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*Research Article*

# Determination of erythromycin in giant freshwater prawn by fast scanning stripping square wave voltammetry at a slowly-dropping mercury electrode

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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*) in particular while exporting to the US, EU, Japan, Canada. There are many methods used for analyzing this antibiotic in giant freshwater prawns such as ELISA, HPLC, LC-MS/MS, GC-MS, etc. However, these methods require costly equipment, long time analysis, as well as pure analytical solutions. A fast scanning stripping square wave voltammetry at the slowly dropping mercury electrode was primarily developed and validated to quantify this antibiotic with simple and short time analysis, not too pure analytical solutions, not high priced equipment. 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 disolving 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 (≥ 90.40 %), high sensitivity (lower limit of detection, LoD = 0.57 µg.kg$^{-1}$) and high precision (RSD ≤ 1.58%) as well as excellent linearity ($r^2_{adjusted}$ ≥ 0.99999).

**Keywords:** aquaculture, chemicals, contamination, antibiotic, seafood export, Vietnam, *Macrobrachium rosenbergii*.

## Introduction

Giant freshwater prawn (*Macrobrachium rosenbergii*) is considered one of the most important species grown in freshwater aquaculture in Viet Nam, especially in the Mekong Delta. Prawn culture in Viet Nam is comprised of several models such as integrated and alternative culture with rice on rice paddy, semi-intensive culture in ponds and intensive culture in pens located along river/canal banks. Increasing demand for this species for domestic consumption and export markets has increased remarkably, leading to culture systems with large scale, high stocking density and intensive feeding. Hence, bacterial necrosis is a common disease observed in adult prawns and inevitable in these uncontrollable culture models.

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* and *Aeromonas sp., Pseudomonas fluorescens* [1, 2, 4, 16, 19, 22, 23].

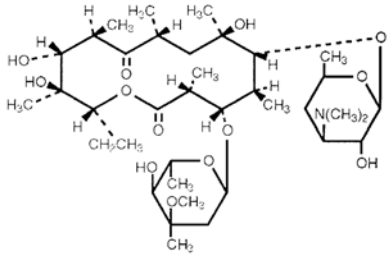

**Figure 1. Chemical structure of erythromycin A.**

According to the regulations of Codex, WHO/FAO, EU, US, Canada, Australia and Japan, erythromycin residue in seafood muscle must be lower than 100 µg/kg. Viet Nam Ministry of Agriculture and Rural Development regulated erythromycin belongs to the group of limited antibiotics with maximum residual limit 200 µg/kg. There have been numerous papers published recently concerning detection of such antibiotics in food (Table 1).

**Table 1. Some typical papers published recently.**

| Year | Author | Sample | Method | LoD (µg/kg) |
|---|---|---|---|---|
| 1994 | Zierfels G [26] | Egg, muscle, milk, liver, kidney of swan | HPLC | <10 |
| 1998 | Yong-Xi Li [25] | Human plasma | LC-MS/MS | LoQ 0.5 |
| 1999 | Kondo T [15] | Human plasma | LC-MS/MS | LoQ 0.05 |
| 2000 | Dreassi E [7] | Plasma: beef, pork, poultry | HPLC-UV | LoQ 250 |
| | | Milk | HPLC-UV | LoQ 25 |
| | | Kidney, liver, muscle, gan, fat of beef, pork, poultry. | HPLC-UV | LoQ 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 | LoQ 50 |
| | | Kidney of beef | LC-MS/MS | LoQ 80 |
| 2003 | Stanley M. Billedeau [20] | Salmon | LC – ESI/MS | LoD: 5, LoQ: 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 | LoD 1.27, LoQ 5.0 |
| 2009 | P. Norouzi [18] | Human plasma, urine. | CV | LoD 2.4, LoQ 7.0 |

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 muscle.

## Materials and Methods

### *Reagents*

The high purity antibiotic standards of erythromycin A, chloramphenicol, furazolidone, florfenicol, ciprofloxacin, colistin, malachite green were purchased from Vietnam Central Institute of Pharmacy. Methanol, acetonitrile (HPLC grade) were obtained from J. T. Baker. All reagents were analytical 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 tissue 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-cm3 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 machine in mode stripping square wave voltammetry.

