Antibiotic residuals in some farmed rainbow trout (*Oncorhynchus mykiss*) of market size in Iran

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Abstract

To assess the hygienic conditions of market rainbow trout (*Oncorhynchus mykiss*), the residuals of three antibiotics consisting of oxytetracycline (OTC), enrofloxacin (EN) and erythromycin (ET) were measured in the muscles of freshly caught fish obtained from 17 trout farming in Charmahal-va-Bakhteyari (CVB) province using high performance liquid chromatography. Totally 266 fish samples were randomly obtained and 798 muscle samples were used for detection of the above antibiotics. The obtained results showed that OTC was detected in one fish farm (5.8%) having a residual of 0.75 to 7.13 µg g^{-1}. EN was detected in 6 fish farms (35%) with a residual of 0.5- 0.73 µg g^{-1}. Also, the lowest and highest residuals of ET were 23.38 and 181.38 µg g^{-1}, respectively in 5 fish farms (29.4%). The obtained results showed that the residual of these antibiotics in trout muscles of some fish farms were higher than the acceptable levels and therefore, requires a serious attention of both the environment and the consumer health care. Also, the detection limit of 0.05 µg g^{-1} used for these antibiotics shows that application of high performance liquid chromatography method used here in this study is a useful tool for a routine screening of these antibiotics in trout farming.

Key words: antibiotic residual, trout, erythromycin, enrofloxacin, oxytetracyline.

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Introduction

Increasing demands upon human societies for animal protein, especially with the origin of aquatic-source have led to the development of aquaculture sector worldwide (Sapkota, Sapkota, Kucharski, Burke, McKenzie, Walker & Lawrence 2008). Evidences show that the uncontrolled use of chemicals such as antibiotics can cause serious problems in the environment as well as humans as the main consumers of aquaculture productions.This adverse effect is more noticeable in the immunocompromised people such as diabetics and infants. Use of food containing antibiotic residuals can also cause allergies, cancers, birth defects and drug resistance to the diseases in humans. The residuals of antibiotics in food products can also cause development of drug resistancein potential pathogenic bacteria in the digestive tracts of animals, including human and fish (Hernández-Serrano 2005). Evencreating drug resistance in non-pathogenic bacteria can led to more drug-resistant causing a development of such resistant genes in to pathogenic bacteriain humans and other animals (FDA. 2009b; Miranda, Tello & Keen 2013).

The widespread use of antibiotics in the aquaculture industry for treatment of bacterial diseases caused by *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Pasteurella pisicida*, *Edwardsiella ictaluri*, *Vibrio angulillarum*, *Yersinia ruckeri*, *Streptococcus iniae*, *Lactococcus garviee* and *Renibacterium salmoninarum* has raised a serious concern for the consumers (Cabello 2006; Park, Hwang, Hong & Kwon 2012). Since some bacterial diseases including streptococcosis/lactococcosis and yersiniosis have been dramatically increased in farmed rain-

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bow trout (*Oncorhynchus mykiss*) in Iran (Soltani, Jamshidi & Sharifpour 2005), the use of some antibiotics have been remarkably increased by the trout farmers recently. This increase in the chemotherapy has caused a serious publich health concern. Therefore, the aim of this study was to assess the residuals of 3 commonly used antibiotics in rainbow trout of market size from 17 farmed trout in Charmahal-va-Bakhteyari (CVB) province the leading state of Iran in trout production in freshwater.

Materials and Methods

A number of 266 carcasses of rainbow trout weighing 300-400 g were randomly sampled from the trout suppliers. The samples were originally obtained from 17 trout farms (15-20 samples each farm) in CVB province. The main target markets of these fish suppliers are Isfahan and Tehran cities. At the time of sampling some historical details including name and location of trout farmers were obtained to follow the previous antibiotic treatment at the culturing stage. Therefore, some historical details of theses trout farms including previous history of streptococcosis/lactococcosis (the common disease in the region) and antibiotic used are show-in in Table 1. Fish samples were transported to the laboratory on ice. About 15 g of lateral muscles of each fish sample was aseptically obtained and immediately transferred to -196°C until used within 3 weeks post-sampling.

