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Simultaneous determination of erythromycin A in giant prawn and tilapia in Mekong region by stripping square wave voltammetry

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Abstract: Erythromycin A (EA) is now one of antibiotics limited in seafood products in general and in giant freshwater prawns (*Macrobrachium rosenbergii*) and tilapia (*Oreochromis niloticus*) in particular while exporting to the US, EU, Japan, Canada. There are many methods used for analyzing this antibiotic in these aquatic species (e.g., ELISA, HPLC, LC-MS/MS, GC-MS). A new, sensitive, analytical approach for determination of erythromycin A using stripping square wave voltammetry at the slowly dropping mercury electrode was developed and validated to quantify this antibiotic with simple and short time analysis; the method is inexpensive and performs best at moderate concentrations of erythromycin A. Electrochemical signals were measured at potential wave -1430 mV. The optimal experimental parameters for the method were: supporting electrolyte ammonium acetate 0.1 M, pH 8.0, the solvents for dissolving erythromycin standard: acetonitril, V_{start} = -400 mV, V_{stop} = -1700 mV, V_{step} = 6 mV, V_{pulse} = 40 mV, T_{drop} = 5000 ms , $V_{elctrolise}$ = -1100 mV, $T_{electrolise}$ = 5 s. The method showed high recovery (85.07 ÷ 96.50%), high sensitivity (lower limit of detection, LoD = 0.57 µg.kg^{-1} in giant prawn and LoD = 0.52 µg.kg^{-1} in tilapia) and high precision (RSD 0.91 ÷ 2.1%) as well as excellent linearity ($r^2_{adjusted} \geq 0.99999$).

Keywords: Erythromycin A, giant freshwater prawn, tilapia, stripping square wave voltammetry, dropping mercury electrode

Introduction

Giant freshwater prawn (*Macrobrachium rosenbergii*) and Nile tilapia (*Oreochromis niloticus*) have been considered two of the most important species of freshwater aquaculture in Viet Nam, especially in the Mekong River Delta. Bacterial necrosis is a common disease observed in adult prawns. Bacterial necrosis has variously been termed as 'black spot', 'brown spot', 'shell disease' or chitinolytic bacterial disease. It is caused by the invasion of chitinolytic bacteria, which break down the chitin of the exoskeleton. *Aeromonas hydrophila*, *A. caviea*, *A. sorbia* and *Aeromonas* sp. All have been isolated from necrosis prawns. *Pseudomonas fluorescens*, *Aeromonas* sp., *Lactococcus garvieae* and *Edwardsiella tarda* were bacteria flora isolated from adult prawns. Meanwhile, the most significant diseases in Nile tilapia (*Oreochromis niloticus*) culture are caused by *Streptococcus iniae*, *Aeromonas hydrophila*, *Trichodina* sp., *Flexibacter* and *Edwardsiella* spp.

Erythromycins are broad spectrum antibiotics that exhibit high activity against nearly all Gram-positive and Gram-negative bacteria. Erythromycin A consists of a polyhydroxylactone and two sugars (Figure 1). Erythromycin is the antibiotic of choice against *Aeromonas hydrophila*, *A. caviea*, *A. sorbia* , *Aeromonas* sp. and *Pseudomonas fluorescens* [1, 2, 4, 16, 19, 22, 23].

According to Codex regulation (e.g., WHO/FAO, EU, US, Canada, Australia), erythromycin residue in seafood muscle must be lower than 100 µg/kg. Viet Namese Ministry of Agriculture and Rural Development regulates erythromycin as a limited antibiotic with maximum residual limit 200 µg/kg. Methods for erythromycin analysis have evolved rapidly in the last 15 years (Table 1).

The aim of this work was to develop a fast scanning square wave voltammetry using a dropping mercury electrode to quantify erythromycin A. The results demonstrated that it could be used as a simple and rapid analytical screening technique for the detection of erythromycin in giant freshwater prawn and tilapia muscle.

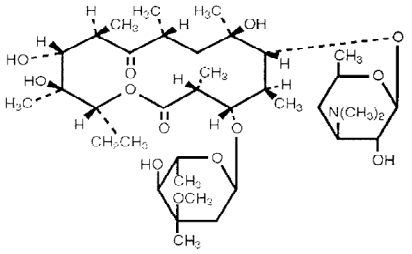

Figure 1. Chemical structure of erythromycin A

Materials and Methods

Reagents

The high purity antibiotic standards of erythromycin A, chloramphenicol, furazolidone, florfenicol, ciprofloxacin, colistin, and malachite

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Table 1. Some published papers on the methods of quantifying Erythromycin A from different samples