## Results and Discussion

### *Voltammetric behaviour of erythromycin at the slowly dropping electrode*

*Effect of Supporting Electrolytes and pH values*

The adsorptive peak current of erythromycin has been strongly affected by the type of supporting electrolyte. To study the adsorptive behaviour of erythromycin, different supporting electrolytes including sodium acetate, ammonium acetate, citrate-phosphate, borax, Tris buffers were examined. Ammonium acetate buffer was recommended to complete these studies where erythromycin showed the highest peak current and the best peak shape (Table 2 and Figure 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 investigation.

**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)*

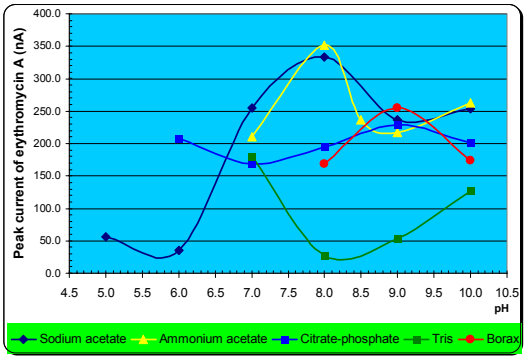


**Figure 2. Effect of supporting electrolytes, pH values on peak current of erythromycin A.**

*Effect of the ionic strength of supporting electrolyte*

This 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 and Figure 3).

**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)

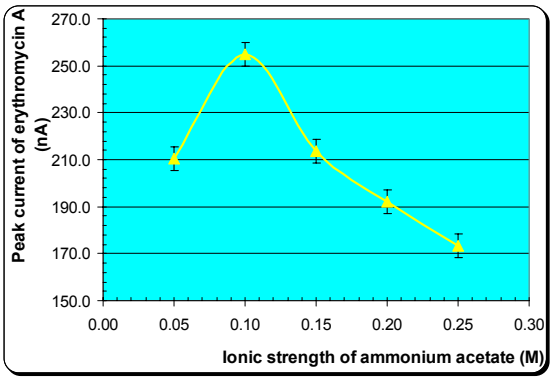


**Figure 3. Effect of ionic strength of ammonium acetate on peak current of erythromycin A**

*Effect of the solvents*

For dissolving erythromycin A standard on the peak current were examined. Among methanol, acetonitril, 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 and Figure 4).

**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)

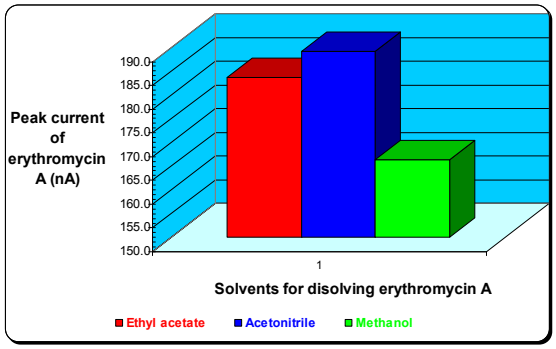


**Figure 4. Effect of solvents for dissolving erythromycin A on peak current.**

***Optimization of measurement conditions***

*Effect of forward scanning (0 to -1800 mV) and reverse scanning (-1800mV to 0)*

The forward scanning (0 to -1800 mV) showed a 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, $V_{pulse}$: 30mV, $T_{drop}$: 3000ms, $V_{electrolise}$: -700mV, $T_{electrolise}$: 6s, $T_{stabilize}$: 1s. Examining $V_{start}$ from -400 mV to -1100 mV. $V_{start}$ was optimum at -400mV ($E_{1/2}$ = -1430 mV, I = 106.5 ± 4.7 nA) (Table 5 and Figure 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 ± | **106.5$^{g}$ ±** | 97$^{f}$ ± | 79.5$^{e}$ ± | 71.5$^{d}$ ± | 93.3$^{f}$ ± | 45.0$^{c}$ ± | 23.4$^{b}$ ± | 14.8$^{a}$ ± |
| SD | **4.7** | 3.4 | 0.8 | 4.3 | 4.0 | 2.6 | 1.0 | 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)

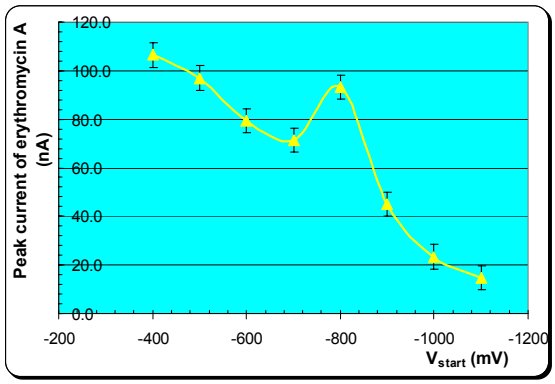


**Figure 5. Effect of $V_{start}$ on erythromycin A peak current.**

*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 and Figure 6).

