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Investigation of square wave voltammetry for ciprofloxacin determination in black tiger shrimp

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Abstract
The antibiotic resistance that is developing globally in disease-causing bacteria is one of the major threats to human medicine. It leads to additional burdens on health systems, to treatment failures and, in the worst cases, to untreatable infections or infections treated too late to save life. Although the over-use of antibiotics in human medicine is the major cause of the current crisis of antibiotic resistance, public-health experts are agreed that the over-use and mis-use of antibiotics in intensive animal production is also an important factor – around half of the world's antibiotic production is used in farm animals. Ciprofloxacin is a case in point. Our study focused on square wave voltammetry or electrochemistry for screening ciprofloxacin residue in black tiger shrimp. This is evaluated as a promising application for small – medium enterprises in controlling antibiotic residues in general and ciprofloxacin in particular.

Keywords: Ciprofloxacin, voltammetry, electrochemistry, black tiger shrimp, promising application

1. Introduction
1.1 Ciprofloxacin
Ciprofloxacin is an antibiotic (second generation quinolones group, also known as fluoroquinolones group) which is used in livestock breeding and fisheries in our country to prevent and treat diseases in harm to the species of shrimp as black disease carrying, red body disease, disease played rong, swollen disease carrying, and for species of fish such as the red spot disease all previous diseases, viscosity, intestinal infections.

Noteworthy is ciprofloxacin and other fluoroquinolones are also used to prevent and treat human diseases because the antibiotic has a very broad antibacterial spectrum, including the bacteria gram (-) and gram (+), especially gram (-). Ciprofloxacin is specified in case of heavily infested as urinary tract inflammation, prostatitis, inflammation of the intestines of heavy infections, osteomyelitis, sepsis. There are also taking ciprofloxacin to prevent brain tissue and infection prevention for people with immune deficiencies. Also need to specify a range of side effects to the use of fluoroquinolones: gastrointestinal disturbances such as nausea, vomiting, diarrhea, abdominal pain, indigestion, causing headaches, insomnia and restlessness. These about ciprofloxacin and fluoroquinolones contributes to explain the countries with big market share of imported farmed seafood such as the us, EU, Japan ... the continuity given the higher requirements of strict control of antibiotic residues, the fluoroquinolones in aquatic products breeding. Recently, the results of control of antibiotic residues in aquatic products breeding, 2007 Department of fisheries shows: fluoroquinolones residues detected frequently in samples of black tiger shrimp and *Pangasius*. Pollution became a major challenge for the output of the country's fisheries industry.

1.2 Analyzer SQF-505
Analyzer SQF-505 is the modern electrochemical device works according to the principle of the square-wave scanning on an extreme slowly drop, running on the Windows XP operating system with the specialized operating software SQF-505. Active principles can be summarized as follows: Soluble substances need to analyze in a solution containing the proper ionic substance called aqueous background. Dipped in a solution of three electrodes: Working electrode is an extreme drop of mercury to flow slowly and steadily, in about 13 seconds for 7 drops. Electrode AgCl/Ag in saturated KCl has not changed. Support as a Platinum electrode. The square waves put on working electrode has two components: the d.c. increase gradually over time as your ladder with steps from 2 to 10mV; the a.c. is the perpendicular pulse frequency to a few hundred Hz and a pulse amplitude 10-40 mV.

How to measure the capacitor line counteract this, enhanced sensitivity. Universal often contain many vertices, each vertex represents a substance or chemical reactions, phase one of a substance. The top position is characterized qualitatively. The height of the top is characterized quantitatively. In the Analyzer measuring 505 SQW SQF-external mode-F (square-wave scanning on an ultra-slow drops) is also the mode of other measurements such as PSA-F (stripping square-wave ultra-fast on scan-drop slow). The principle of this technique is to: conduct physical measure contribution area for about 5 seconds of the life of a drop of mercury and proceed to measure the concentration of the substance that was in the area, the next two seconds. With the stage area of contributions are usually made metal ion reduction into metal and melts into the mercury in the form of amalgam, also with the organic process of donations made possible by the adsorption of organic substances to the surface drops of mercury. Thanks to this technique can increase the signal strength meter to range from 4 to 9 times.