Year	Author	Sample	Method	LoD (µg/kg)
1994	Zierels G[26]	Egg, muscle, milk, liver, kidney of swan	HPLC	<10
1998	Yong-Xi Li [25]	Human plasma	LC-MS/MS	0.5*
1999	Kondo T[15]	Human plasma	LC-MS/MS	0.05*
2000	Dreassi E [7]	Plasma: beef, pork, poultry	HPLC-UV	250*
		Milk	HPLC-UV	25*
		Kidney, liver, muscle, gan, fat of beef, pork, poultry.	HPLC-UV	125*
2000	Huaisheng Wang [12]	Drug, urine.	ASV-PGCE	5
LC-FL	Carmen Leal [3]	Chicken		400
ELISA	R. Draisci [8]	Beef		0.4
		Muscle and liver of beef	LC-MS/MS	50*
		Kidney of beef	LC-MS/MS	80*
2003	Stanley M. Billedeau [20]	Salmon	LC – ESI/MS	5, 16*
2003	Horie Masakazu [10]	Meat and seafood	LC- ESI-MS	10
2003	Michael P. Sche [17]	Manure	HPLC-MS/MS	0.4-11
2005	W. Xiao [24]	Drugs (propionate, base)	HPLC-ESI-MS	1
2006	A. Deubel [5]	Muscle	LC-MS/MS	0.25
2006	Hui Yun – Hua [13]	Tilapia	HPLC	400
2006	Jian Wang [14]	Fresh milk	LC-ESI/MS/MS	0.07
2006	Tang HP [21]	Meat	LC-MS/MS	0.1
2007	Deng B [6]	Rat plasma	ECL	0.35
2008	Berrada Houda [11]	Meat and seafood	LC-ESI/MS	25
2009	Granja R [9]	Honey	LC-MS/MS	1.27, 5.0*
2009	P. Norouzi [18]	Human plasma, urine.	CV	2.4, 7.0*

* LoQ

green were purchased from Vietnam Central Institute of Pharmacy. Methanol and acetonitrile (HPLC grade) were obtained from J. T. Baker. All reagents were analytic grade and all solutions were prepared by dissolving appropriate weights in bi-distilled water.

Apparatus

A fast scanning, stripping, square wave voltammetry at the slowly dropping mercury electrode was performed in the ANALYZER SQF-505. The mercury dropping electrode was used as a working electrode, silver/silver chloride (saturated KCl) as a reference electrode, and a platinum wire as an auxiliary one.

Sample extraction and clean-up procedure

Primary extraction

A 5 g aliquot of a blank or spiked minced muscle sample was mixed with a small volume of erythromycin standard. After a 15 min equilibration period, the tissues were mixed vigorously for 15 min with 25 ml Tris buffer (0.1M; pH 10.5). After a 10 min centrifugation at 3000 g and 4°C, the supernatant was transferred to a polypropylene tube and the solid residue extracted a second time with 25 ml Tris buffer. Acetic acid (600 µl) and 5 ml sodium tungstate buffer (0.15M) were added to precipitate the proteins. After equilibration for one hour at 4°C, the samples were centrifuged at 3000 g for 10 min. The supernatants were further filtered through a plug of glass wool.

Solid phase extraction

The 6-cm^3 HLB OASIS extraction cartridges (200 mg) were prepared and conditioned with 10 ml methanol and 10 ml water. The biological samples were placed at the top of the column. Two wash solution volumes were applied before erythromycin elution: 20 ml methanol-water (5:95, v/v) and 5 ml hexane. After the last washing step, the OASIS columns were vacuum-dried for 10 min. Erythromycin was finally eluted with 5 ml methanol-ammonia 30% (95:5, v/v) and evaporated dry under a nitrogen flow. The extracts were dissolved in 500 µl NH_4AC-ACN (80/20 v/v), transferred to Eppendorf tubes and centrifuged at 3000 g for 10 min. Aliquots of the supernatant were transferred into the voltammetric cell with 2,500 mL of ammonium acetate 0.1 M, pH 8.0 before being quantified by Analyzer SQF-505 in mode stripping, square wave, voltammetry.

Results and Discussion

Voltammetric behavior of erythromycin at the slowly dropping electrode

Effect of supporting electrolytes and pH values on the adsorptive peak current of erythromycin has been strongly affected by the type of supporting electrolyte. To study the adsorptive behavior of erythromycin, different supporting electrolytes including sodium acetate, ammonium acetate, citrate-phosphate, borax, and Tris buffers were examined. Ammonium acetate buffer is recommended to complete these studies because erythromycin showed the highest peak current and the best peak shape (Table 2).

The effect of pH of ammonium acetate buffer on the peak current was examined from 7.0 to 10.0. Erythromycin showed highest peak current at pH 8.0 ($E_{1/2}$ = -1438 mV, I = 351.7 ± 5.7 nA). Hence ammonium acetate buffer (pH 8.0) was selected for further investigations

The effect of the ionic strength of supporting electrolyte was examined at pH 8.0 over the range from 0.05÷0.25 M. Erythromycin showed highest peak current at ammonium acetate 0.1M ($E_{1/2}$ = -1438 mV, I = 254.8 ± 10.2 nA). So this value was selected for further studies (Table 3).

