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Research Article

ACCUMULATION OF ACTIVE PHARMA INGREDIENTS IN POND RARED *LABEO ROHITHA*

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ABSTRACT

Objective: The present study aimed to estimate the amount of Active Pharma Ingredients in fish tissues of pond rared *Labeo rohitha,* in and around ponds of Nandivada Mandal, Krishna (Dt), Andrapradesh.

Materials and methods: With the aid of Isocratic PEAK HPLC-LC 7000 we understood drastic situation and undependable use of antibiotics or drugs in culture practices.

Result: When consuming farm raised fish from above said locality, consumer may not be aware that with every bite they are getting a dose of antibiotic residue per gram of fish tissue, Albendazole-0.26µg\g, Doxycyclin-0.35µg\g, Enrofloxacin-0.39µg\g, Sulphamethaxazole-0.21µg\g, Furazolidine-0.45µg\g, and Ivermectin-0.036µg\gram of fish tissue respectively.

Conclusion: The public health hazards related to antimicrobial use in aqua culture include the development and spread of antimicrobial- resistant bacteria and resistant genes and the presence of antimicrobial residues in aqua culture products and the environment.

Keywords: Bioaccumulation, API, HPLC, Resistant bacteria, Public Health Hazards.

INTRODUCTION

Fish culture involves controlled cultivation and harvesting of fishes in either fresh, marine, lagoon waters. Ponds were selected like diversed, parallel, intensive culture type, and poly culture tanks [1], of Nandivada mandal, Krishna (Dt), Andrapradesh. Aquaculture farmers above said locality seed their tanks with zero size <250g *Labeo rohitha* 2,500 to 3,000 and *Catla catla* 300 to 350 number in the range per acre[2], and fed with de oiled brawn, some are giving floating feed[3], to get Maximum Sustainable Yield (MSY). Proper pond and water quality management is essential for successful and quality fish production. Maintaining a good culture environment through use of proper management practices will reduce the risk of disease and increase production, fish quality and marketability. Due to lack of quality and quantity of water, water sanitizers have got pivot importance.

Disinfection of ponds is of a big importance in prevention and elimination of fish pathogens. The above said farmers using Oxidizing agents like Halogens (Chlorine, Iodine, and Bromine), Peroxides- (Hydrogen Peroxide, Calcium Peroxide) [4], Kmno4, [5] Reducing agents- Formaldehyde[6], and Detergents- Quaternary ammonium compounds like Benzalkonium Chloride. If not treated well or not continuous or accumulation of fish catabolic substances, low dissolved oxygen, over crowding, sudden changes in water temperature and weather, high nitrite, high free ammonia, sudden drop or raise of pH, alkalinity and hardness changes of water, poses the fish to stress[7]. It is generally accepted that all out breaks of a communicable disease are the result of interaction between the host, pathogen and the environment. Carps are susceptible to many communicable and non-communicable diseases; the most significant among them are being described.

In case of communicable diseases, viral diseases- spring viremia of carp, epithelioma papillosum, and gill necrosis are frequent diseases in aquaculture. [8] While in case bacterial diseases such as - columnaris, red spot, bacterial hemorrhagic septicemia, bacterial gill disease, bacterial kidney disease, dropsy, fin rot, pasteurellosis, epitheliocystis, tuberculosis, lateral line erosion, pop eye, and carp erythro dermatitis. Fungal diseases are very common like- saprolegniosis, branchiomycosis. In general protozoan infections like ich, trichodiniais, and myxosporidian diseases are very common. Parasitic diseases in fishes like dactylogyrus, gyrodactylus and diplostomum. The common crustacean parasites are argulus, lernaea has been reported [9] In case of non communicable diseases liver lipoid diseases and gas bubble disease have been reported in *Labeo rohitha* fish. The above mentioned bacterial diseases were caused by *Flexibacter columnaris, Aeromonas hydrophila, Pseudomonas flourescens, Aeromonas liquifaceans, Streptococci, Renibacterium salmonarum, Pasteurella, Mycobacterium, Rickettsiae, and Chlamydiae.*

For the treatment of fishes a number of antibiotics or has been used by fish farmers these could cure the disease, but cause some other problems like development of resistance, and some residues accumulate in the tissues of treated fishes. The drugs which are commonly used by farmers in culture are Albendazole, Ivermectin, Doxycyclin, Enrofloxacin, Sulpha methaxazole, and Furazolidine raw materials in the range of 10g, 1 to 5g, 8g, 10g, 10g, 10 to20g per ton of fishes respectively. Medicate fishes via application of antibiotic powders directly to water or , feed pellets or DOB mixed with artificial binders like, gel or starch with above mentioned chemotherapeutic agents, to prevent chemical wastage and to reach every fish, selection of antibiotic based on the disease and it' s sevearity, for effective elimination of pathogens. Therefore in the present paper efforts have been made to detect the various drugs or antibiotics in tissues of treated fishes.

