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Ciprofloxacin residue analysis in pangasius by electrochemical voltammetry

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Abstract
A simple, sensitive electrochemical method (voltammetry) for the detection and quantification of ciprofloxacin in Pangasius has been developed. The assay consisted of recovery, limit of detection, analyzed ciprofloxacin concentration, regression equation, correlation coefficient when examining ciprofloxacin stock solution added at 1.52745, mg/L, 1.0183mg/L, and 0.50915 mg/L. Results show high recovery (74.5, 77.5, and 78.2% in equivalent), low LoD (48.7, 20.1, 54.9 µg/L in equivalent). Then Pangasius fillets are sampled in local markets to confirm the detection of the analysis. This method is sufficiently sensitive for screening analysis strongly supporting for other confirmation ones that small and medium enterprises of fish processing can be approached.

Keywords: electrochemical method, detection, quantification, ciprofloxacin, Pangasius, screening analysis

1. Introduction
Antibiotic in a broader sense is a chemotherapeutics agent that capable of inhibit or abolishes the growth of microorganisms such as bacteria, fungi and protozoa. Microbial effects of drug residues can have an effect on human intestinal flora which can diminish the activity of intestinal bacteria. Another effect of antibiotic drug residues can be the development of transient resistant populations. For example, the unapproved use of fluoroquinolones, such as ciprofloxacin, poses the risk of increasing antibiotic resistant bacteria with the potential for serious human health consequences from untreatable infections. In addition, chronic dietary exposure to high concentrations of fluoroquinolone residues, particularly during early growth, may result in a number of toxicities including joint and testicular lesions. The use of this compound not approved in the US, or the misuse of FDA approved new animal drugs, will have an impact on the safety of aquaculture products for consumers. Nowadays, antimicrobial resistance is a growing public health threat and has been designated by the WHO as an emerging public health problem [3].

Fig 1: Vietnam Pangasius

Andrieu M *et al.* (2014) conducted ecological risk assessment of the antibiotic enrofloxacin applied to Pangasius catfish farms in the Mekong Delta, Vietnam. In the present study, we assessed the ecological risks posed by the use of the antibiotic enrofloxacin (ENR), and its main metabolite ciprofloxacin (CIP), in a Pangasius catfish farm in the Mekong Delta region, Vietnam. Water and sediment samples were collected in a stream receiving effluents from a Pangasius catfish farm that had applied ENR. The toxicity of ENR and CIP was assessed on three tropical aquatic species: the green-algae Chlorella sp. (72h - growth inhibition test), the micro-invertebrate *Moina macrocopa* (48h - immobilization test), and the Nile tilapia (*Oreochromis niloticus*). The toxic effects on *O. niloticus* were evaluated by measuring the cholinesterase (ChE) and catalase (CAT) activities in the fish brain and muscles, respectively, and by considering feed exposure and water exposure separately. Ecological risks were assessed by comparing maximum exposure concentrations with predicted no effect concentrations for cyanobacteria, green algae, invertebrates and fish derived with available toxicity data. The results of this study showed that maximum antibiotic concentrations in Pangasius catfish farm effluents were $0.68 \mu gL^{-1}$ for ENR and $0.25 \mu gL^{-1}$ for CIP (dissolved water concentrations). Antibiotics accumulated in sediments down-stream the effluent discharge point at concentrations up to $2590 \mu gkg^{-1}$ d.w. and 592 μgkg^{-1} d.w. for ENR and CIP, respectively. The calculated EC50 values for ENR and CIP were 111000 and 23000 μgL^{-1} for Chlorella sp., and 69000 and 71000 μgL^{-1} for M. macrocopa, respectively. Significant effects on the ChE and CAT enzymatic activities of O. niloticus were observed at $5 gkg^{-1}$ feed and 400-50000 μgL^{-1}, for both antibiotics. The results of the ecological risk assessment performed in this study indicated only minor risks for cyanobacteria communities, suggesting that residual concentrations of ENR and CIP after medication are not likely to result in severe toxic effects on exposed aquatic ecosystems. However, more studies should be performed by considering other antibiotic treatments used in Pangasius catfish production and the potential ecotoxicological effects of relevant antibiotic mixtures on sediment communities [3].