Effect of the solvents for disolving erythromycin A standard on the peak current were examined. Among methanol, acetonitril, and ethyl acetate, the peak current increased with a maximum at acetonitril ($E_{1/2}$ = -1438 mV, I = 189.2 ± 3.5 nA). So acetonitril was selected for subsequent work (Table 4).

Table 2. Peak current of erythromycin A was affected by supporting electrolytes, pH values

pH	5.0		6.0		7.0		8.0		9.0		10.0	
Supporting electrolytes	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)
Natri acetate	56.67^{b} ± 1.1	1.9	35.5^{a} ± 2.3	6.6	255.5^{d} ± 7.3	2.8	333.4^{e} ± 4.7	1.4	236.2^{c} ± 7.7	3.3	254^{d} ± 4.7	1.9
Ammonium acetate					210.3^{a} ± 5.4	2.6	**351.7^{d} ± 5.7**	1.6	216.5^{b} ± 2.7	1.2	263.1^{c} ± 1.6	0.6
Citrat-phosphate			207.7^{d} ± 6.0	2.9	168.2^{a} ± 2.3	1.4	194.4^{b} ± 2.6	1.4	229.4^{e} ± 2.1	0.9	200.9^{c} ± 2.0	1.0
Tris					180.0^{d} ±13.1	7.3	27.5^{a} ± 0.3	1.1	53.4^{b} ± 3.6	6.7	126.4^{c} ± 9.7	7.7
Borax							168.1^{a} ± 1.6	0.9	255.5^{c} ± 6.5	2.6	173.9^{b} ± 1.8	1.0

* Each value was the mean of 5 samples (n = 5).

Table 3. Erythromycin A peak current was affected by ionic strength of ammonium acetate

Ionic strength (M)	0.05		0.1		0.15		0.2		0.25	
	Mean ±SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)
	210^{c} ± 7.1	3.4	**254.8^{d} ± 10.2**	4.0	213.7^{c} ± 10.1	4.7	192.1^{b} ± 2.2	1.1	173.2^{a} ± 1.8	1.0

* Each value was the mean of 5 samples (n = 5).

Table 4. Peak current of erythromycin A was affected by solvents

Ethyl acetate		Acetonitril		Methanol	
Mean± SD	RSD (%)	Mean± SD	RSD (%)	Mean ± SD	RSD (%)
183.6^{b} ± 1.5	0.8	**189.2^{c} ± 3.5**	1.9	166.3^{a} ± 5.5	3.3

* Each value was the mean of 5 samples (n = 5).

Table 5. Peak current of erythromycin A was affected by V_{start}

V_{start} (mV)	-400	-500	-600	-700	-800	-900	-1000	-1100
Mean ± SD	**106.5^{g} ± 4.7**	97^{f} ± 3.4	79.5^{e} ± 0.8	71.5^{d} ± 4.3	93.3^{f} ± 4.0	45.0^{c} ± 2.6	23.4^{b} ± 1.0	14.8^{a} ± 0.9
RSD (%)	4.4	3.5	1.0	6.0	4.3	5.8	4.3	6.2

* Each value was the mean of 5 samples (n = 5).

Optimization of measurement conditions

Effect of forward scanning (0 to -1800 mV) and reverse scanning (-1800mV to 0) on the peak current signal was examined. The forward scanning (0 to -1800 mV) showed high peak. Meanwhile, the peak current of the reverse scanning (-1800mV to 0) was too low. So the forward scanning (0 to -1800 mV) was chosen for further investigations.

Effect of V_{start}. Mode PSA-F, forward scanning, V_{stop}: -1800 mV, V_{step}: 4 mV, V_{pulse}: 30 mV, T_{drop}: 3000 ms, $V_{electrolise}$: -700 mV, $T_{electrolise}$: 6 s, $T_{stabilize}$: 1 s. Examining V_{start} from -400 mV to -1100 mV. V_{start} was optimum at -400 mV ($E_{1/2}$ = -1430 mV, I = 106.5 ± 4.7 nA) (Table 5).

Effect of V_{stop}. Mode PSA-F, forward scanning, V_{start}: -400 mV, V_{step}: 4 mV, V_{pulse}: 30 mV, T_{drop}: 3000 ms, $V_{electrolise}$: -700 mV, $T_{electrolise}$: 6 s, $T_{stabilize}$: 1 s. Examining V_{stop} from -1700 mV to -2000 mV. V_{start} was optimum at -1700 mV ($E_{1/2}$ = -1430 mV, I = 136.7 ± 3.9 nA) (Table 6).

Effect of V_{step}. Mode PSA-F, forward scanning, V_{start}: -400 mV, V_{stop}: -1700 mV, V_{pulse}: 30 mV, T_{drop}: 3000 ms, $V_{electrolise}$: -700 mV, $T_{electrolise}$: 6 s, $T_{stabilize}$: 1 s. Examining V_{step} from 4 mV to 10 mV. V_{step} was optimum at 6.0 mV ($E_{1/2}$ = -1430 mV, I = 214.6 ± 13.1 nA) (Table 7).