MATERIALS AND METHODS

Collection of fish sample

For the purpose of present study fish samples were collected, after 30 days, having recovered, from cultured ponds of Nandivada Mandal, Krishna (Dt), Andhra Pradesh, brought to the laboratory, dissected and removed the muscle tissue and analyzed, by Isocratic PEAK HPLC-LC 7000 instrument with Zodiac C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

Sample Preparation & Extraction Procedure

The muscle (0.2 g) of fish was homogenized with phosphate buffer (2 mL). Dicholoro methane (8 mL) was added to the homogenate, vortexed for 1 min and centrifuged at 4000 rpm for 20 min. The upper aqueous layer was discarded, the organic phase was

transferred to a clean tube and the tissue was again extracted with 6 mL of dichloromethane. Organic layers were combined and evaporated at 30 ^{0}C under nitrogen stream. The extract was re dissolved with 1 ml of mobile phase and 20 µL was used for HPLC analysis. The quantification in µg of antibiotic/ g of muscle was performed in relation to the correspondent peak area of the standard curve. The method conditions for all the antibiotics mentioned in Table 1.

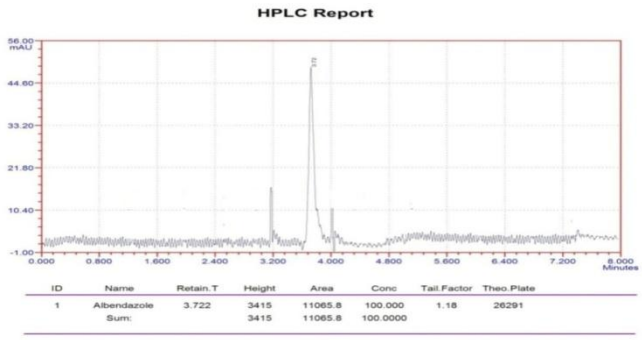
By using the simple formula sample concentration is calculated:

Sample concentration: Sample Area * Standard Concentration
Standard Area

According to standard retention time, sample area was taken, interpret ate in above formula we get sample concentration\ ml. Half of the value of sample concentration per ml give amount of API accumulated per gram tissue.

RESULTS AND DISCUSSION

These were the different conditions applied for the samples, to get sample concentration in fish tissues by using HPLC, adapted standard testing protocols have been reported by different authors, like Anil Waldia 2008 [10], Muralidharan Selvadurai 2010[11]; H. Garcia Ovando 2004[12]; Sayar E 2010[13]; K Na- Bang Chang *et al* 2006 [14]; Dhirender Singh Mittan *et al* 2008 [15].



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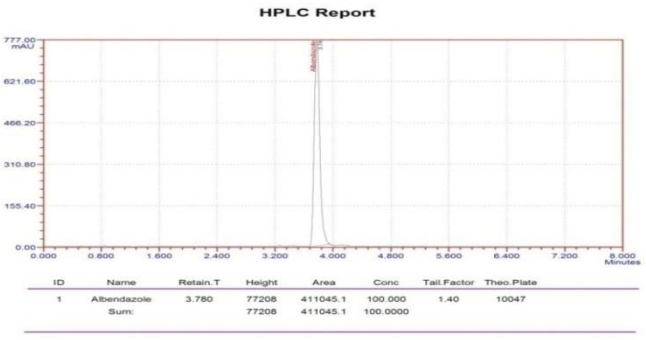

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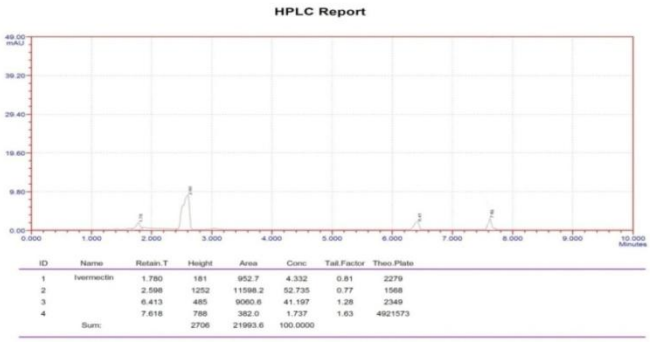