1.3 Some notable studies about ciprofloxacin determination

Marika Kamberi *et al*. (1998) determined ciprofloxacin in plasma and urine by HPLC with ultraviolet detection. A simple, sensitive isocratic method for the detection and quantification of ciprofloxacin in plasma and urine has been developed. The assay consisted of reversed-phase HPLC with ultraviolet detection. Plasma proteins were removed by a fast and efficient procedure. For the urine samples, the only required sample preparation was dilution. Separation was achieved on a C_{18} reversed-phase column. The quantification limit was 0.01 mg/L in plasma and 0.5 mg/L in urine. This method was sufficiently sensitive for pharmacokinetic studies.

Najla Mohamad Kassab, *et al* (2005) conducted the quantitative determination of ciprofloxacin and norfloxacin in pharmaceutical preparations by high performance liquid chromatography.The objective of this research was to develop and validate an analytical method for quantitative determination of ciprofloxacin (CIP) and norfloxacin (NOR) in pharmaceutical preparations. A simple and rapid chromatographic method was developed and validated for quantitative determination of two fluoroquinolone antibiotics in tablets and injection preparations. The quinolones were analyzed by using a LiChrospher® 100 RP-18 column (5 µm, 125 x 4 mm) and a mobile phase consisted of water:acetonitrile:triethylamine (80:20:0.3 v/v/v). The pH of final mixture was adjusted to 3.3 with phosphoric acid. The flow rate was 1.0 mL/min and UV detection was made at 279 nm. The analyses were performed at room temperature (24 ± 2 ºC). CIP and NOR were eluted within 5 min. The calibration curves were linear (r > 0.9999) over a concentration range from 4.0 to 24.0 µg/mL. The relative standard deviation (RSD) was < 1.0% and the mean recovery was 101.85%.

A. Dincel *et al*. (2005) determined ciprofloxacin in human gingival crevicular fluid by high-performance liquid chromatography. A simple and selective high-performance liquid chromatographic (HPLC) method has been developed for determination of ciprofloxacin in human gingival crevicular fluid (GCF). GCF samples were collected with paper strips. Ciprofloxacin was extracted from the pooled strips with methanol–water, 50:50 (v/v), and separated on a cartridge column (Radial-pak C18, 100 mm × 8 mm, 10 µm) with acetonitrile–sodium dihydrogen phosphate, 2:8 (v/v), as mobile phase at a flow rate of 2 mL min−1. The effluent was monitored with a fluorescence detector at 280 nm (excitation) and 455 nm (emission). The retention times of ciprofloxacin and internal standard quinine sulphate were 4.55 and 13.25 min, respectively. The within-day and day-to-day precision were less than 9% for ciprofloxacin at 0.05, 0.1 and 0.4 µg mL−1 (n = 6), the within-day and day-to-day accuracy values were in the range 96.33–102.67% for ciprofloxacin at the concentrations given above and the detection limit corresponding to a signal-to-noise ratio of 3:1 was 3 ng mL−1. This method was suitable and selective for determination of ciprofloxacin levels in human GCF.

Anunciación Espinosa-Mansilla *et al*. (2006) applied HPLC determination of ciprofloxacin, cloxacillin, and ibuprofen drugs in human urine samples. This paper reports, for the first time, a liquid chromatographic method for the simultaneous determination of three frequently co-administered active principles, two antibiotics, ciprofloxacin (CIPRO) and cloxacillin (CLOXA) belonging to the fluoroquinolones and β-lactam families, respectively, and ibuprofen (IBU), a non-steroidal anti-inflammatory drug. The chromatographic separation was performed on a C-18 analytical column, using isocratic elution with methanol-acetonitrile-pH 3 formate buffer (C_T = 0.1 M) (15: 12: 73, v/v/v) for 3 min and, after that, a linear gradient with methanol-acetonitrile (88: 12, v/v) for 8 min. Several absorption spectra were obtained for each peak using a DAD detector. Chromatograms at the maximum absorption wavelength for each analyte, 220 nm for both IBU and CLOXA, and 280 nm for CIPRO were selected as the most suitable. The proposed chromatographic method requires about 15 min per sample. The presence of a urine background was tested and no interference was found. The method was satisfactorily applied to the determination of CIPRO, CLOXA, and IBU, in fortified urine, and in urine samples from a patient undergoing treatment with these three active principles, among others. Limits of quantification in urine were 1.00, 1.70, and 2.87 µg/mL for CIPRO, CLOXA, and IBU, respectively.