Effect of V_{pulse}. Mode PSA-F, forward scanning, V_{start}: -400 mV, V_{stop}: -1700 mV, V_{step}: 6.0 mV, T_{drop}: 3000 ms, $V_{electrolise}$: -700 mV, $T_{electrolise}$: 6 s, $T_{stabilize}$: 1 s. Examining V_{pulse} from 10 mV to 40mV. V_{pulse} was optimum at 40 mV ($E_{1/2}$ = -1430 mV, I = 692.6 ± 14.9 nA) (Table 8).

Effect of T_{drop} Mode PSA-F, forward scanning, V_{start}: -400 mV, V_{stop}: -1700 mV, V_{step}: 6 mV, V_{pulse}: 40 mV, $V_{electrolise}$: -700 mV, $T_{electrolise}$: 6 s, $T_{stabilize}$: 1 s. Examining T_{drop} from 1000 ms to 5,000 ms. T_{drop} was optimum at 5,000 ms ($E_{1/2}$ = -1430 mV, I = 381.3 ± 2.9 nA) (Table 9).

Effect of $T_{electrolise}$ Mode PSA-F, forward scanning, V_{start}: -400 mV, V_{stop}: -1700 mV, V_{step}: 6 mV, V_{pulse}: 40 mV, T_{drop}: 5,000 ms, $V_{electrolise}$: -700 mV, $T_{stabilize}$: 1 s. Examining $T_{electrolise}$ from 3s to 6s. $T_{electrolise}$ was optimum at 5 s ($E_{1/2}$ = -1430 mV, I = 1717.0 ±13.7 nA) (Table 10).

Effect of $V_{electrolise}$ Mode PSA-F, forward scanning, V_{start}: -400 mV, V_{stop}: -1700 mV, V_{step}: 6 mV, V_{pulse}: 40 mV, T_{drop}: 5,000 ms, $T_{electrolise:}$ 5 s, $T_{stabilize}$: 1 s. Examining $V_{electrolise}$ from -400 mV to -1400 mV.

Table 6. Peak current of erythromycin A was affected by V_{stop}

V_{stop} (mV)	-1500.0	-1600.0	-1700.0	-1800.0
Mean ± SD	104^{a} ± 1.0	118.2^{b} ± 1.4	**136.7^{c} ± 3.9**	120.1^{b} ± 1.1
RSD (%)	0.9	1.2	2.9	0.9

* Each value was the mean of 5 samples (n = 5).

Table 7. Peak current of erythromycin A was affected by V_{step}

V_{step} (mV)	4.0	6.0	8.0	10.0
Mean ± SD	162.8^{a} ± 5.8	**214.6^{c} ± 13.1**	176.9^{b} ± 8.3	165.0^{ab} ± 10.9
RSD (%)	3.5	6.1	4.7	6.6

* Each value was the mean of 5 samples (n = 5).

Table 8. Peak current of erythromycin A was affected by V_{pulse}

V_{pulse} (mV)	10	20	30	40
Mean ± SD	230.6^{a} ± 2.7	388.4^{b} ± 12.7	528.0^{c} ± 8.0	**692.6^{d} ± 14.9**
RSD (%)	1.2	3.3	1.5	2.2

* Each value was the mean of 5 samples (n = 5).

Table 9. Peak current of erythromycin A was affected by T_{drop}

T_{drop} (ms)	1,000	2,000	3,000	4,000	5,000
Mean ± SD	128.3^{a} ± 1.2	197.9^{b} ±1.9	269.1^{c} ± 12.9	323.2^{d} ±3..3	**381.3^{e} ± 2.9**
RSD (%)	0.9	1.0	4.8	1.0	0.8

* Each value was the mean of 5 samples (n = 5)

Table 10. Peak current of erythromycin A was affected by $T_{electrolise}$

$T_{electrolise}$ (s)	3	4	5	6
Mean ± SD	1353.6^{a}± 10.8	1555.4^{b} ±13.0	**1717.0^{d} ±13.7**	1655.4^{c} ±5.0
RSD (%)	0.8	0.8	0.8	0.3

* Each value was the mean of 5 samples (n = 5)

Table 11. Peak current of erythromycin A was affected by $V_{electrolise}$

$V_{electrolise}$ (mV)	-400.0	-900.0	-1100.0	-1400.0
Mean ± SD	1709.0^{b}± 17.3	1815.0^{c} ±3.7	1863.2^{c} ± 24.1	1593.4^{a} ±13.5
RSD (%)	1.0	0.2	1.3	0.8

* Each value was the mean of 5 samples (n = 5)

$V_{electrolise}$ was optimum at -1100 mV ($E_{1/2}$ = -1438 mV, I = 1863.2 ± 24.1 nA) (Table 11).