**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)

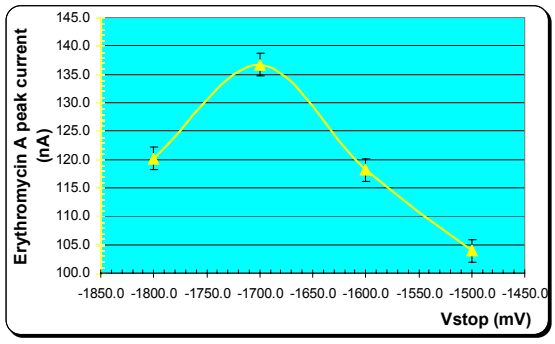


**Figure 6. Effect of $V_{stop}$ on erythromycin A peak current.**

*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 and Figure 7).

**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)

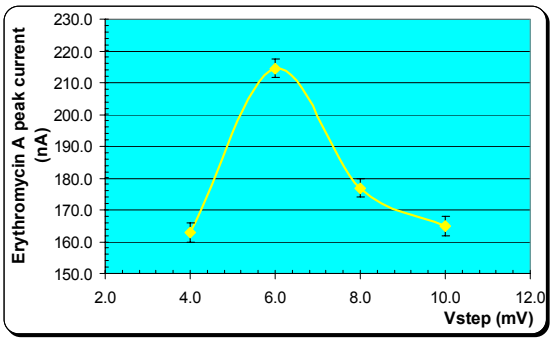


**Figure 7. Effect of $V_{step}$ on erythromycin A peak current.**

*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 and Figure 8).

**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)

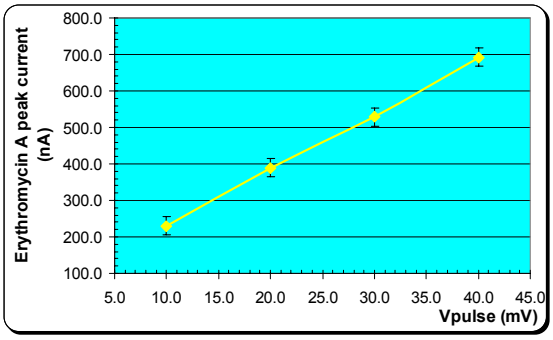


**Figure 8. Effect of $V_{pulse}$ on erythromycin A peak current.**

*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 and Figure 9).

**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)

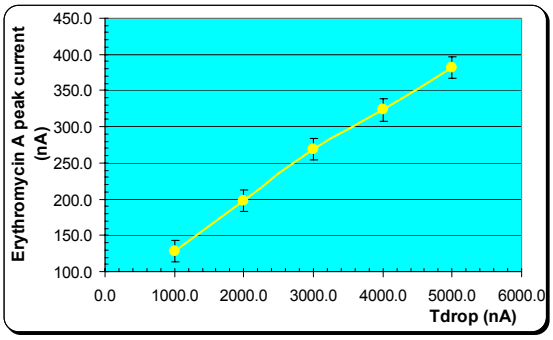


**Figure 9. Effect of $T_{drop}$ on erythromycin A peak current.**

*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. $\mathbf{T}_{electrolise}$ was optimum at **5s** ($E_{1/2}$ = -1430 mV, I = 1717.0 ±13.7 nA) (Table 10 and Figure 10).