Extraction of fish muscle samplesfor OTC

Muscle samples were first homogenized and a 5±0.01 g was then mixed in a sterile polypropylene container containing 15 mL of sterile phosphate buffered solution (PBS) (0.02 mol, pH = 2.25) plus 50% (W/V)aceticacid (Merck) prepared in pure distilled water. The homogenizing process was repeated three times with homogenizer each time 30 s prior to centrifugation at 3000 rpm for 20 min. The supernatants were collected in to a sterile container. The extraction procedure was repeated for the rest of muscle sample. The final supernatant was passed through the cartridge activated with passing 4 mL of methanol (Merck) and 4 mL of buffer. The

Table 1 Farmed trout from which the trout samples were obtained plus their history of chemotherapy used in this study;OTC= oxytetracycline, EN= enrofloxacin, ET=erythromycin, FP= florphenicol

No of fish farm	Name of fish farm	No of fish samples (g)	History of previous antibiotic therapy.
1	CH_ME	20 (350)	ET, FP
2	CH_MD	20(300)	EN, FP
3	CH_AB	15(400)	ET, FP,
4	CHTN	15(300)	EN, FP, ET
5	CHAN	15(380)	ET
6	S1DB	16 (350)	EN
7	SH_ZI	15 (400)	EN, FP
8	SH_DA	15(340)	EN, FP
9	S2_SHB	15(400)	EN, FP
10	S2_HE	15(350)	EN, FP
11	G_FE	15(400)	FP
12	SA_TSH	15(350)	ET
13	SAMH	15(350)	EN
14	SA_AR	15(300)	-
15	SA_MF	15(100)	OTC
16	SA_YA	15(200)	-
17	GM_KAJ	15(300)	-

OTC was washed out from the cartridge using 7 mL methanol and driedup by nitrogen at 40°C. The samples were then filtered using Milipor filter and were maintained at -20°C. Positive control samples were included in using trout muscle samples containing pure OTC (Sigma) at known concentration. The OTC content was then measured using HPLC system (Salte & Liestøl 1983; Esposito, Fabrizi, Lucchetti, Marvasi, Coni & Guandalini 2007).

Extraction of fish muscle samples for EN

Muscle samples were first homogenized and a 5±0.01 g was then mixed in a polypropylene container containing 1.5 mL of sterile PBS (0.02, mol, pH=9.1). After15 min, a volume of 5 mL acetonenitrile (Merck) was added, mixed well and the sample was then placed in the ultrasonic as an energy source. The homogenizing samples were centrifuged at 3000 rpm for 10 min. The supernatants were then collected in to a sterile container and the organic phase was evaporated by nitrogen at 40°C. The extraction procedure was repeated for the rest

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Table 2 Residuals of ET and EN (µg g^{-1}) in rainbow trout muscle samples, ND = not detectable

Number of trout farm										
1	3	4	4	5	6	7	8	9	10	12
ET	ET	ET	EN	ET	EN	EN	EN	EN	EN	ET
103.45	25.31	132.29	0.29	80.40	0.08	0.09	0.62	0.09	0.07	125.17
93.53	23.34	123.46	0.30	66.42	0.07	0.06	0.55	0.09	0.08	118.14
99.28	23.85	152.22	ND	59.23	0.09	ND	0.59	0.06	0.06	135.78
99.56	24.22	111.30	0.20	68.70	0.08	0.08	0.65	0.08	0.05	ND
132.56	22.86	134.55	0.23	63.88	0.09	ND	0.80	0.06	0.05	123.65
97.54	ND	100.55	0.30	52.89	0.09	0.07	0.55	0.08	0.05	103.43
91.67	35.23	121.26	0.27	67.25	0.08	0.07	0.50	0.05	0.05	100.10
102.42	22.10	114.69	0.27	76.32	0.09	0.06	0.53	0.05	0.05	151.39
93.45	28.90	121.88	0.22	64.53	0.09	ND	0.74	ND	0.05	122.7
89.28	22.72	102.30	0.22	71.90	0.09	0.07	0.60	ND	ND	ND
68.68	22.55	130.26	0.22	ND	0.09	0.07	0.53	ND	0.05	ND
89.53	23.70	124.90	0.22	82.25	0.09	ND	0.53	ND	0.09	ND
95.83	28.42	126.53	0.24	64.22	0.08	ND	0.55	ND	ND	ND
97.69	23.99	134.25	0.21	89.33	0.07	ND	0.69	ND	ND	ND
100.30	ND	ND	ND	ND	ND	ND	0.67	ND	ND	ND
60.83	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