Calibration

For the calibration curves and detection limit, a 25 mL supporting electrolyte ammonium acetate 0.1M, pH 8.0 was transferred to the cell and spiked with 5 µL, 10 µL, 20 µL, 30 µL, 40 µL, 50 µL, 60 µL, 70 µL, 80 µL, 90 µL of stock 250 ppm solution of erythromycin in pure acetonitril. The concentrations of erythromycin in the cell were 50 µg/kg, 100 µg/kg, 200 µg/kg, 300 µg/kg, 400 µg/kg, 500 µg/kg, 600 µg/kg, 700 µg/kg, 800 µg/kg, 900 µg/kg respectively. Mode PSA-F, V_{start}: -400 mV, V_{stop}: -1700 mV, V_{step}: 6 mV, V_{pulse}: 40 mV, T_{drop}: 5000 ms, $T_{electrolise}$: 5 s, $V_{electrolise}$: - 1100 mV, $T_{stabilize}$: 1 s.

A detection limit of 0.57 µg/kg was obtained for erythromycin. A linear behavior was also observed with a correlation coefficient r^2_{adjust} = 1.0 (Figure 2).

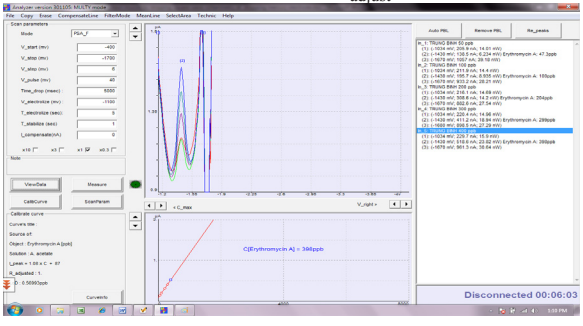

Figure 2. Calibration curve of erythromycin A

Interference

Effect of Interferences on the effect of co-existing ions was examined by introducing different concentrations of selected ions to the voltametric cell and recording the corresponding voltammogram using the conditions selected above. It was observed that the additions of 0÷5 ppm K^+, Na^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cl^-, SO_4^{2-}, HPO_4^{2-} ions had no effect (< ±5%) on the peak response.

Sample analysis

In giant freshwater prawn samples, the recovery rate were: 90.40 ÷ 96.50 %, LoD: 0.80 µg.kg^{-1}, R^2_{adjust}: 0.99999, RSD: 0.91 ÷ 1.58 %. (Figure 3). In tilapia samples the recovery rate were: 85.07 ÷ 88.56%, MDL: 0.52 µg.kg^{-1}, R_adjust: 1.0, RSD: 0.80 ÷ 2.10 %. (Fig. 4)

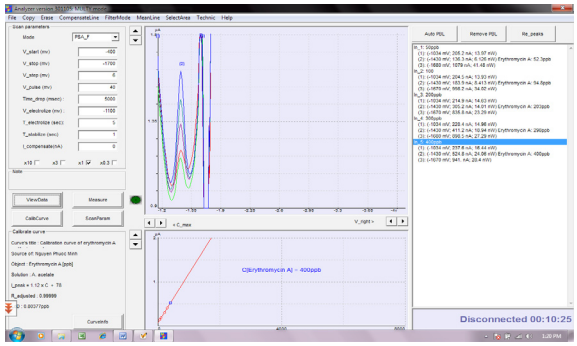

Figure 3. Calibration curve of erythromycin A in giant prawn muscle

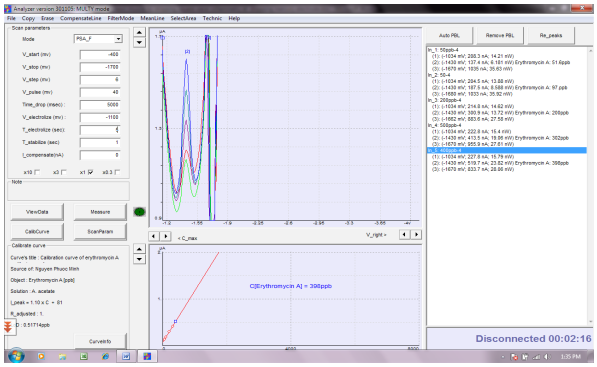

Figure 4. Calibration curve of erythromycin A in tilapia sample

For the comparison results between SWV and LC-MS/MS method, dosed giant prawn and tilapia samples were obtained through medication at 100 mg erythromycin·kg^{-1} prawn body weight^{-1}·d^{-1} for 7 days; sampled at 7, 8, and 9 days post-dosing. These dosed samples (high, medium, low) were divided in two groups: samples in group A were analyzed by Square Wave Voltammetry, samples in group B were controlled by LC-MS/MS via Intertek Vietnam Ltd. Close homogeneity could be seen between the two analyzing methods (Table 12, 13).

Validation of quantification method

Linearity

The linearity was evaluated by least squares, linear regression. The calibration curves constructed for erythromycin were linear over the concentration range of 50 ÷ 400 µg/kg. Peak areas of erythromycin were plotted versus its concentration and linear regression analysis performed on the resultant curve. A correlation coefficient of R^2_{adjust} = 1.0 with %R.S.D. values ranging from 0.4 ÷ 3.7 % across the concentration range studied were obtained (Table 14).The regression equation for the calibration curve was Y=1.08*X + 87.

