Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol.11, pp.129-140, December, 2010

ISSN: 0975-833X

RESEARCH ARTICLE

A FAST SCANNING STRIPPING SQUARE WAVE VOLTAMMETRY ANALYSIS OF ERYTHROMYCIN A IN TILAPIA (*Oreochromis niloticus)* WITH THE DROPPING MERCURY ELECTRODE

Nguyen Phuoc Minh

Department of Food Technology, Ho Chi Minh City University of Technology, 268 Ly Thuong Kiet Street, District 10, Ho Chi Minh City, Vietnam

ARTICLE INFO

Article History:

Received 18th September, 2010
Received in revised form
17th October, 2010
Accepted 15th November, 2010
Published online 16th December, 2010

Key words:

Erythromycin A;
Tilapia;
Stripping square wave voltammetry;
Dropping mercury electrode.

ABSTRACT

A new sensitive analytical approach for determination of erythromycin A in tilapia (*Oreochromis niloticus)* by a fast scanning stripping square wave voltammetry using the dropping mercury electrode (PSA-F) was developed and validated. Optimum parameters for erythromycin A quantification were: Ammonium acetate buffer 0.1 M, pH 8.0 as supporting electrolyte; acetonitrile as the solvents for disolving erythromycin standard; forward scanning; V_{start}: -400 mV; V_{stop}: -1700 mv; V_{step}: 6 mV; V_{pulse}: 40 mV; T_{dop}: 5000 ms; $V_{electrolide}$: -1100 mV; $T_{electrolide}$: 5s. Peak of erythromycin A appeared at $E_{1/2}$ = -1430 mV, separated and distinguished with peaks of other antibiotics. This assay showed high recovery (> 85.07 %), high sensitivity (detection limit 0.52 µg/kg), high precision (RSD, 0.8 ÷ 2.1 %), high accuracy (relative error - RE, 85.07÷ 88.56 %) as well as excellent linearity ($r^2_{adjusted}$ = 1.0). Simpler, reagent-saving and time-saving were other advantages of this assay method. An equivalence of analyzing results between PSA-F and LC-MS/MS could be obviously seen.

INTRODUCTION

Tilapias are known to have been an important component of fisheries in the Mekong River Delta. The most significant diseases in Nile tilapia (*Oreochromis niloticus)* culture are caused by *Streptococcus iniae, Aeromonas* hydrophila**,** Trichodina *sp.***,** Flexibacter *Columnaris, Edwardsiella* spp. *Streptococcocus* spp, gram positive bacteria, have become a major problem for tilapia farmers and there is still no effective commercial vaccine available that can be used to prevent *Streptococcocus* spp. in tilapia. They can cause mass death in tilapia farms, and unlike many other tilapia diseases it will affect even large and otherwise healthy fish. The macrolide antibiotic erythromycin has long been the chemotherapeutant of choice to prevent and control *Streptococcocus* spp. Erythromycins are broad spectrum antibiotics that exhibit high activity against nearly all Gram positive and Gram-negative bacteria. Erythromycin

***Corresponding author:** *stapimex@gmail.com*

A consists of a polyhydroxylactone and two sugars. The aglycone part of all erythromycin molecules, the erythronolide, is a 14-membered lactone ring (Fig. 1). Depending on the type of erythromycin this lactone ring is substituted via 4-position with a cladinose in case of erythromycin A, erythromycin B, erythromycin E, erythromycin F and with a mycarose in case of erythromycin C and erythromycin D. All erythromycin molecules contain the aminosugar d-desosamine, which is β-glycosidic linked to the 6 position of the lactone ring. The minimum inhibited concentration of erythromycins A, B, C, and D and some of their derivatives were determined against 21 gram-positive and 15 gram-negative microorganisms. Antibacterial activity was confined to gram-positive and very few gram-negative bacteria. Erythromycin B was somewhat less active than erythromycin A, and erythromycin C and D showed about half that activity or even less (Isaac Ongubo Kibwage *et al.,* 1985). Owing to their extensive use in infectious disease therapy, several procedures have been reported for its determination (Table 1). The aim of this work was to determine the feasibility of square wave voltammetry for the direct detection and quantification of erythromycin A in tilapia tissue.

MATERIALS AND METHODS

Reagents and materials

All chemicals and reagents were HPLC grade or p.a. Double-distilled water (DDW) was used throughout the study. The high purity antibiotic standards (>99%) of erythromycin A, erythromycin A, chloramphenicol, furazolidone, florfenicol, ciprofloxacin, colistin, malachite green were purchased from Vietnam Central Institute of Pharmacy.

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 behavior of erythromycin at the slowly dropping electrode

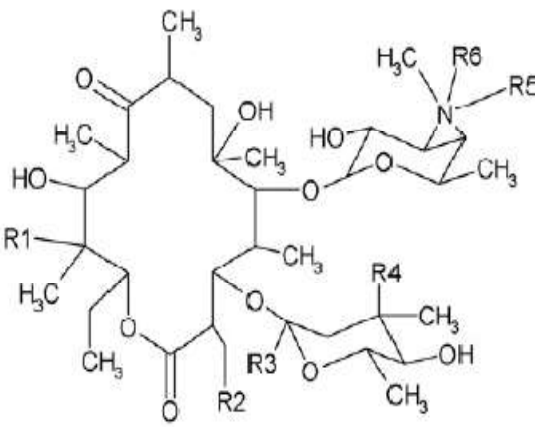
Effect of Supporting Electrolytes and pH values.

The adsorptive peak current of erythromycin has been strongly affected by the type of supporting electrolyte. To study the adsorptive behavior of erythromycin, different supporting electrolytes including sodium acetate, ammonium acetate, citrate-phosphate, borax, Tris buffers were

erythromycin showed the highest peak current and the best peak shape (Table 2 and Fig. 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.

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 [illegible]w. So the forward



Erythromycin	Formula	Molecular mass	R_1	R_2	R_3	R_4	R_5
A	$C_{37}H_{57}NO_{13}$	734	OH	H	H	OCH_3	CH_3
B	$C_{37}H_{57}NO_{12}$	718	H	H	H	OCH_3	CH_3
C	$C_{38}H_{55}NO_{13}$	720	OH	H	H	OH	CH_3
D	$C_{36}H_{65}NO_{12}$	704	H	H	H	OH	CH_3
E	$C_{37}H_{67}NO_{13}$	748	OH	-O-		OCH_3	CH_3
F	$C_{37}H_{67}NO_{14}$	750	OH	OH	H	CH_3	CH_3

Figure 1. Structural formula of erythromycin A and related substances

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 and Figure 3). Effect of the solvents for disolving 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). 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 -400mV to -1100mV. V_{start} was optimum at -400mV ($E_{1/2}$ = -1430 mV, I = 106.5 ± 4.7 nA; Table 5 and Fig. 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 ($E_{1/2}$ = - mV, V_{stop}: -1700 mV, V_{step}: 6.0 mV, T_{drop}: 3000

Table 1. Some typical papers published in recently

Author	Sample	Method	LoD (µg/kg)
Zierfels and Petz, 1994	Egg, muscle, milk, liver, kidney of swan	HPLC	<10
Yong-Xi Li *et al.,* 1998	Human plasma	LC-MS/MS	LoQ 0.5
Kondo *et al*., 1999	Human plasma	LC-MS/MS	LoQ 0.05
Dreassi *et al.,* 2000	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
Huaisheng Wang *et al.,* 2000	Drug, urine.	ASV-PGCE	5
Carmen Leal *et al.,* 2001	Chicken	LC-FL	400
Draisci *et al.,* 2001	Beef	ELISA	0.4
	Muscle and liver of beef	LC-MS/MS	LoQ 50
	Kidney of beef	LC-MS/MS	LoQ 80
Stanley M. Billedeau *et al., 2003*	Salmon	LC - ESI/MS	LoD: 5, LoQ: 16
Horie Masakazu *et al.,* 1999.	Meat and seafood	LC- ESI-MS	10
Michael P. Sche *et al.,* 2003	Manure	HPLC-MS/MS	0.4-11
Xiao *et al.*, 2005	Drugs (propionate, base)	HPLC–ESI-MS	1
Deube *et al.,* 2006	Muscle	LC-MS/MS	0.25
Hui Yun – Hua *et al.,* 2006	Tilapia	HPLC	400
Jian Wang *et al.,* 2006	Fresh milk	LC-ESI/MS/MS	0.07
Tang *et al*., 2006	Meat	LC-MS/MS	0.1
Deng *et al.,* 2007	Rat plasma	ECL	0.35
Berrada Houda *et al.,* 2008	Meat and seafood	LC-ESI/MS	25
Granja *et al.,* 2009	Honey	LC-MS/MS	LoD 1.27, LoQ 5.0
P. Norouzi *et al.,* 2009	Human plasma, urine.	CV	LoD 2.4, LoQ 7.0

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
					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
Tris							168.1[a] ± 1.6	0.9	255.5[c] ± 6.5	2.6	173.9[b] ± 1.8	1.0
Borax												

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

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 Fig. 7). Effect of V_{pulse}. Mode PSA-F, forward scanning, V_{start}: -400 optimum at 40 mV ($E_{1/2}$ = -1430 mV, I = 692.6 ± 14.9 nA) (Table 8 and Fig. 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)

mV, V_{step}: 6 mV, V_{pulse}: 40 mV, T_{drop}: 5,000 ms, $V_{electrolise}$: -700 mV, $T_{stabilize}$: 1s. Examining $T_{electrolise}$ from 3s to 6s. $T_{electrolise}$ was optimum at 5s ($E_{1/2}$ = -1430 mV, I = 1717.0 ±13.7 nA) (Table 10 and Fig. 10). Effect of $V_{electrolise}$ Mode PSA-F, forward scanning, V_{start}: -400 mV, V_{stop}: -1700 mV, V_{step}:6mV, 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 Fig. 11).

Calibration

Calibration curves and detection Limit: A 25mL supporting electrolyte ammonium acetate 0.1M, pH 8.0 was transferred to the cell and spiked with 5µL, 10µL, 20µL, 30µL, 40µL, 50µL, 60µL, 7µL, 80µL, 90µL of stock 250ppm solution of erythromycin in pure acetonitril. The concentrations of erythromycin in the cell were 50 µg/kg, 100µg/kg, 200µg/kg, 300µg/kg, 400µg/kg, 500µg/kg, 600µg/kg, 700µg/kg, 800µg/kg, 90µg/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 behavior was also observed with a correlation coefficient r^2_{adjust} = 1.0 (Fig. 12).

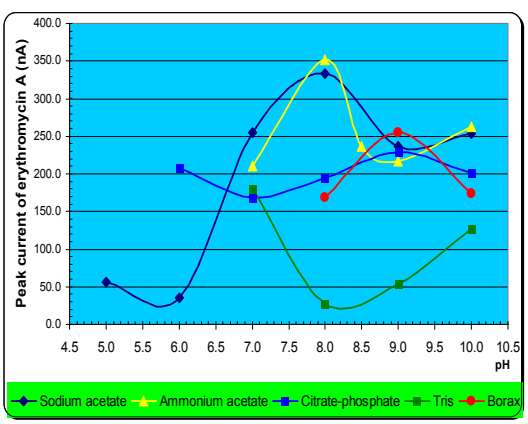


Figure 2. Effect of supporting electrolytes, pH values on peak current of erythromycin A

ions was examined by introducing different

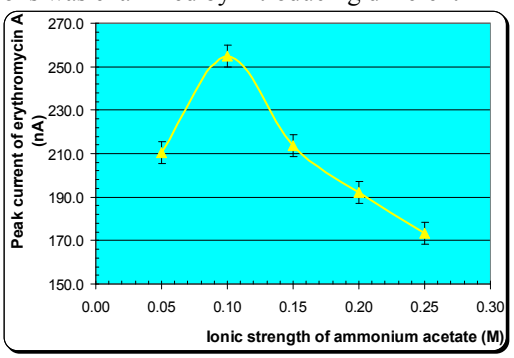


Figure 3. Effect of ionic strength of ammonium acetate on peak current of erythromycin A

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)*

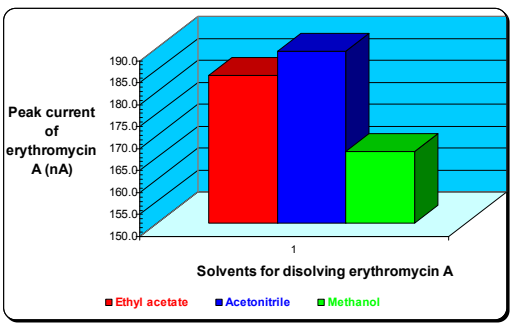
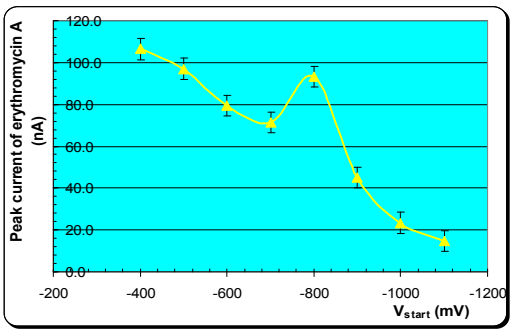


Fig. 4. Effect of solvents for disolving erythromycin A on peak current



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.

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)*

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)*

Sample analysis

In tilapia samples: Recovery rate: 85.07 ÷ 88.56%, MDL: 0.52 µg.kg^{-1},R_adjust: 1.0, RSD: 0.80 ÷ 2.10 %. (Fig. 13). Comparison results between

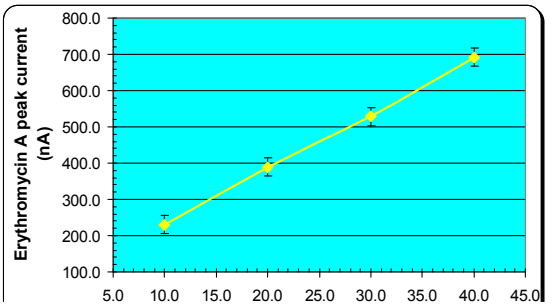


Fig. 8. Effect of V_{pulse} on erythromycin A peak current

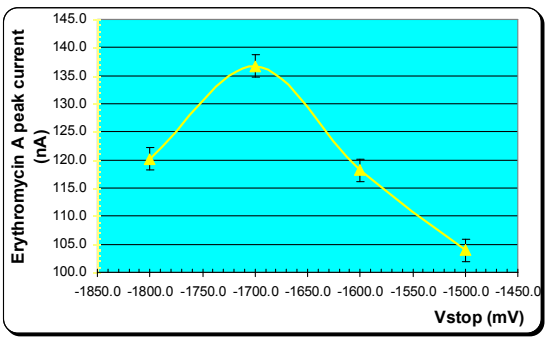


Fig. 6. Effect of V_{stop} on erythromycin A peak current

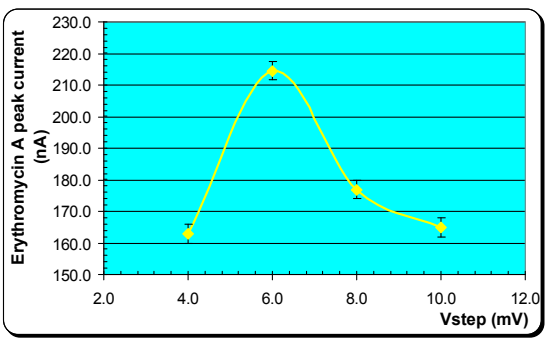


Fig. 7. Effect of V_{step} on erythromycin A peak current

Incurred tilapia samples were obtained through medication at 100 mg erythromycin·kg^{-1} fish body weight^{-1}·d^{-1} for 7 days; sampled at 7, 8 and 9 days post-dosing. These incurred samples (high, medium, and 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

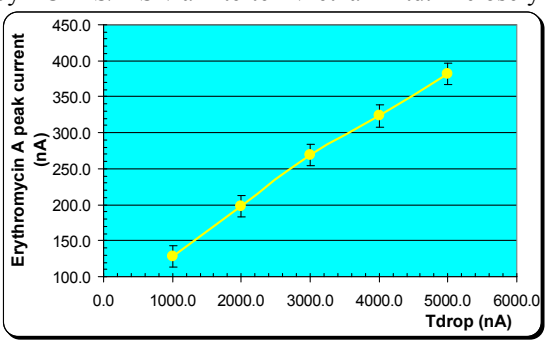


Fig. 9. Effect of T_{drop} on erythromycin A peak current

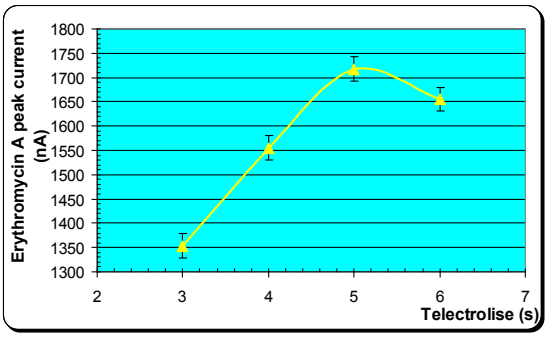


Fig.10. Effect of $T_{electrolise}$ on erythromycin A peak current

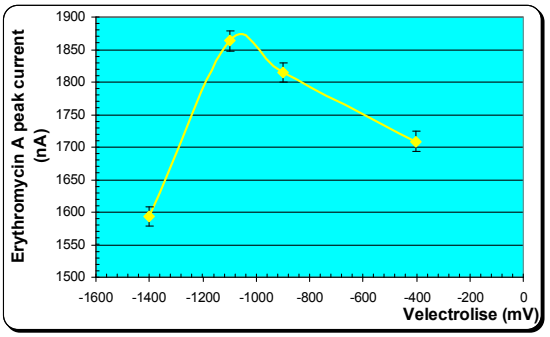


Fig.11. Effect of $V_{electrolise}$ on erythromycin A peak current

homogeneity could be obviously seen in comparison between two analyzing methods. SWV seems to be superior and more accurate to LC-MS/MS (Table 12)

Validation of quantification method

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 = 1.0 with %R.S.D. values

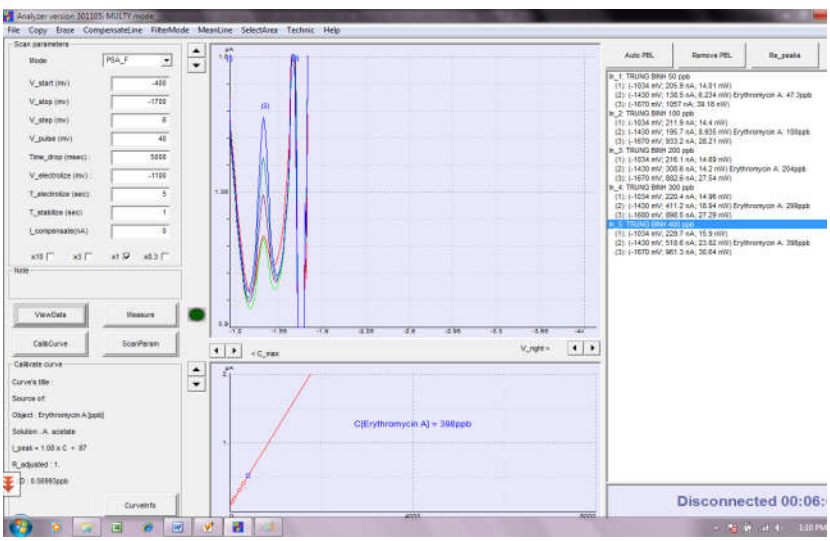
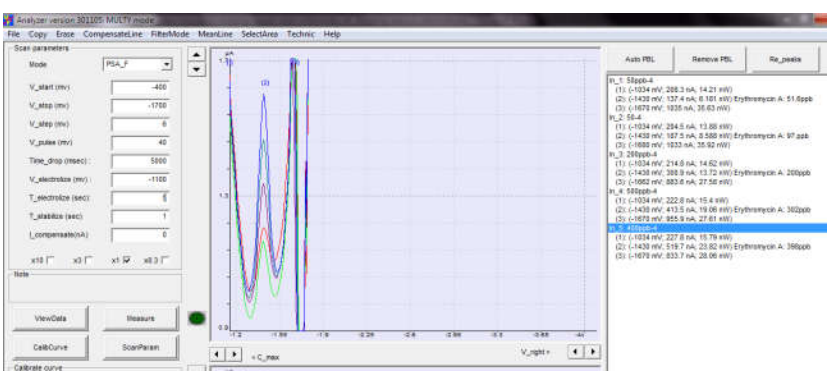


Fig.12. Calibration curve of erythromycin A



range studied were obtained following linear regression analysis (Table 13). Typically, the regression equation for the calibration curve was found to be Y=1.08*X + 87.

LOD

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-

Table 12. Comparison homogeneity of two analyzing methods

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	1.29^{a} ± 0.02	1.61	1.23	1.26^{a} ± 0.03	2.10
TL – I	1.27			1.28		
TL – I	1.30			1.27		
TL – II	1.95	2.02^{b} ± 0.07	3.47	3.14	2.72^{b} ± 0.43	15.64
TL – II	2.09			2.72		
TL – II	2.02			2.29		
TL – III	2.80	2.78^{c} ± 0.03	1.25	2.80	2.80^{b} ± 0.01	0.21
TL – III	2.80			2.80		
TL – III	2.74			2.81		

**LOD: Limit of detection; **N.D: Not detected*

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*

Table 14. 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

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).

Differentiation

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 ($E_{1/2}$ = -1152 mV), florfenicol ($E_{1/2}$ = -78 mV),

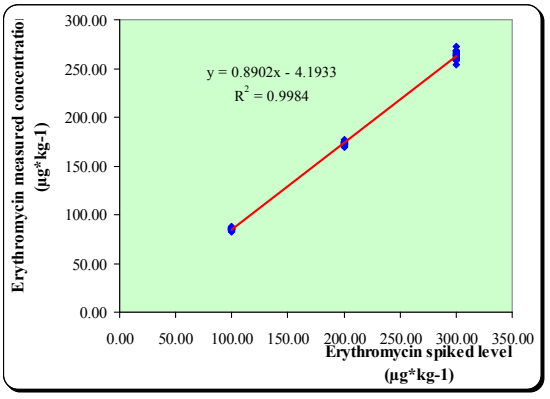


Fig. 14. Erythromycin concentration was detected on fish samples at different times and levels

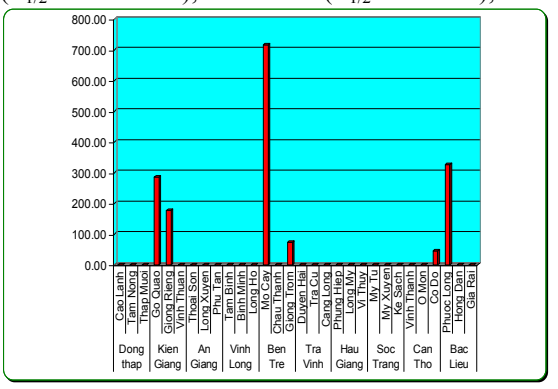


Fig. 15. Erythromycin surveillance in tilapia aquaculture at ten provinces, three districts in each province of Mekong River Delta, VN

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

No	Province	District	Tilapia						
			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

enrofloxacin, ciprofloxacin ($E_{1/2}$ = -1336 mV), colistin ($E_{1/2}$ = -1120 mV) malachite green ($E_{1/2}$ = -1228 mV). Hence, the determination of

Application

Tilapia 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 the Table 15 and Figure 15.

Conclusion

A new analytical procedure based Square Wave Voltammetry had been developed for determination of erythromycin in tilapia. The proposed method was simple, quick, economical, and sensitive. It should be extensively used for veterinary drug residue screening in food surveillance programs.

Acknowledgements

This work was supported by Dr Tran Bich Lam – HCM University of Technology, Dr Nguyen Trong Giao – Viet Nam_Russia Tropical Center, South Branch, Viet Nam

REFERENCES

Carmen Leal, Rosa Codony, Ramon Compañó, Mercè Granados and M. Dolors Prat, 2001. Determination of macrolide antibiotics by liquid chromatography. *Journal of Chromatography A*, 910: 285-290.

Deng B, Kang Y, Li X and Xu Q. 2007. Determination of Erythromycin in rat plasma with capillary electrophoresis-electro chemiluminescence detection of tris (2, 2'-bipyridyl) ruthenium (II). *Journal of chromatography B*, 857: 136-141.

Deubel A, Fandiño AS, Sörgel F and Holzgrabe U. 2006. Determination of Erythromycin and related substances in commercial samples using liquid chromatography/ion trap mass spectrometry. *Journal of Chromatography A*, 1136: 39-47.

Draisci R, Delli Quadri F, Achene L, Volpe G, Palleschi L and Palleschi G. 2001. A new electrochemical enzyme-linked immunosorbent 1946.

Dreassi E, Corti P, Bezzini F and Furlanetto S. 2000. High-performance liquid chromatographic assay of erythromycin from biological matrix using electrochemical or ultraviolet detection. *Analyst.,* 125: 1077-1081.

Granja R, Niño AM, Zucchetti R, Niño RM, Patel R and Salerno AG. 2009. Determination of erythromycin and tylosin residues in honey by LC-MS/MS. *J AOAC Int.*, 92: 975-980.

Horie Masakazu, Ishii Rie, Yoshida Terumitsu, Hoshino Yoji and Nakazawa Hiroyuki, 1999. Determination of erythromycin and oleandomycin in meat and fish by LC/MS. *Journal of the Food Hygienic Society of Japan*, 40: 309-313.

Houda Berrada, Francesc Borrull, Guillermina Font and Rosa Maria Marcé, 2008. Determination of macrolide antibiotics in meat and fish using pressurized liquid extraction and liquid chromatography–mass spectrometry. *Journal of Chromatography A*, 1208: 83-89.

Huaisheng Wang, Aimei Zhang, Hui Cui, Daojie Liu and Renmin Liu, 2000. Adsorptive stripping voltammetric determination of erythromycin at a pretreated glassy carbon electrode. *Microchemical Journal*, 64: 67-71.

Hui Yun-Hua, Yu Hui-juan, Cai You—Xiong and Zhou Pei—Ge 2006. Residual determination of erythromycin in tilapia by high performance liquid chromatography. *Marine Fisheries*, 28: 321-325.

Isaac Ongubo Kibwage,Jos Hoogmartens, Eugene Roets, Hubert Vanderhaeghe, Ludo Verbist, Maurice Dubost,V Claude Pascal, