analytical Methods . Antibiotics (Basel), 2017; 6(4): 34.

Reyes-Herrera I. and Donoghue DJ. Antibiotic residues distribute uniformly in broiler chicken breast muscle tissue. Journal of Food Protection, 2008;71(1):223-225.

Reyes-Herrera I., Schneider MJ., Cole K., Farnell MB., Blore PJ. and Donoghue DJ. Concentrations of antibiotic residues vary between different edible muscle tissues in poultry. Journal of Food Protection, 2005;68(10):2217-2219.

[DOR: 20.1001.1.2717378.2018.2.5.5.6]

Reyes-Herrera I., Schneider MJ., Blore PJ. and Donoghue DJ. The relationship between blood and muscle samples to monitor for residues of the antibiotic enrofloxacin in chickens. Poultry Science, 2011;90(2):481-485.

Rezaee Moghadam N., Arefhosseini SR., Javadi A., Lotfipur F., Ansarin M., Tamizi E. and Nemati M. Determination of Enrofloxacin and Ciprofloxacin Residues in Five Different Kinds of Chicken Tissues by Dispersive Liquid-Liquid Microextraction Coupled with HPLC. Iranian Journal of Pharmacology and Research, 2018;17(4):1182-1190.

Salama NA., Abou-Raya SH., Shalaby AR., Emam WH. and Mehaya FM . Incidence of tetracycline residues in chicken meat and liver retailed to consumers. Food Additive and Contaminant Part B Surveill, 2011;4(2):88-93.

San Martin B., Cornejo J., Lapierre L., Iragüen D., Pérez F., Hidalgo H. and Andre F. Withdrawal time of four pharmaceutical formulations of enrofloxacin in poultry according to different maximum residues limits. Journal of Veterinary Pharmacology Therapeutic, 2010;33(3):246-251.

Sarker Y., Hasan M., Paul T., Rashid S., Alam M. and Sikder M. Screening of antibiotic residues in chicken meat in Bangladesh by thin layer chromatogram phy . Journal of Advanced Veterinary and Animal Research, 2018;5 (2): 140-145.

Sarmah AK., Meyer MT. and Boxal AB. Environmental Assessment of Tetracycline's Residues Detected in Pig Slurry and Poultry Manure. A Global Perspective on the Use, Sales, Exposure Pathways, Occurrence, Fate and Effects of Veterinary Antibiotics (VAs) in the Environment. Chemosphere, 2006, 5: 725-759

Shim JH., Shen JY., Kim MR., Lee CJ. and Kim IS. Determination of the fluoroquinolone enrofloxacin in edible chicken muscle by supercritical fluid extraction and liquid chromatography with fluorescence detection. Journal of Agriculture and Food Chemistry, 2003;51(26):7528-7532.

Tajick M. and Shohreh B. Detection of Antibiotics Residue in Chicken Meat Using TLC International Journal of Poultry Science, 2006, 5 (7): 611-612.

Tajik H., Malekinejad H., Razavi-Rouhani SM., Pajouhi MR., Mahmoudi R. and Haghnazari A. Chloramphenicol residues in chicken liver, kidney and muscle: a comparison among the antibacterial residues monitoring methods of Four Plate Test, ELISA and HPLC. Food and Chemical Toxicology, 2010;48(8-9):2464-2468.

Tavakoli HR., Safaee Firouzabadi MS., Afsharfarnia S., Joneidi Jafari N. and Saadat S. Detecting antibioticresidues by HPLC method in chicken and calves meat in diet of a military center in Tehran . Acta Medica Mediterranea, 2015; 31: 1427

Vahedi N., Motaghedi A. and Golchin M. Determination of antibiotic residues in industrial poultry carcass by means of F.P.T (four –plate – test) method in Mazandaran province Journal of Food Science and Tecnology, 2011; 8(1):65-72(in Persian)

Yibar A., Cetinkaya F. and Soyutemiz GE. ELISA screening and liquid chromatography-tandem mass spectrometry confirmation of chloramphenicol residues in chicken muscle, and the validation of a confirmatory method by liquid chromatography-tandem mass spectrometry. Poultry Science, 2011;90(11):2619-26.

[DOR: 20.1001.1.2717378.2018.2.5.5.6]

Zhang S., Zhou J., Shen J., Ding S. and Li J. Determination of chloramphenicol residue in chicken tissues by immunoaffinity chromatography cleanup and gas chromatography with a microcell electron capture detector. Journal of AOAC Internatinal, 2006;89(2):369-373.

[DOR: 20.1001.1.2717378.2018.2.5.5.6]