M. Seifrtova *et al*. (2008) determined fluoroquinolone antibiotics in hospital and municipal wastewaters in Coimbra by liquid chromatography with a monolithic column and fluorescence detection. The main goal of this work was determination of residues of the antibiotics ofloxacin (OFLO), norfloxacin (NOR), ciprofloxacin (CIPRO), and enrofloxacin (ENRO) in wastewater samples. The samples, after acidification to pH 4.5 and addition of EDTA, were extracted on an anion-exchange cartridge in tandem with an Oasis HLB cartridge. The LC–FD method, developed in previous studies, was based on application of a monolithic C18 column. The limit of quantification (LOQ) of the method was 250 ng L for OFLO,25ng/L for NOR and CIPRO, and 50 ng/L for ENRO. Mean recovery ranged between 75 and 121% for OFLO,NOR,CIPRO, and ENRO.

Shihn-Sheng Wu *et al*. (2008) examined the analysis of ciprofloxacin by a simple high-performance liquid chromatography method. A simple and sensitive high-performance liquid chromatographic method is described for the quantitative analysis of ciprofloxacin in pharmaceuticals and human plasma. The method employs reversedphase chromatography using an RP-C18 column with an isocratic mobile phase of acetonitrile–2% acetic acid aqueous solution (16:84, v/v), umbelliferone as an internal standard, and a

flow rate of 1.0 mL/min. The UV detector is set at 280 nm. The limit of detection is 0.25 µM (S/N = 3, injection volume = 10 µL). The regression equations are linear (r > 0.9999) over a range between 0.51~130 µM for the pharmaceutical analysis of ciprofloxacin and 0.51~64.8 µM for the biological analysis of ciprofloxacin in human plasma. The intra- and inter-day relative standard deviation and relative error are less than 3.39% and 5.71%, respectively. All the recoveries are greater than 93.8%. This method is successfully applied in a pharmacokinetic study of a volunteer who receives a 500 mg ciprofloxacin tablet.

Christel Grondin *et al*. (2011) verified the determination of ciprofloxacin in plasma by micro-liquid chromatography–mass spectrometry: An adapted method for neonates. After a simple protein precipitation, analytes were separated on a micro-liquid chromatography and quantified by mass spectrometry, with D8-ciprofloxacin as internal standard. The calibration range was linear from 25 to 3000 ng/mL. Intra- and inter-day precision was less than 2.4 and 4.1%, respectively. The acceptance criteria of accuracy (between 85 and 115%) were met in all cases. A plasma volume of 150 µL was required to achieve the limit of quantification of 25 ng/mL. The method was successfully applied to routine monitoring of ciprofloxacin in pediatric patients and also used in preclinical studies. It will be used to determine the population pharmacokinetic parameters of ciprofloxacin in neonates.