LOD

The LOD was the lowest amount of measured analyte that may be detected to produce a response which is significantly different from that of a blank. Limit of detection was obtained by calculations based on the standard deviation of the response (δ) (here the current) which is obtained from blank with 5 replicas and (S) is the slope of the calibration curve according to equation LOD=3.3(δ/S). The LOD for erythromycin was 0.57 µg/kg (Figure 2).

Precision, accuracy and recovery

Precision was investigated by the intra- and inter-day (n = 6) assays at three different concentrations with respect to both repeatability and reproducibility. Repeatability was investigated by injecting six replicate samples of each of the 100, 200, 300 µg/kg standards. Inter-day precision was assessed by injecting the same three concentrations over 3 consecutive days. Accuracy (relative error, RE, %) was calculated by assessing the agreement between measured and nominal concentrations of the fortified samples. Recovery was assessed as erythromycin A concentrations of 100, 200, 300 µg/kg and the mean value was calculated (Table 15, 16; Figure 5, 6).

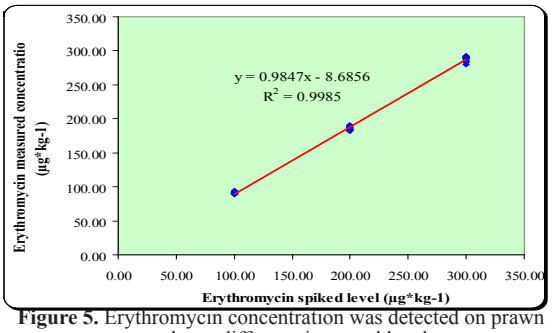

Figure 5. Erythromycin concentration was detected on prawn samples at different times and levels

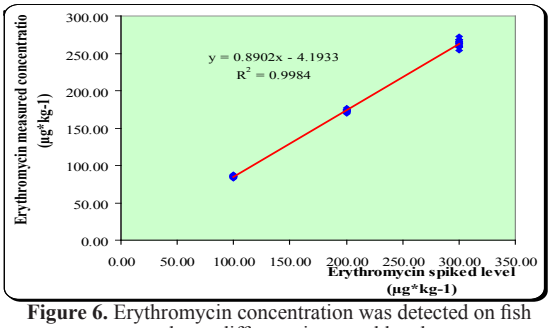

Figure 6. Erythromycin concentration was detected on fish samples at different times and levels

Differentiation

The differentiation of the method was checked by monitoring standard solutions of erythromycin A in the presence of other antibiotic components. The peak response ($E_{1/2}$) of erythromycin A ($E_{1/2}$ = -1430 mV) was separated, independent and distinguished from ones obtained in chloramphenicol ($E_{1/2}$ = -196 mV), furazolidone ($E_{1/2}$ = -1152 mV), florfenicol ($E_{1/2}$ = -78 mV), enrofloxacin, ciprofloxacin ($E_{1/2}$ = -1336 mV), colistin ($E_{1/2}$ = -1120 mV) malachite green ($E_{1/2}$ = -1228 mV). Hence, the determination of erythromycin by SWV was considered having not only "screening" but also "confirming" abilities.

Application

Giant freshwater prawn and tilapia samples from ten provinces in the Mekong River Delta were analyzed to survey the erythromycin residue. Residual results are displayed figures 7 and 8.

Conclusion

A new analytical procedure based Square Wave

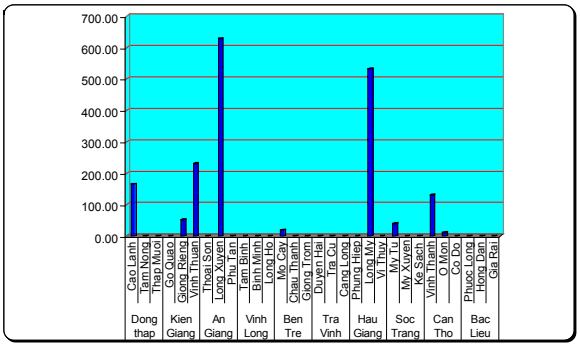

Figure 7. Erythromycin surveillance in prawn aquaculture at ten provinces, three districts in each province of Mekong Region, Vietnam

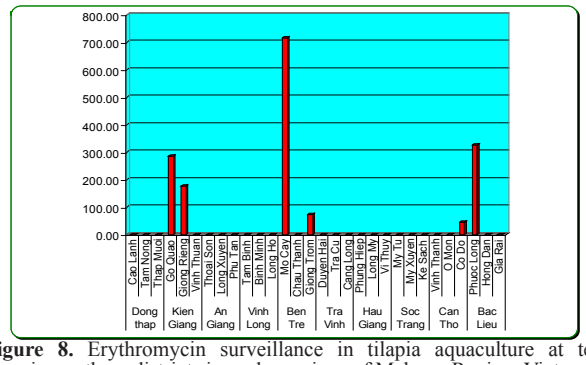

Figure 8. Erythromycin surveillance in tilapia aquaculture at ten provinces, three districts in each province of Mekong Region, Vietnam