Antibiotic pollution has many reasons, but directly by the enterprises of breeding aquatic products (especially local) no analytical equipment to tight control of antibiotic residues immediately after each use. To analyse residues of antibiotics in general and in particular ciprofloxacin in aquatic products breeding, the country imported aquatic products often favour using a number of techniques such as chromatography analysis technique to HPLC [4, 11, 12] or technique LC/MS/MS [8, 10]. These techniques but for ciprofloxacin analysis results very reliable but requires rather expensive equipment, reagents must Z be very pure, hard to current deployment in Vietnam. Reasonable outlet is seeking a modern analysis techniques capable of replacing the equivalent but less expensive. The current trend is looking for a technical analysis of cell culture, because according to J. Wang [17], electrochemical analysis device has three main advantages: high-performance, compact and cheap. That will be more feasible if the device is manufactured in Vietnam. In Vietnam, on the scene, after a period of understanding and preliminary testing, we decided to opt for a chemical analysis equipment, modern Vietnam production, model SQF-505 to conduct research on method of determination of pesticide residues in farmed seafood Ciprofloxacin. Model SQF-505 are designed to make the analysis of the substance by two square-wave polarographic techniques are modern: 1) Square wave sweep on pole drops slowly Stripping square wave and 2) scans on an extreme drops slowly. Machine with dedicated software to choose parameters of the machine, display the results by using the measurements and parameters in the form line, handling and storage of measurement results. In our country, both techniques in model SQF-505 was once used to analyze a number of antibiotics in the actual object. These are the basis to form the premise for this research

Some methods of determination of ciprofloxacin:
Chromatography methods [4, 8, 11, 16 23, 24]

To analyze the ciprofloxacin by method is often used in chromatography HPLC techniques and LC/MSn. Conduct analysis conditions are as follows:

- Column chromatography non-polar stationary phase with C8 or C18.
- Mixed with high polarity as orthophosphoric acid mix (pH = 3) and acetonitrile (18/82, v/v), mixture of formic acid (2%) and acetonitrile (86, 14, v/v), or the mixture of ammonium formate (pH = 3.5 mm) and acetonitrile (85/15, v/v) of
-UV wavelengths 275nm probes or probes MS. UV detector less sensitive probes MS, (UV probes have LOD about 2ng/ml, while the MS is less than 1ng/ml).Techniques of LC/MS/MS has the nose of m/z for ciprofloxacin is 332, 288. While LC/MS3, for the m/z is 245, 268, 288.

Electrochemical method [13, 14, 15]

To analysis of ciprofloxacin by electrochemical method can use the technique of linear sweep stripping ămpe-volt (linear sweep stripping voltammetry), differential pulse polarographic techniques, or may use the electrochemical titration method. With volts-ămpe stripping the condition to analyze ciprofloxacin are: electrode working electrode is the hanging Hg (HMDE), electrode Ag/AgCl is comparison, while the electrode wire is supported to contributing area PT. is-1000mV, time area of contributions is 30s, scan speed is 140 mV/s, an aqueous buffer solution is background Britton-Robinson (pH = 3.78). This technique for the LOD value is 0.011 µg/g/ml. With differential pulse techniques, analytical conditions ciprofloxacin is identified as pH = 8.5; in which the two waves is reduced in-1.44 V and-1.64 V, this method allows the determination of ciprofloxacin in concentration from 5×10^{-7} to 3×10^{-5} M, with the RSD < 4%

Photometry method [1, 5, 6, 7, 18, 19]