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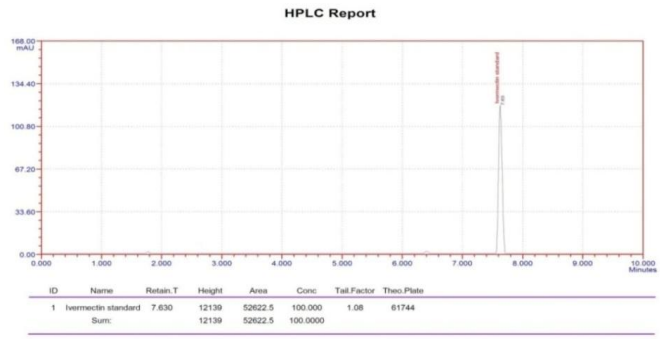

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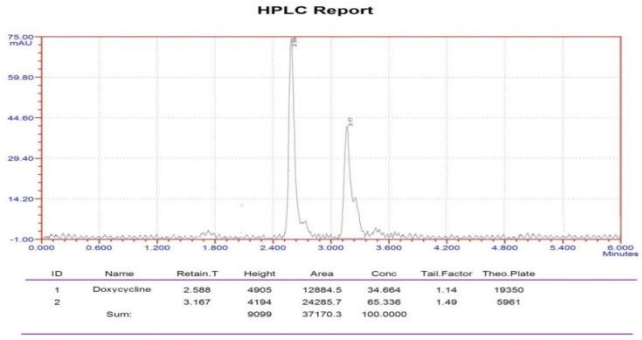

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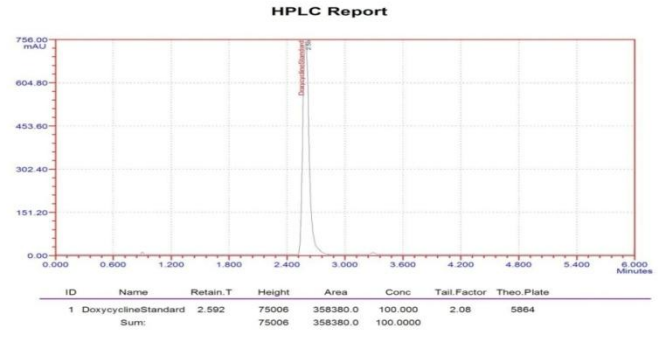

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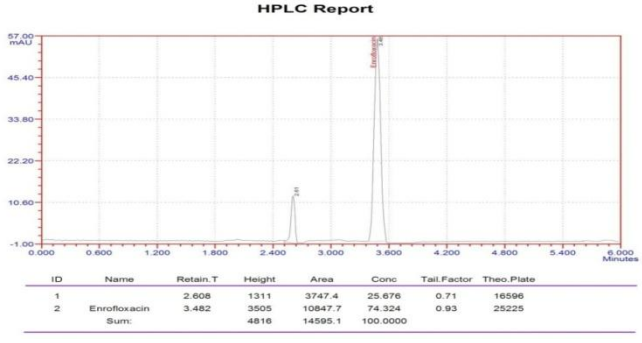

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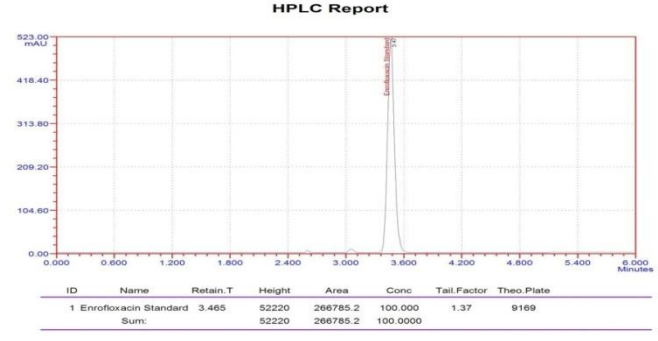

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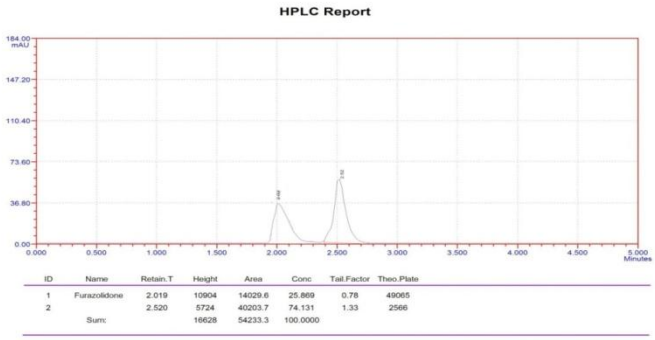

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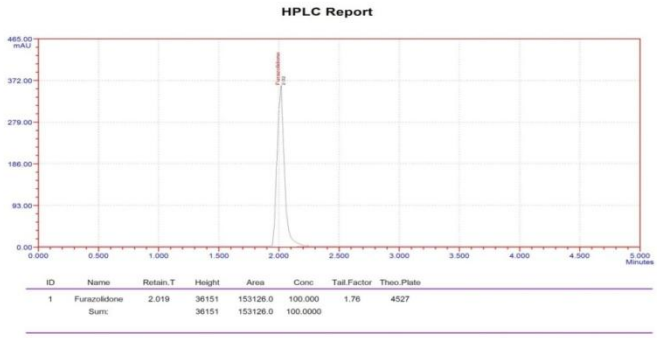

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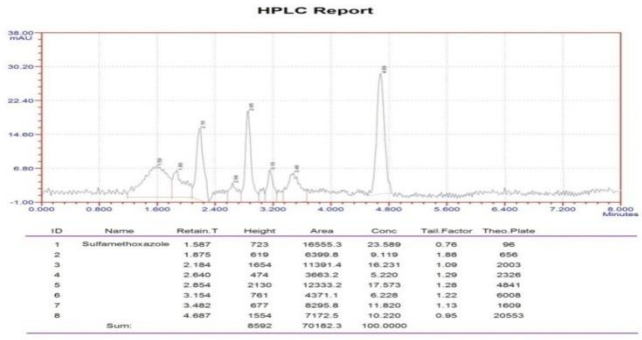

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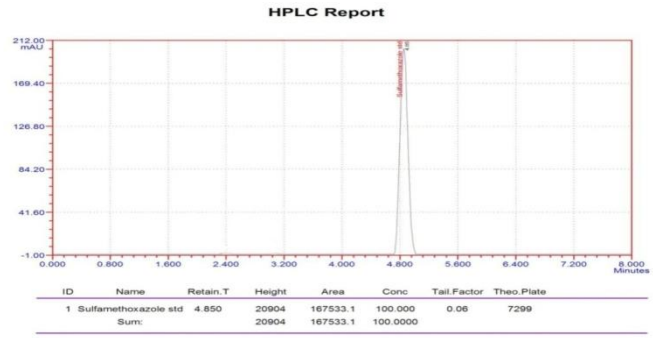

7(B)

Table 1: Method conditions for all the Antibiotics

S. No	Parameter	Albendazole	Ivermectin	Doxycyclin	Enrofloxacin	Furazolidine	Sulpha methaxazole
1	Mobile phase	Acetonitrile: Methanol: Water (60:30:10 v\v\v)	aceto-nitrile, methanol, water 50:45:5 (v/v/v)	Acetonitrile: Ammonium Acetate: (80:20 v\v)	Water: Acetonitrile: Triethylamine (80. 19. 1v\v\v)	Methanol: Acetonitrile (90: 10 v\v)	Potassiumhydrogen phosphate, acetonotrile, methanol.(50:30:20 v\v\v)
2	Pump mode	Isocratic	Isocratic	Isocratic	Isocratic	Isocratic	Isocratic
3	Column	C18	C18	C18	C18	C18	C18
4	Column temp	Ambient	Ambient	40°c	Ambient	Ambient	Ambient
5	Wave length	245nm	365-475 nm	350nm	295nm	259nm	230nm
6	Injection volume	20µl	20µl	20 µl	20 µl	20 µl	20 µl
7	Flow rate	1.8ml\ min	1ml\ min	0.3ml\ min	1.2ml\ min	1ml\ min	1ml\ min
8	Run time	8min	5min	6min	6min	5 min	8min
9	Retention time	3.7min	7.6 min	2.5 min	3.46 min	2.01min	4.8min
10	Standard concentration	20µg\ ml	10 µg\ml	20 µg\ml	10 µg\ml	20 µg\ml	10 µg\ml