Brisa Marisol Flores-Miranda et a. (2012) demonstrated the accumulation and elimination of enrofloxacin and ciprofloxacin in tissues of shrimp *Litopenaeus vannamei* under laboratory and farm conditions. This study aimed to quantify the accumulation and elimination of Enrofloxacin (ENRO) and Ciprofloxacin (CIPRO) in cultivated Litopenaeus vannamei under controlled laboratory and farm conditions. Laboratory- and farm-raised shrimp were given feed supplemented with 200 mg/kg ENRO for 14 days, followed by a 16-day diet without antibiotics. The levels of ENRO and CIPRO were analyzed by High Performance Liquid Chromatography (HPLC). In the laboratory, ENRO concentrations in the muscle and hepatopancreas reached a maximum (Cmax) of 0.54 ± 0.26 µg/g and 3.52 ± 1.9 µg/g, respectively; Cmax values for CIPRO in the laboratory were 0.18 ± 0.13 µg/g (muscle) and 1.05 ± 0.20 µg/g (hepatopancreas). In farmed shrimp, Cmax values for ENRO were 0.36 ± 0.17 µg/g muscle and 1.60 ± 0.82 µg/g in the hepatopancreas; CIPRO Cmax values were 0.03 ± 0.02 µg/g (muscle) and 0.36±0.08 µg/g (hepatopancreas). Two to fourteen days were necessary to eliminate both antibiotics from muscular tissue and four to more fourteen days for complete elimination of the antibiotics from the hepatopancreas. These results should be considered in terms of minimum concentrations necessary to inhibit Vibrio bacteria to determine whether the current use of this antibiotic is effective in controlling disease. Purpose of our search is to apply square wave voltammetry to determine ciprofloxacin residue in shrimp tissue. This technique will create an applicable performance for seafood enterprises in screening their raw shrimp before processing.

2. Material & 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 solution are mixed from the solid or liquid concentrate with distilled twice to the concentration required.

3. Result & Discussion

3.1 Different factors affecting to analysis

Shrimp body, in addition to the basic chemical ingredients such as water, proteins, lipids etc., also contain small amounts of metal salts such as Ca, K, Fe, Al, Na, Mg..., in which the salt, K, Na, Mg, Ca constitute most. So in this paper Ireview the effects of the four elements above for the determination of ciprofloxacin in shrimp.

3.1.1 Effect of K^+

Mix a solution of K+ has a concentration of 500 mg/L; Sucked into the measuring cup 25 ml liquid background, add 100 µ L of original standard solution cipro into the measuring cup, then add each 100 µg/L solution of K+ into the measuring cup, to have the concentration of K+ in turn is 2, 4, 6, 8, 10mg/L, shake well and proceed with the measurement of parameters in optimal conditions. The results obtained in the table 1. From the results of the above table we noticed almost no K + ions affect the measuring result cipro.

Table 1: measuring results cipro without and with K^+

$[K^+]$ mg/L	E_{peak} (mV)	Current intensity, I (nA)				
		1st measure	2nd measure	3rd measure	Mean	s_i^2
0	-1336	388.5	395.4	397.5	393.8	22.170
2	-1336	386.1	391.4	390.8	389.4	8.423
4	-1336	387.6	388.1	388.0	387.9	0.070
6	-1336	392.8	389.4	384.2	388.8	18.760
8	-1336	381.3	389.1	384.2	384.9	15.543
10	-1336	380.8	378.2	384.5	381.2	10.023

3.1.2 Effect of Na+

Mix a solution of Na+ has a concentration of 500 mg/l. sucked into measuring cup 25 ml liquid background, add 100 µ l of original standard solution cipro into the measuring cup, then add each 100 µg/l solution of Na + into the measuring cup, to have the concentration of Na+ is 2, 4, 6, 8, 10 mg/l,

shake well and proceed with your parameters in optimal conditions. The results obtained in table 2. From the results obtained in the above table 2, we get influenced by Na+ ions not significant.

Table 2: Measurable results cipro with and without Na^+

$[Na^+]$ (mg/L)	E_{peak} (mV)	Current intensity, I (nA)				
		1st measure	2nd measure	3rd measure	Mean	s_i^2
0	-1336	391.5	391.7	401.5	394.9	32.680
2	-1336	396.8	398.2	400.9	398.6	4.343
4	-1336	400.7	396.4	390.3	395.8	27.310
6	-1336	387.1	391.9	394.8	391.3	15.123
8	-1336	393.1	387.0	404.6	394.9	79.870
10	-1336	393.1	389.7	387.6	390.1	7.703

3.1.3 Effect of Ca2+

Mix a solution of Ca^{2+} concentration was 500 mg/l. sucked into measuring cup 25 ml liquid background, add 100 µ l of original standard solution cipro into the measuring cup, then add each 100 µg/l solution of Ca^{2+} into the measuring cup to have the concentration of Ca^{2+} in turn is 2, 4, 6, 8, 10 mg/L, shake well and proceed with the measurement of parameters in optimal conditions. The results obtained in table 3. From the results obtained showed the concentration of Ca^{2+} in the survey do not affect the measuring result cipro.