To determine what ciprofloxacin can use UV absorption spectrometry, fluorescence spectrometry or emitting chemical method. For the UV absorption spectrum method, proceed to the determination of ciprofloxacin in buffered HCO2H/NaOH (1 m, pH = 2.2), proceed to measure A (in comparison to the white form) at 277 nm and get the maximum signal, or is that gauge Δ A at the two wavelengths is 277 nm and 380 nm and retrieved Δ signal A = A277-A380.LOD = 35ppb. As for the fluorescent spectrum method can define ciprofloxacin by measuring directly or after forms complexes with ion Sc3+. If a direct measure of the wavelength excites the fluorescent wavelength 270nm is 415 nm. If forms complexes with ion Sc3+, made in buffers CH3COOH/NaOH (pH = 4.2). Wavelength excites the fluorescent wavelength and 280 nm is 430 nm. LOD=0.5Nm (=0.166 ppb). For emitting chemical methods, the determination of ciprofloxacin in progress on luminescent luminal-hydrogen peroxide-gold (Au). Luminescent intensity increases with levels of ciprofloxacin. Or is carried one mitting system KMnO4-Na2S2O¬-Tb3 +-ciprofloxacin. Allows rapid and highly sensitive analysis, applied to multiple objects. The luminal-hydrogen peroxide-gold (Au) have LOD = 9.5 ng/mL (= 9.5 ppb), while KMnO4 system-Na2S2O4-Tb3 +-ciprofloxacin have LOD = 4.4 × nmol/L (= 1.46 ppb). Purpose of our search is to apply square wave voltammetry to determine ciprofloxacin residue in Pangasius tissue. This technique will create an applicable performance for seafood enterprises in screening their raw fish before harvesting and processing.

2. Material and Method

2.1 Equipment: Multifunction Analyzer ANALYZER SQF-505 in Tropical Vietnam-Russia Center.

2.2 Chemical: Ciprofloxacin hydrochloride standard from the pharmaceutical Institute-Ministry of health. Solvent: ethanol, Methanol, Acetone (China); Acetonitrile (Merck). Others: CH_3COONH_4, CH_3COONa, NaOH, NH_4OH condensed, CH_3COOH condensed, H_3PO_4, H_3BO_3, borax, etc (China).

2.3 Preparation: The original standard solution are mixed from standard ciprofloxacin hydrochloride 120 mg (purity was 94.18%) in 100 ml of distilled twice (the concentration of cipro is 1018.3 mg/L) and stored in the refrigerator. The smaller concentrations of cipro were mixed from the solution in the original standard, distilled twice. The other solutions are mixed from the solid or liquid concentrate with distilled twice to the concentration required.

3. Result & Discussion

3.1 Recovery, limit of detection of testing method

Pangasius with templates we also made similar processes in model fish. And we also conducted a survey of experimental performance recovery for the three cases that the concentration of the standard for Pangasius form more ciprofloxacin (cipro) is different. In the case of Pangasius fish samples, samples are used to determine the recovery performance with cipro have known as 7.34 µ g/kg according to the method of LC/MS/MS (defined at the Center for training and development of chromatography Ho Chi Minh City (Vietnam). So with the amount of the initial balance model is 5 gam then the last weight measurement solution cipro was available was 0.734 x 10-3mg/L.

3.1.1 Cipro stock solution added at 1.52745mg/L

As for 150 µg/L standard solution to the original fish form, then follows the process of extracting the concentration of cipro is 1.52745 mg/L, so the total concentration of cipro in the solution measured finally ($1.52745 + 1.52818 \times 10^{-3} = 0.734$ mg/L) if recovery efficiency of 100%. Recovery performance results shown in table 1

Table 1: Cipro stock solution added at 1.52745mg/L.

Addition of ciprofloxacin (mg/L)	Intensity, I (nA)		
	1st measure	2nd measure	3rd measure
0	220.36	214.5	230.9
0.203	243.6	236.2	266.8
0.407	291.6	282.2	311.0
0.611	330.7	326.0	353.3
0.815	373.3	354.8	387.3
Regression equation	y = 213.1 + 193.3x	y = 208.66 + 181.87x	y = 229.46 + 196.946x
Coefficient relation (R^2)	0.996	0.994	0.999
Analyzed ciprofloxacin concentration Co=a/b	1.1024	1.1473	1.165
Recovery R_{th} = Co/1.52818	72.14%	75.08% R_{th}tb ≈ 74.5	76.24%
LOD (µg/L)	55.7	64.9 LODtb = 48.7	25.4

3.1.2 Cipro stock solution added at 1.0183mg/L

The concentration of cipro in last solution for the measurement is (1.0183 + 0.734 x 10-3 = 1.0190 mg/L) if recovery efficiency of 100%. The result is shown in table 2.

Table 2: Cipro stock solution added at 1.0183mg/L.