**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)

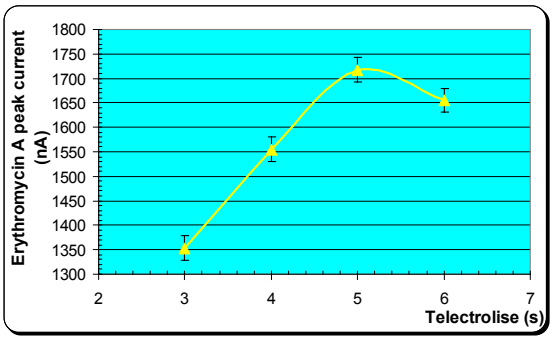


**Figure 10. Effect of $T_{electrolise}$ on erythromycin A peak current.**

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

**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)

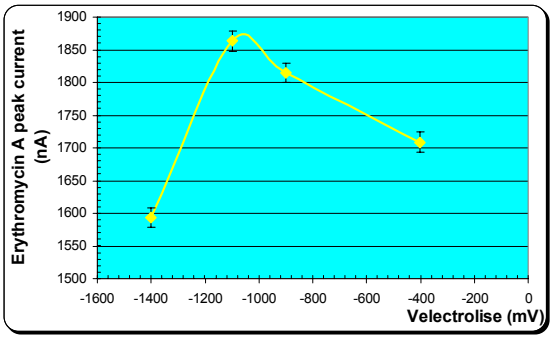


**Figure 11. Effect of $V_{electrolise}$ on erythromycin A peak current.**

## *Calibration*

*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, $V_{start}$: -400mV, $V_{stop}$: -1700 mV, $V_{step}$: 6 mV, $V_{pulse}$: 40 mV, $T_{drop}$: 5000 ms, $T_{electrolise}$: 5s, $V_{electrolise}$: - 1100 mV, $T_{stabilize}$: 1s.

A detection limit of 0.57 µg/kg was obtained for erythromycin. A linear behaviour was also observed with a correlation coefficient $r^2_{adjust}$ = 1.0 (Figure 12).

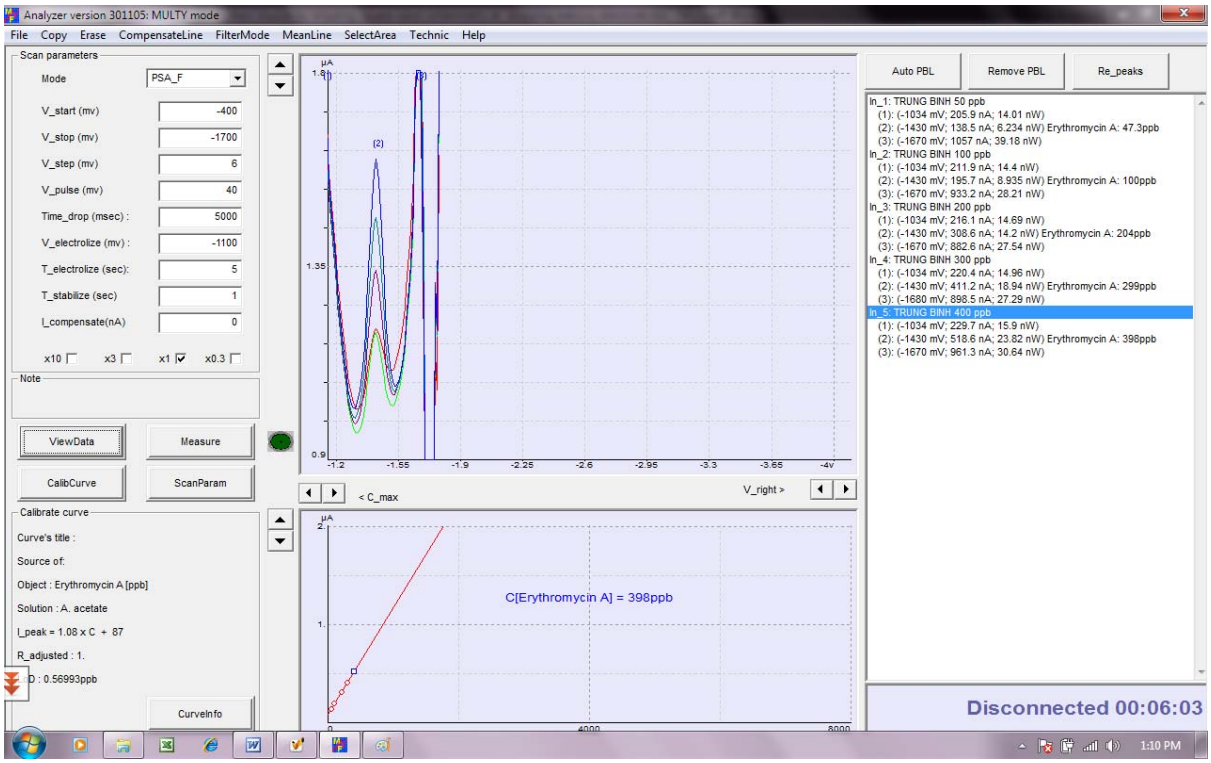


**Figure 12. Calibration curve of erythromycin A.**

***Interference***

*Effect of interferences*

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

***Sample analysis***

*In giant freshwater prawn samples*

Recovery rate: 90.40 ÷ 96.50 %, LoD: 0.80 µg.kg$^{-1}$, $R^2_{adjust}$: 0.99999, RSD: 0.91 ÷ 1.58 %. (Figure 13).

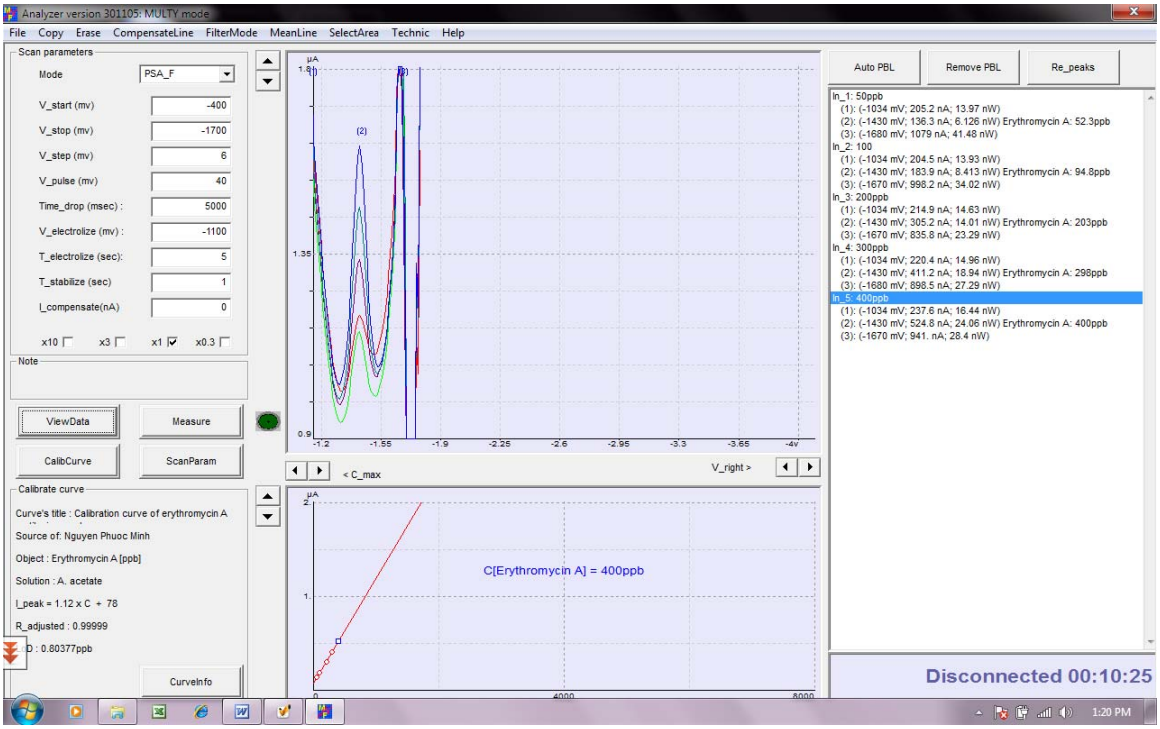


**Figure 13. Calibration curve of erythromycin A in giant prawn muscle.**

*Comparison of results between SWV and LC-MS/MS methods*

Incurred giant prawn 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 incurred 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. A closely homogeneity could be obviously seen in comparison between the two methods of analysis (Table 12).

**Table 12. Comparison of homogeneity of two analyzing methods.**

| 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.D | | |
| GP – Blank | N.D | N.A | N.A | N.D | N.A | N.A |
| 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

### *Validation of quantification method*

*Linearity*

The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. 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 following linear regression analysis (Table 12). Typically, the regression equation for the calibration curve was found to be Y=1.08*X + 87.

**Table 13. 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

*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 approved 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 13).

*Precision, Accuracy & 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 at

erythromycin A, concentrations of 100, 200, 300 µg/kg and the mean value was calculated (Table 14 and Figure 14).