Table 3: measurable results cipro without and with Ca^{2+}

$[Ca^{2+}]$ (mg/L)	E_{peak} (mV)	Current intensity, I (nA)				
		1st measure	2nd measure	3rd measure	Mean	s_i^2
0	-1336	430.0	432.5	434.7	432.4	5.293
2	-1336	429.3	432.5	440.9	434.2	35.893
4	-1336	437.5	434.9	420.2	430.9	87.023
6	-1336	435.5	438.1	431.0	434.9	12.903
8	-1336	436.4	427.7	435.8	433.3	23.610
10	-1336	423.6	433.7	423.0	426.8	36.143

3.1.4 Effect of Mg^{2+}

Mix a solution of Mg^{2+} concentration was 500 mg/l. sucked into measuring cup 25 ml of the solution in the background, add 100 µ l of original standard solution cipro into the measuring cup, then add one 100 µg/l solution of Mg^{2+} into the measuring cup to have the concentration of Mg^{2+} in turn is 2, 4, 6, 8, 10 mg/L, shake well and proceed with the measurement of parameters in optimal conditions. The results obtained in table 4. From the results obtained showed between the concentration of Mg^{2+} ions, the survey does not have the influence to measurable results cipro.

Table 4: Measurable results cipro without and with Mg^{2+}

$[Mg^{2+}]$ (mg/L)	E_{peak} (mV)	Current intensity, I (nA)				
		1st measure	2nd measure	3rd measure	Mean	s_i^2
0	-1326	412.3	412.6	418.9	414.6	13.890
2	-1326	421.1	411.9	415.3	416.1	21.640
4	-1326	430.0	424.1	423.6	425.9	12.670
6	-1326	427.7	415.4	420.2	421.1	38.430
8	-1326	417.5	423.9	426.7	422.7	22.240
10	-1326	422.2	420.4	430.0	424.2	26.040

3.2 Survey procedures and recovery performance

3.2.1 The survey of solvent extraction

3.2.1.1 Solvent extraction as ethanol (C_2H_5OH)

When the solvent extraction of ethanol is the ultimate solution to measure are fairly in. The result is shown in table 5.

Table 5: Recovery performance measurement results when filling with pure ethanol

Cipro (mg/L)	Current intensity, I (nA)		
	1st measure	2nd measure	3rd measure
0	234.47	281.9	284.57
0.203	273.9	338.4	338.6
0.407	313.6	396.1	395.97
0.611	357.9	445.47	444.4
0.815	395.63	491.03	490.9
Regression equations	y= 233.8+ 199.5x	y = 285.514+ 257.945x	y=287.196+ 254.57x
Correlation coefficients (R^2)	0.9998	0.997	0.998
The concentration of cipro are obtained (C_0=a/b)	1.1721	1.1069	1.128
Recovery performance $R_{th}=C_0/1.52745$	76.74%	72.47% R_{th} tb ≈ 74.4%	73.86%

3.2.1.2 Solvent extraction as methanol

When the solvent extraction is the final solution of methanol to measure is in compared to the case of solvent extraction of ethanol. The result is shown in table 6.

Table 6: Recovery performance measurement results when filling with pure methanol.

Cipro (mg/L)	Current intensity, I (nA)		
	1st measure	2nd measure	3rd measure
0	317.03	306.4	314.9
0.203	371.1	360.5	361.17
0.407	426.53	412.67	410.7
0.611	472.2	460.23	461.47
0.815	516.2	505.7	506.3
Regression equations	y= 320.604 + 245.47x	y = 309.434 + 244.68x	y = 314.288+ 237.2x
Correlation coefficients (R^2)	0.997	0.9986	0.9996
The concentration of cipro are obtained (C_0=a/b)	1.306	1.2646	1.3249
Recovery performance R_{th} =C_0/1.52745	85.5%	82.79% R_{th} tb ≈ 85.0%	86.74%

Table 7: Recovery performance measurement results when extracting with methanol/ acetone (1/1, v/v).

Cipro (mg/L)	Current intensity, I (nA)		
	1st measure	2nd measure	3rd measure
0	268.8	254.1	274.77
0.203	313.4	310.53	313.1
0.407	365.23	355.4	363.63
0.611	408.43	399.37	419.5
0.815	445.87	436.8	453.6
Regression equations	y = 270.29 + 220.9x	y = 260.392 + 223.04x	y = 272.108 + 227.86x
Correlation coefficients (R^2)	0.997	0.9945	0.994
The concentration of cipro are obtained (C_0=a/b)	1.2236	1.1675	1.1942
Recovery performance R_{th} =C_0/1.52745	80.1%	76.43% R_{th} tb ≈ 78.2%	78.18%

3.2.1.4 Solvent extraction as methanol/acetonitrile (5/1, v/v)

With solvent extraction is the mixture of Methanol and Acetonitrile in volume ratio is 5: 1, the ultimate solution to measure quite in and measuring results obtained shown in table 8.

Table 8: Recovery performance measurement results when extracting with methanol/acetonitrile (5/1, v/v)

Cipro (mg/L)	Current intensity, I (nA)		
	1st measure	2nd measure	3rd measure
0	334.77	337.77	347.03
0.203	388.17	383.6	394.43
0.407	436.57	438.73	438.27
0.611	486.07	487.57	483.77
0.815	529.87	529.8	547.3
Regression equations	y= 337.47 + 239.66x	y = 337.888 + 239.63x	y = 346.362 + 240.53x
Correlation coefficients (R^2)	0.9997	0.999	0.997
The concentration of cipro are obtained (C_0=a/b)	1.408	1.41	1.44
Recovery performance R_{th} =C_0/1.52745	92.19%	92.3% R_{th} tb ≈92.9%	94.27%

Remarks: after examining a number of different extraction solvents are found with solvent extraction is the mixture of methanol and acetonitrile in volume ratio is 5: 1, the recovery performance of the process of extracting the best, so in the next experiment, I choose this solvent system to proceed. The final solution used to measure just the pretty in (i.e. also the lubricant). To reduce viscosity, then at that stage (4) 1 ml of hexane extraction process more well, giving centrifuge to remove the hexane layer, then proceed period (5) as the other experiments. The results show that the solution ultimately is out but not back higher performance, perhaps in part the substance hexane layer diffusion cipro removed should be losing a part. Concrete results are presented in table 9.

Table 9: Recovery performance measurement results when extracting with methanol/ acetonitrile (1/5, v/v) Hexane has added to the fatty removal

Cipro (mg/L)	Current intensity, I (nA)		
	1st measure	2nd measure	3rd measure
0	348.1	344.2	347.2
0.203	428.9	429.5	428.8
0.407	484.4	488.1	484.3
0.611	547.5	545.7	547.2
0.815	608.1	607.5	612.2
Regression equations	y = 355.68 + 313.56x	y = 354.44 + 315.92x	y = 354.26 + 318.37x
Correlation coefficients (R^2)	0.996	0.9936	0.9965
The concentration of cipro are obtained (C_0=a/b)	1.134	1.1223	1.1127
Recovery performance R_{th}= Co/1.52745	74.26%	73.52% R_{th} tb ≈ 73.5%	72.85%

Therefore, the following experiments were conducted with mixed solvents methanol and acetonitril proportion by volume is 5: 1. To evaluate the sensitivity of our analysis process based on regression equations to calculate the limit of detection (LOD). With solvent extraction is methanol and acetonitril proportion by volume is 5: 1 have LOD based on the results of the table 8.

Particular: *Experiment #1: Regression equation: y= 337.47 + 239.66x and R2 = 0.9997; Sresidue = 2.934; choose m = 4 so f = N + m -2 = 5 + 4 -2 =7; t0.95,f=7 = 2.36
LOD = 0.0194 mg/L = 19.4µg/L.
With the calculation of similar for us the following results:
* Experiment #2: LOD = 0.0246 mg/L = 24.6 µg/L.
* Experiment #3: LOD = 0.0434 mg/L = 43.4 µg/L.
So the average value of LOD after three times the experiment is: (19.4 + 24.6 +43.4)/3 = 29.1 µg/g/l. From this result shows the limit of detection values smaller than 100 µ g/L is the limit of cipro in aquatic products breeding.