Table 12. Comparison homogeneity of two analyzing methods in giant prawn muscle

Sample I.D	SWV (LOD = 0.8 µg*kg^{-1})			LC-MS/MS (LOD = 10 µg*kg^{-1})		
	Result (µg*kg^{-1})	Mean ± SD (µg*kg^{-1})	RSD (%)	Result (µg*kg^{-1})	Mean ± SD (µg*kg^{-1})	RSD (%)
GP – Blank	N.D	N.A	N.A	N.D	N.A	N.A
GP – Blank	N.D			N.D		
GP – Blank	N.D			N.D		
GP – I	51.37			50.73		
GP – I	50.19	50.75^{a} ± 0.59	1.17	56.89	52.61^{a} ± 3.72	7.06
GP – I	50.68			50.21		
GP – II	65.45			72.82		
GP – II	64.97	65.48^{b} ± 0.53	0.80	68.00	70.49^{b} ± 2.41	3.42
GP – II	66.02			70.65		
GP – III	80.61			74.60		
GP – III	79.16	80.00^{c} ± 0.75	0.94	85.12	80.23^{c} ± 5.30	6.61
GP – III	80.22			80.98		

*LOD: Limit of detection.
** N.D: Not detected.
*** N.A: Non application.
****Each value was the mean of 3 samples.

Table 13. Comparison homogeneity of two analyzing methods in tilapia muscle

Sample I.D	SWV (LOD = 0.52 µg*kg^{-1})			LC-MS/MS (LOD = 10 µg*kg^{-1})		
	Result (mg*kg^{-1})	Mean ± SD (mg*kg^{-1})	R.S.D (%)	Result (mg*kg^{-1})	Mean ± SD (mg*kg^{-1})	R.S.D (%)
TL – Blank	N.D	N.A	N.A	N.D	N.A	N.A
TL – Blank	N.D			N.D		
TL – Blank	N.D			N.D		
TL – I	1.31			1.23		
TL – I	1.27	1.29^{a} ± 0.02	1.61	1.28	1.26^{a} ± 0.03	2.10
TL – I	1.30			1.27		
TL – II	1.95			3.14		
TL – II	2.09	2.02^{b} ± 0.07	3.47	2.72	2.72^{b} ± 0.43	15.64
TL – II	2.02			2.29		
TL – III	2.80			2.80		
TL – III	2.80	2.78^{c} ± 0.03	1.25	2.80	2.80^{b} ± 0.01	0.21
TL – III	2.74			2.81		

*LOD: Limit of detection.
**N.D: Not detected.
***N.A: Non application.
****Each value was the mean of 3 samples.

Table 14. Linear range in regression analysis of erythromycin

Erythromycin A concentration (ppb)	50.0	100.0	200.0	300.0	400.0
Peak current (Mean ± SD)	**138.5^{a} ± 5.1**	194.0^{b} ± 3.9	305.2^{c} ± 5.2	411.2^{d} ± 1.8	524.8^{e} ± 14.3
RSD (%)	3.7	2.0	1.6	0.4	2.7

* Each value was the mean of 5 samples.

Table 15. Precision (RSD %), accuracy (RE %) and recovery of erythromycin A in giant freshwater prawn muscles

Day	Spike level (µg*kg^{-1})	Measured concentration (mean ± SD, µg*kg^{-1})	RSD (%)	RE (%)
1	100	91.45^{a} ± 1.44	1.58	91.45
2	100	90.40^{a} ± 1.25	1.38	90.40
3	100	90.92^{a} ± 1.43	1.57	90.92
1	200	187.23^{b} ± 2.79	1.49	93.61
2	200	184.76^{b} ± 1.97	1.07	92.38
3	200	185.18^{b} ± 2.43	1.31	92.59
1	300	286.03^{c} ± 4.01	1.40	95.34
2	300	288.27^{c} ± 2.71	0.94	96.09
3	300	289.50^{c} ± 2.65	0.91	96.50

Table 16. Precision (RSD %), accuracy (RE %) and recovery of erythromycin A in tilapia muscles

Day	Spike level (µg*kg^{-1})	Measured concentration (mean ± SD, µg*kg^{-1})	RSD (%)	RE (%)
1	100	85.07^{a} ± 1.26	1.48	85.07
2	100	85.34^{a} ± 1.79	2.10	85.34
3	100	85.31^{a} ± 1.24	1.45	85.31
1	200	173.25^{b} ± 2.34	1.35	86.63
2	200	173.03^{b} ± 2.09	1.21	86.51
3	200	172.82^{b} ± 1.39	0.80	86.41
1	300	261.62^{c} ± 4.53	1.73	87.21
2	300	262.57^{c} ± 3.42	1.30	87.52
3	300	265.68^{c} ± 5.38	2.02	88.56

* Each value was the mean of 6 samples.

Table 17. Erythromycin residue of prawn samples from ten provinces, three districts in each province in the Mekong River Delta

			Giant Freshwater Prawn						
No	**Province**	**District**	**M1**	**M2**	**M3**	**M4**	**M5**	**Mean (μg*kg^{-1})**	**RSD (%)**
1	Dong Thap	Cao Lanh	169.00	164.00	171.00	159.00	162.00	165.00^{f}	3.00
		Tam Nong	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Thap Muoi	N.D	N.D	N.D	N.D	N.D	N.D	N.D
2	Kien Giang	Go Quao	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Giong Rieng	49.60	52.10	48.90	51.00	51.30	50.58^{d}	2.58
		Vinh Thuan	232.40	235.10	230.20	228.70	229.50	231.18^{g}	1.12
3	An Giang	Thoai Son	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Long Xuyen	626.10	630.40	628.90	631.80	627.30	628.90^{i}	0.36
		Phu Tan	N.D	N.D	N.D	N.D	N.D	N.D	N.D
4	Vinh Long	Tam Binh	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Binh Minh	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Long Ho	N.D	N.D	N.D	N.D	N.D	N.D	N.D
5	Ben Tre	Mo Cay	18.00	18.70	19.20	17.90	18.60	18.48^{b}	2.90
		Chau Thanh	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Giong Trom	N.D	N.D	N.D	N.D	N.D	N.D	N.D
6	Tra Vinh	Duyen Hai	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Tra Cu	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Cang Long	N.D	N.D	N.D	N.D	N.D	N.D	N.D
7	Hau Giang	Phung Hiep	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Long My	531.70	529.50	533.10	528.90	541.00	532.84^{h}	0.91
		Vi Thuy	N.D	N.D	N.D	N.D	N.D	N.D	N.D
8	Soc Trang	My Tu	38.90	40.00	39.30	39.60	38.90	39.34^{c}	1.20
		My Xuyen	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Ke Sach	N.D	N.D	N.D	N.D	N.D	N.D	N.D
9	Can Tho	Vinh Thanh	129.30	130.10	128.90	128.20	130.40	129.38^{e}	0.69
		O Mon	10.50	10.30	11.10	10.70	10.90	10.70^{a}	2.96
		Co Do	N.D	N.D	N.D	N.D	N.D	N.D	N.D
10	Bac Lieu	Phuoc Long	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Hong Dan	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Gia Rai	N.D	N.D	N.D	N.D	N.D	N.D	N.D

* N. D: Not dedected

Table 18. Erythromycin residue of tilapia samples from ten provinces, three districts in each province in the Mekong River Delta

			Tilapia						
No	**Province**	**District**	**M1**	**M2**	**M3**	**M4**	**M5**	**Mean (μg*kg^{-1})**	**RSD (%)**
1	Dong Thap	Cao Lanh	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Tam Nong	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Thap Muoi	N.D	N.D	N.D	N.D	N.D	N.D	N.D
2	Kien Giang	Go Quao	289.4	287.5	290.3	279.9	280.8	285.6^{d}	1.71
		Giong Rieng	175.2	177.9	176.3	175.9	177.8	176.6^{c}	0.67
		Vinh Thuan	N.D	N.D	N.D	N.D	N.D	N.D	N.D
3	An Giang	Thoai Son	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Long Xuyen	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Phu Tan	N.D	N.D	N.D	N.D	N.D	N.D	N.D
4	Vinh Long	Tam Binh	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Binh Minh	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Long Ho	N.D	N.D	N.D	N.D	N.D	N.D	N.D
5	Ben Tre	Mo Cay	713.0	719.2	720.0	715.8	716.9	716.9^{f}	0.39
		Chau Thanh	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Giong Trom	72.5	73.1	72.8	74.0	72.7	73.0^{b}	0.81
6	Tra Vinh	Duyen Hai	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Tra Cu	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Cang Long	N.D	N.D	N.D	N.D	N.D	N.D	N.D
7	Hau Giang	Phung Hiep	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Long My	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Vi Thuy	N.D	N.D	N.D	N.D	N.D	N.D	N.D
8	Soc Trang	My Tu	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		My Xuyen	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Ke Sach	N.D	N.D	N.D	N.D	N.D	N.D	N.D
9	Can Tho	Vinh Thanh	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		O Mon	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Co Do	45.6	47.1	44.9	45.2	45.0	45.6^{a}	1.98
10	Bac Lieu	Phuoc Long	327.4	328.4	319.7	326.6	326.9	325.8^{e}	1.07
		Hong Dan	N.D	N.D	N.D	N.D	N.D	N.D	N.D
		Gia Rai	N.D	N.D	N.D	N.D	N.D	N.D	N.D

* N. D: Not dedected.

Voltammetry had been developed for simultaneous determination of erythromycin in giant prawn and tilapia. The proposed method was simple, quick, economical, and sensitive. It should be extensively used for veterinary drug residue screening in food surveillance programs.

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