**Table 14. 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 |

* Each value was the mean of 6 samples

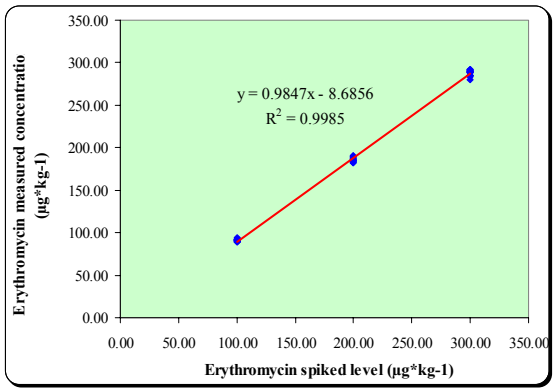


**Figure 14. Erythromycin concentration was detected on prawn samples at different times and levels.**

*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 samples from ten provinces in the Mekong River Delta were taken and analyzed to survey the erythromycin residue. Residual results could be obviously seen in Table 15 and Figure 15.

**Table 15. Erythromycin residue of prawn samples from ten provinces, three districts in each province in the Mekong River Delta**

| No. | Province | District | Giant Freshwater Prawn | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 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 |

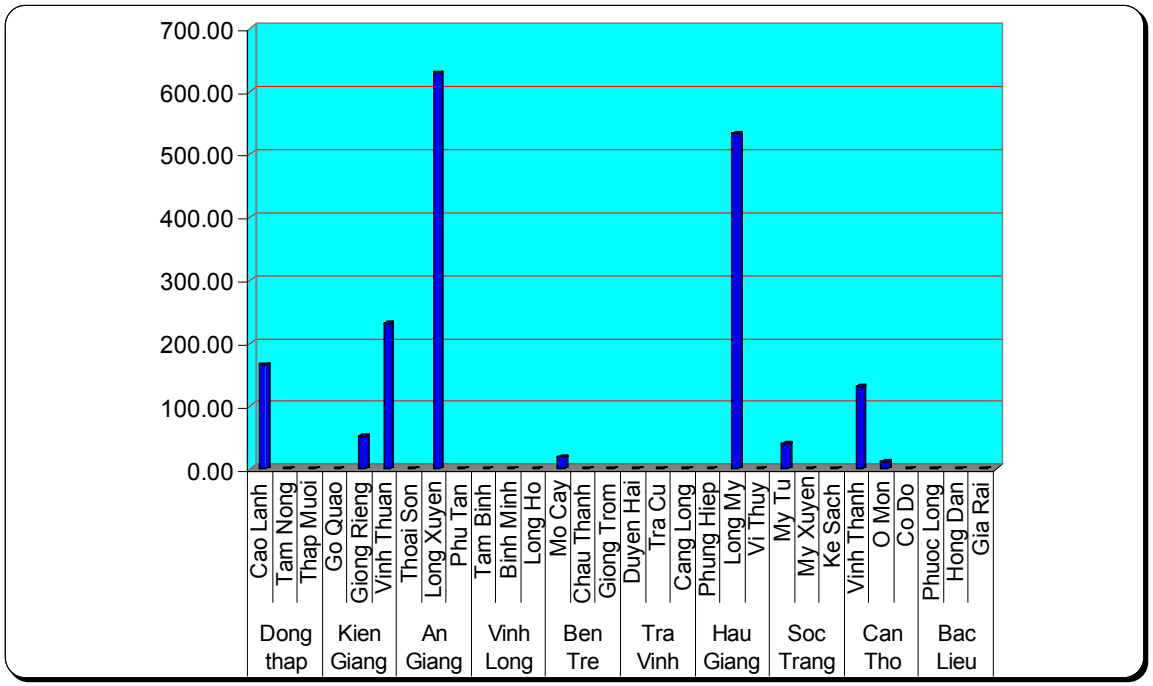


**Figure 15. Erythromycin surveillance in prawn aquaculture at ten provinces, three districts in each province of Mekong River Delta, Viet Nam.**

## Conclusion

A fast scanning stripping square wave voltammetry at the slowly dropping mercury electrode (PSA-F) was achieved in ammonium buffer for determination of erythromycin A with limit of detection – LoD 0.57 µg/kg, recovery 90.40 ÷ 96.50 %, method detection limit - MDL 0.80 µg/kg. The analysing results by PSA-F also compared with LC-MS/MS method. It provides high selectivity, sensitivity, precision and accuracy to quantify erythromycin A.

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