of muscle sample. The final supernatant was passed through the cartridge activated with passing 4 mL of methanol and 4 mL of PBS EN was washed out from the cartridge using 5 mL methanol containing 2% hydrochloric acid (Merck) and dried up by nitrogen at 40°C.The samples were then filtered using Milipor filter and were maintained at -20°C. Negative control and positive control samples were considered using trout muscle samples containing pure EN (Sigma) at known concentration was included. The EN content was then measured using HPLC system (Ramirez, Mottaleb, Brooks & Chambliss 2007).

Extraction of fish muscle samples for ET

Muscle samples were first homogenized and a 5±0.01 g was then mixed in a polypropylene container containing 15 mL acetonitrile prior to shaking for 10 min. The samples were then ultrasonicated and centrifuged at 3000 rpm for 10 min to separate the organic phase in another container. A volume of 3 mL hexane (Merck) was then added before shaking for 1 min. The acetonitrile phase was removed and a volume of 20 mL methylene chloride (Merck) plus 2 mL sodium hydroxide (1 M) (Merck) and 20 mL PBS (1%) was added. The mixture was shaked for 10 min prior to 2 gsodium chloride (Merck) added. The methylene chloride phase was isolated by rotary evaporationat 37°C. The samples were then washed out with a minimum volume of acetonitrile and filtered using Milipor filter before kept at -20°C until subjecting to HPLC system. Positive control samples were considered using trout muscle samples containing pure ET (Sigma) (Lucchetti, Fabrizi, Esposito, Guandalini, Di Pasquale & Coni 2005). According to the ratio of signal to noise (5/1), the maximum amount for each OTC and EN was calculated at 50 ng per one g of tissue, and the recovery of the method was then calculated to be 74.4% for both test and standard samples. In all experiments, initially the calibration and standardization of methods were performed prior to examine the test samples.

Results

A calibration curve in trout muscle free of the antibiotics is shown in Fig. 1-3. Minimum detectable for all three antibiotics was 0.05 µg g^{-1} trout muscle.The residuals of OTC were detectablein range 0.07-7.13 µg g^{-1} in 6 muscle samples in one

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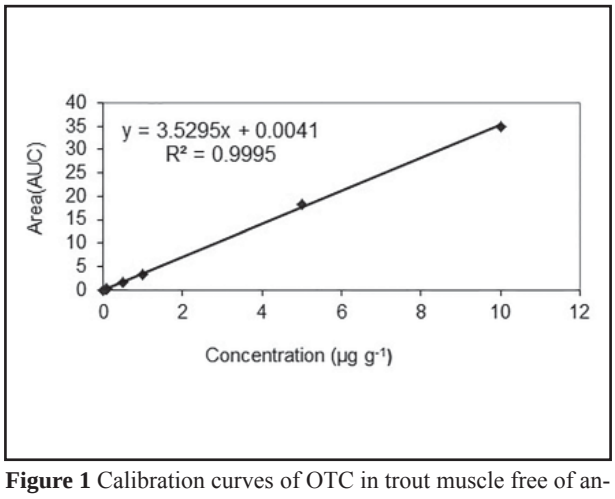

Figure 1 Calibration curves of OTC in trout muscle free of antibiotics.

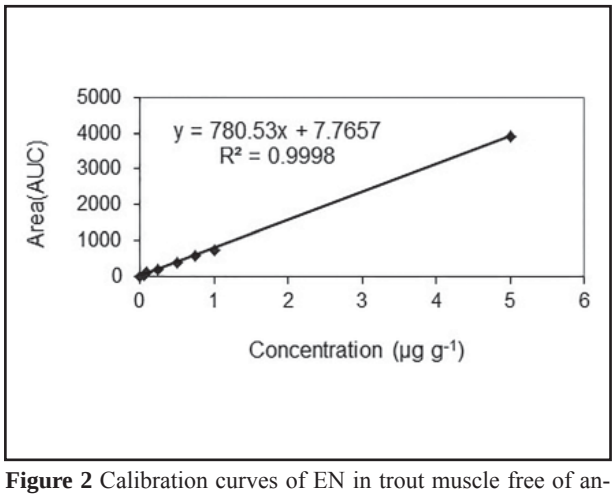

Figure 2 Calibration curves of EN in trout muscle free of antibiotics.

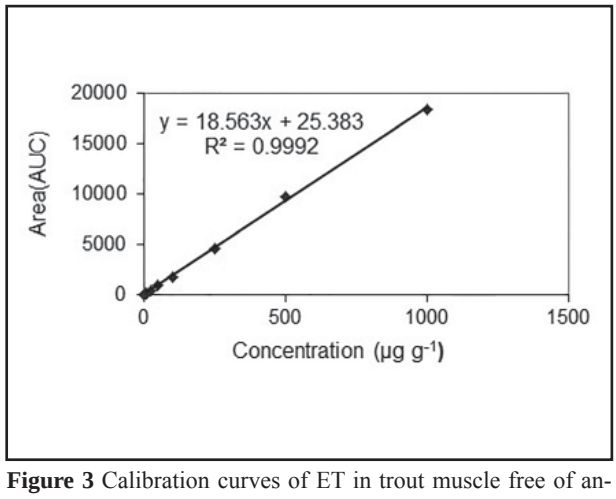

Figure 3 Calibration curves of ET in trout muscle free of antibiotics.

fish farm (farm no 15). The residuals of EN were detectable in range 0.05-0.90 µg g^{-1} in 75 muscle samples obtained from 6 fish farms (Table 2). The detectable values were 0.19-0.29 µg g^{-1} for farm 4 (15 samples), 0.07-0.09 µg g^{-1}, for farm 6 (9 samples), 0.05-0.80 µg g^{-1} for farm 7 (15 fish samples), 0.5-0.8 µg g^{-1} for farm 8 (8 fish samples), 0.05-0.09 µg g^{-1} for farm 9 (11samples) and 0.05-0.08 µg g^{-1} for farm 10 (Table 2). Also, the residuals of ET were detectable in range 23.38-181.38 µg g^{-1} in 69 muscle samples obtained from 5 fish farms (Table 2). The detectable values were 60.82-132.5 µg g^{-1} (20 samples) in farm 1, 23.38-35.22 µg g^{-1} (13 samples) for farm 3, 100.54-154.21 µg g^{-1} (15 samples) for farm 4, 51.21-89-32 µg g^{-1} (15 samples) for farm 5 and 100.02-181.38 µg g^{-1} (14 samples) for farm 12 (8 sample).

Discussion

Results of this study show that the residuals of OTC, EN and ET measured by HPLC in 798 muscle samples of cultured trout obtained from17 fish farms were detectable. Generally a number of 151 (19%) muscle samples were positive for the residuals of these antibiotics. Maximum detectable levels for EN, ET and OTC were 0.9,152 and 0.7.4 µg g^{-1}muscle sample. The recommended levels of maximum residual limits (MRL) reported by the Codex, EU and FDA for these antibiotics are100, 200 and 200 µg g^{-1} fish tissues, respectively (Council of the European Communities, Council Regulation 2377/90/EC 1990; European Commission, Council Directive 96/23/EC 1996; FDA 2009a,b,c). Although in this study the residual levels of these antibiotics are below MRLs, Two issues may raised from these data. Firstly, the fish samples were obtained randomly from the fish farms, although an attempt was undertaken to select the fish farm with a previous history of antibiotic therapy. Secondly, the fish samples used in this study hadnotbeen specified for their exactperiod of chemothera pyprior to the sample collection. Thus, in such circumstances there is a possibility for collecting the untreated fish samples and /or collecting the fish samples which been treated a long time ago e.g above 2 month before being sampled resulting in undetectable residuals of these antibiotics in their muscle tissues. Also, by comparing the maximum detectable range of the residuals of these three antibiotics, one might show that the use

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of ET was more common than OTC and EN in the examined trout farms. Because the maximum values for ET was 181 µg g^{-1} muscle where as for OTC and EN was 0.8-0.9 µg g^{-1} muscle. Perhaps, one of the most common reasons forusing ET in these trout farms was its application as an antibiotic of choice for treatment of streptococcois/lactococcosis that is the most common and serious bacterial diseases in the region. Moreover, the findings of this study revealed that the use of EN in trout farming is increasing as its residuals were detectable in approximately 29% of the examined fish farms. However, the amount of an antibiotic residual in the tissues of fish is influenced by several factors including type of antibiotic, duration time for storage of tissues/ sample, temperature of storage period, type of tissue, fish species, route of drug administration and some environmental factors such as water quality parameters especially water temperature of the pond fish. Recently, these concerns have led to the standardizaton of measurement methods of residual drugs in the tissues of aquatic animals, which should be considered for scientific and official authorities (Canada-Canada & Munoz de laPena 2009). In this regard, some official authorities such as the Europe Union (EU), World Food Organization, (FAO) World Health Organization (WHO), American Drug Administration (FDA) (FDA. 2009a,b,c), Canada Food and DrugInspection Administration (CFIA), and the Australian Bureau of veterinary drugs and pesticides (ADVMA) were going to develop guidelines, including the Maximum Residual Limits (MRL), antibiotics used in aquaculture, especially in edible and breeding fish. The outstanding point here is that there are significant differences between countries and the afore-mentioned references far as the defined MRL for some antibioticsis concerned. For instance, FP fish consumption is only given by the EU (Commission Decision 2003/181/EC of 13 March 2003, Council Directive 96/23/EC of 29 April 1996). In some countries such as Chile, the amount of MRL FLQ in tissues of rainbow trout estimated 500 µg kg^{-1} (Hernández-Serrano 2005). Also, the MRL values for tetracycline in fish in the countries of Europe Union (EU) such as Canada, Australia and America, were expressed 100, 200, 2000 µg kg^{-1} of tissue respectively (Council of the European Communities, Council Regulation 2377/90/EC1990; European Commission, Council Directive 96/23/EC 1996; European Commission, Commission Decision 2003/181/EC 2003; FDA 2009a,b,c). These differences and prohibited use of some antibiotics and chemical agents in aquaculture have led to the equalization of the methods, especially in terms of sensitivity, quantification, maximum residual limits, and types of drugs. In conclusion, result of this study shows that some trout farmers in Iran are currently using different antibiotics including OTC, ET and EN in their own trout farms. The main reason for such antibiotic application is mainly due to the morbidity and mortality by both *Streptococcus iniae* and *Lactococcus garvieae*. Therefore, use of other protective measures such as vaccination is highly recommended.

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References

Cabello F.C. (2006) Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental Microbiology* **8,** 1137-44.

Canada-Canada E., Munoz de laPena A. & Espinosa-Mansilla A. (2009) Analysis of antibiotics in fish samples. *Analitical Bioanalitical Chemistry* **395,** 987-1008.

Council of the European Communities (1990) Council Regulation 2377/90/EC laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. *Official Journal of the European UnionL* **224,** 1-8.

Esposito A., Fabrizi L., Lucchetti D., Marvasi L., Coni E. & Guandalini E. (2007) Orally Administered Erythromy-

[Downloaded from ijaah.ir on 2026-01-29]

[DOR: 20.1001.1.2345315.2015.1.1.9.2]
[DOI: 10.18869/acadpub.ijaah.1.1.71]

cin in Rainbow Trout (*Oncorhynchus mykiss*): Residues in Edible Tissues and Withdrawal Time. *Antimicrobial Agents and Chemotherapy* **51,** 1043–1047.

European Commission (1996) Council Directive 96/23/EC on measures to monitor certain substances and residues in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/18/EEC and 91/664/EEC. *Official Journal of the European CommunitiesL* **125,** 10-32.

European Commission (2003) Commission Decision 2003/181/EC amending Decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin. *Official Journal of the European CommunitiesL* **71,** 17-18.

FDA (2009a)*Tolerances for residues of new animal drugs in food*. Part 556. Section 556.226- Enrofloxacin. http://www.accessdata.fda.gov.

FDA (2009b) *Tolerances for residues of new animal drugs in food.* Part 556. Section 556.230- Erythromycin. http://www.accessdata.fda.gov.

FDA (2009c) *Tolerances for residues of new animal drugs in food.* Part 556. Section 556-500. Oxytetracycline. http://www.accessdata.fda.gov.

Hernández-Serrano P. (2005) *Responsible use of antibiotics in aquaculture.* FAO, Rome.

Lucchetti D., Fabrizi L., Esposito A., Guandalini E., Di Pasquale M. & Coni E. (2005) Simple confirmatory method for the determination of erythromycin residues in trout: a fast liquid-liquid extraction followed by liquid chromatography-tandem mass spectrometry. *Journal of Agriculture and Food Chemistry* **53,** 9689-9694.

Miranda C.D., Tello A. & Keen P. (2013) Mechanisms of antimicrobial resistance in finfish aquaculture environments. *Frontiers in Microbiology* **4,** 233.

Park Y.H., Hwang S.Y., Hong M.K., Kwon K.H. (2012) Use of antimicrobial agents in aquaculture. *International Office of Epizootics* **31,** 189-97.

Ramirez A.J., Mottaleb M.A., Brooks B.W. & Chambliss C.K. (2007) Analysis of pharmaceuticals in fish using liquid chromatography-tandem mass spectrometry. *Analytical Chemistry* **79,** 3155–3163.

Salte R. & Liestøl K. (1983) Drug withdrawal from farmed fish. Depletion of oxytetracycline, sulfadiazine and trimethoprim from muscular tissue of rainbow trout (*Salmo gairdneri*). *Acta Veterinaria Scandinavica* **24,** 418–43.

Sapkota A., Sapkota A.R., Kucharski M., Burke J., McKenzie S., Walker P. & Lawrence R. (2008) Aquaculture practices and potential human health risks: current knowledge and future priorites. *Environment Internationa* **l34,** 1215–1226.

Soltani M., Jamshidi SH. & Sharifpour I. (2005) Streptococcosis caused by *Streptococcus iniae* in farmed rainbow trout (*O. mykiss*) in Iran: biophysical characteristics and pathogensis. *Bulletin of European Association of Fish Pathologists* **25,** 95-107.

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باقی مانده آنتی بیوتیکی در برخی مزارع قزل‌آلای سایز بازاری ایران

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چکیده

به منظور ارزیابی بهداشـــتی ماهی قزل‌آلای سایز بازاری، باقی‌مانده سه آنتی‌بیوتیک شـــامل اکسی‌تتراسایکلین، انروفلوکساسین و اریترومایسین در عضلات ماهیان تازه صید شده از ۱۷ مزرعه پرورش ماهی در استان چهارمحال–وبختیاری به روش کروماتوگرافی مایع سنجش شد. برای این‌کار تعداد ۲۶۶ ماهی به صورت تصادفی از ماهیان ۱۷ مزرعه تهیه و تعداد ۷۹۸ نمونه عضله آن‌ها مورد سنجش قرارگرفت. نتایج حاصله نشان داد که اکسی‌تتراسایکلین در ماهیان یک مزرعه (۵/۸٪) و به میزان ۷/۱۳–۰/۷۵ میکروگرم در گرم عضله قابل سنجش بود. به‌علاوه انروفلوکساسین در ۶ مزرعه (۳۵٪) و به میزان ۰/۷۳–۰/۵ میکروگرم در گرم نمونه عضله قابل اندازه‌گیری بود. همچنین اریترومایسین در ماهیان ۵ مزرعه (۲۹/۴٪) و به میزان ۱۸۱/۳۸–۲۳/۳۸ میکروگرم در گرم نمونه‌های عضله قابل ردیابی بود. نتایج این داده‌ها نشـــان می‌دهد که باقی‌مانده اریترومایســـین و اکسی‌تتراسایکلین بالاتر از حد مجاز بوده و لذا سنجش مرتب باقی‌مانده این داروها در مزارع ماهیان امری ضروری است. به‌علاوه با توجه به حداقل میزان سنجش این آنتی‌بیوتیک‌ها در این مطالعه (۰/۰۵ میکروگرم در گرم نمونه) استفاده از این روش کروماتوگرافی مایع می‌تواند یک روش مفیدی برای سنجش باقی‌مانده آنتی‌بیوتیک‌ها در مزارع ماهیان باشد.

واژه‌های کلیدی: باقی‌مانده آنتی‌بیوتیکی، قزل‌آلا، اریترومایسین، اکسی‌تتراسایکلین، انروفلوکساسین.

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