Addition of ciprofloxacin (mg/L)	Intensity, I (nA)		
	1st measure	2nd measure	3rd measure
0	168.7	178.4	187.9
0.203	211.0	224.8	235.6
0.407	245.9	275.6	284.4
0.611	298.3	321.4	328.2
0.815	339.3	365.4	380.1
Regression equation	y = 166.94 + 210.4x	y = 179 + 231.07x	y = 187.84 + 234.2x
Coefficient relation (R^2)	0.998	0.9997	0.9997
Analyzed ciprofloxacin concentration Co = a/b	0.793	0.775	0.802
Recovery R_{th} = Co/1.0190	77.86%	76.05% R_{th}tb ≈ 77.5	78.70%
LOD (µg/L)	33.3	14.2 LODtb = 20.1	12.9

3.1.3 Cipro stock solution added at 0.50915 mg/L

The concentration of cipro in last solution for the measurement is (0.50915 + 0.734 x 10^{-3} = 0.5099 mg/L) if recovery efficiency of 100%. The result is shown in table 3.

Table 3: Cipro stock solution added at 0.50915mg/L

Addition of ciprofloxacin (mg/L)	**Intensity, I (nA)**		
	1st measure	**2nd measure**	**3rd measure**
0	104.3	89.09	95.69
0.203	135.2	150.0	156.5
0.407	197.6	172.8	204.8
0.611	247.8	242.4	246.5
0.815	293.0	281.9	304.9
Regression equation	y = 97.58 + 240.59x	y = 91.634 + 234.7x	y = 99.994 + 249.64x
Coefficient relation (R^2)	0.996	0.992	0.998
Analyzed ciprofloxacin concentration Co= a/b	0.4056	0.390	0.400
Recovery R_{th} = Co/0.5099	79.53%	76.57% R_{th}tb ≈ 78.2	78.56%
LOD (μg/L)	52.2	76.0 LODtb = 54.9	36.4

So to calculate recovery efficiency when surveying the Pangasius with procedures proposed are: Rthtb = (74.5% + 77.5% + 78.2%)/ 3 ≈ 76.8%. Pangasius samples with high fat measurement solution should eventually be opaque than sample the shrimp, so the results measured Rthtb comparable to sample the shrimp. To the last measurement solution I tried to add hexane is like the case of shrimp. Results are measured very in but low-back performance.

3.2 Real fish sample measurement result

Pangasius forms used for experiments are bought in markets, and the results obtained on two samples bought are non-detected cipro in the template.

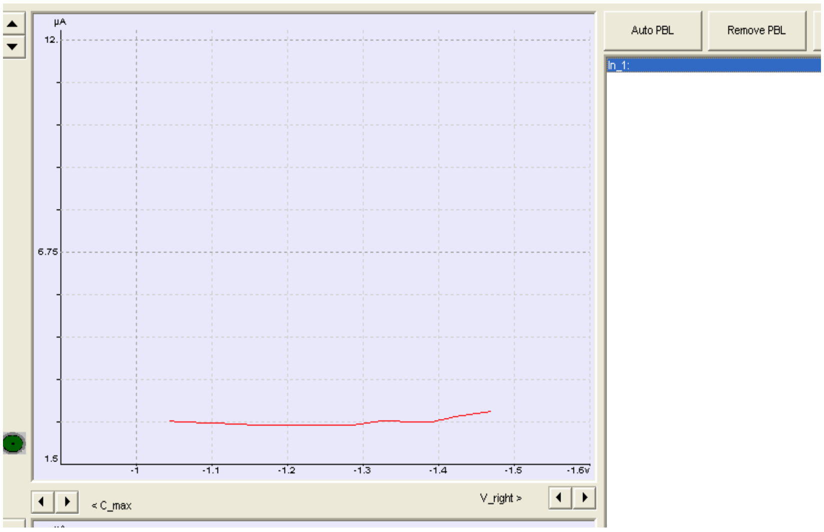

Fig 2: Measurable results cipro in model fish buying markets.

4. Conclusion

Antibiotics are still commonly used in Pangasius farms. Analysis of antibiotic residues in general and ciprofloxacin in particular with qualitative analysis using rapid sensitive voltammetry which can be considered as a potential application for future application. Analysis of freshwater aquaculture fish from farms, analysis of freshwater aquaculture fish from markets and food premises, chemical risk assessment from farm to table are necessary actions for a sustainability of Vietnam Pangasius production.

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