Table 2: Albendazole 2(A) & 2(B)

Parameter	Standard	Sample
Retention time	3.7min	3.7min
Area	411045	11066
Concentration	20µg/ml	0.53µg/ml
Gram tissue	Antibiotic concentration	0.26µg\ g

Table 3: Ivermectin 3(A) & 3(B)

Parameter	Standard	Sample
Retention time	7.63 min	7.61 min
Area	52622	382
Concentration	10 µg/ml	0.07µg/ml
Gram tissue	Antibiotic concentration	0.036µg\ g

Table 4: Doxycyclin 4(A) & 4(B)

Parameter	Standard	Sample
Retention time	2.59min	2.58min
Area	358380	12884
Concentration	20 µg/ml	0.71µg/ml
Gram tissue	Antibiotic concentration	0.35µg\ g

Table 5: Enrofloxacin 5(A) &5(B)

Parameter	Standard	Sample
Retention time	3.46min	3.48min
Area	266785	10847
Concentration	10 µg/ml	0.40µg/ml
Gram tissue	Antibiotic concentration	0.39 µg\ g

Table 6: Furazolidine 6(A) & 6(B)

Parameter	Standard	Sample
Retention time	2.01min	2.01min
Area	153126	14029
Concentration	10 µg/ml	0.91µg/ml
Gram tissue	Antibiotic concentration	0.45µg\ g

Table 7: Sulpha methaxazole 7(A) & 7(B)

Parameter	Standard	Sample
Retention time	4.85min	4.68min
Area	167533	7172
Concentration	10 µg/ml	0.42µg/ml
Gram tissue	Antibiotic concentration	0.21µg\ g

The results of present study indicated that accumulation of Active Pharma Ingredients in tissue of fishes were Albendazole- 0.26µg\g, Doxycyclin-0.35µg\g, Enrofloxacin-0.39µg\g, SMX-0.21µg\g, Furazolidine-0.45µg\g, and Ivermectin-0.036µg\g respectively. The sulpha methaxazole was accumulated in the different fish and prawn samples reported by [16] from Beljium 0.3 to 4µg\g (sea bass), 1 to 42 µg\g (trout), and 4 to 38 µg\g (white shrimp), the similar results were observed in the present study. The investigators [17] reported from Italy that the long term depletion of Enrofloxacin from trout (onchorynchus mykiss), bearing 170 µg\Kg body weight, where as in present study the Enrofloxacin accumulation was in double concentration. The authors [22] reported Ivermectin and Doramectin in the concentration of 200mg\ kg body weight showed adverse reactions like black pigmentation, lethargy and poor appetite in *Labeo rohitha.* Moreover, undetected consumption of antibiotics in food can generate problems of allergy and toxicity, in consumers which are difficult to diagnose because of a lack of previous information on antibiotic ingestion reported by [21]

Supplementing medicated feed to treat diseases, leads to possible modifications in gastrointestinal micro biota of farmed fish, developing of antibiotic resistance after the use of antibiotics. The high selection pressure in gut, permit the growth of only resistant strains, to the concerned antibiotic. The unconsumed food, and fish feces containing antibiotics reach the sediment, possibly change the microbial community of sediment have been reported by [18]. The organisms which were acquired determinants of resistance are able to transfer their resistance genes to bacteria of the terrestrial environment, including human and animal pathogens were reported. For example, strong epidemiological and molecular evidence exists indicating that fish pathogens such as *Aeromonas* can transmit and share determinants for resistance to antibiotics with pathogens such as *Escherichia coli* isolated from humans. The sulfonamide-resistant determinant *Sull* has also been found in plasmids present in *A. salmonicida* and bacteria of other niches including *Erwinia* (a plant pathogen), *Vibrio cholerae* and *E. coli*, reported by [19- 20].

CONCLUSION

In correct disease diagnosis, selection of in appropriate drug, high dosage or in correct dosage frequencies for long time, all these parameters play major role in microbial resistance. Responsible use of chemotherapeutics and their judicious usage is needed, other wise their residues remained in them transferred to higher trophic levels. Prohibition or severe limitation of the use of human antibiotics in food producing animals, prevent use of "off- label" antibiotics in culture practices, Government authorities keep tabs on open sellers of chemotherapeutics, and need to evaluate antibiotic load and regimen, exposure of animals and humans to antibiotic resistant bacteria and exposure of bacteria to antibiotic resistant genes time to time, other wise we will see fish less tanks in near by feature.

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