3.2.2 Recovery performance of cipro in blank sample

In the experiments on above was for a blank sample of 150 µg/L standard stock solution and cipro eventually measure the concentration of cipro respectively as 1.52745 mg/l. Far more cipro the original standard in turn was 100 µg/L and 50 µg/L for aqueous concentrations finally metering 1.0183 mg/L and 0.50915 mg/L if the recovery performance of the process reaches 100%.

3.2.2.1 Cipro addition with concentration 1.52745mg/L

Have conducted experiments in the 3.2.1.4 with R_{th} tb ≈ 92.9%.

3.2.2.2 Cipro addition with concentration 1.0183mg/L

Table 10: Performance measurement result to recover the cipro added is 1.0183mg/L.

Cipro (mg/L)	Current intensity, I (nA)		
	1st measure	2nd measure	3rd measure
0	203.8	200.1	184.3
0.203	244.4	236.1	222.8
0.407	285.2	285.5	262.6
0.611	324.9	327.3	304.4
0.815	373.0	369.0	344.9
Regression equations	y = 201.702 + 206.89x	y= 197.74 + 210.74x	Y= 183.24 + 197.78x
Correlation coefficients (R^2)	0.9989	0.999	0.9999
The concentration of cipro are obtained (C_0=a/b)	0.9749	0.9383	0.926
Recovery performance R_{th} = Co/1.0183	95.74%	92.14% R_{th} tb ≈ 93.0%	90.98%
LOD (µg/L)	20.8	24.1 LODtb = 17.8	8.6

3.2.2.3 Cipro addition with concentration 0.50915mg/L

The average value of performance recovered after adding white form three different concentration values of the original standard solution are: Rthtb = (92.9% + 93.0% + 92.7%)/3 ≈ 92.9%. On the other hand we also have the LOD value on average after adding a blank sample with three different concentration values of the original standard solution are: LODtb = (29.1 + 17.8 + 27.6)/3 = 24.8 µg/L <100 µg/L.

Table 11: Performance measurement results to recover the cipro added 0.50915 mg/L.

Cipro (mg/L)	Current intensity, I (nA)		
	1st measure	**2nd measure**	**3rd measure**
0	88.67	97.76	**99.06**
0.203	136.0	134.0	**137.7**
0.407	173.7	184.3	**184.7**
0.611	215.6	225.4	**226.0**
0.815	248.4	263.0	**265.0**
Regression equations	y = 92.662 + 195.94x	y = 96.516 + 207.15x	**y= 98.456 + 206.31x**
Correlation coefficients (R^2)	0.998	0.999	**0.999**
The concentration of cipro are obtained (C_0=a/b)	0.4729	0.466	**0.4772**
Recovery performance R_{th} = Co/0.50915	92.88%	91.51% R_{th} tb ≈ 92.7%	**93.72%**
LOD (µg/L)	34.4	29.8 LODtb = 27.6	**18.5**

3.3 Cipro analysis in shrimp sample

Table 12: Cipro analysis in shrimp sample

Black tiger shrimp sample	Result
Gia Dinh Ltd.Co, packed 17/06/13	Not detected
An Vinh Ltd. Co, packed 29/08/13	Not detected
Kim Anh Ltd. Co, packed 24/08/13	Not detected

4. Conclusion

One of the major food safety concerns of the imported markets is the presence of unapproved antibiotics and antifungal chemicals in imported seafood. This is of particular concern because consumption of seafood has continued to increase to the point where currently. They have found evidence that several unapproved antibiotics inclusing ciprfloxacin have been used in aquaculture in some countries. Finding a simple technique as voltammetry is very essential and applicable for small and medium enterprise in Vietnam to overcome challenges of technical barriers from imported countries.

5. References

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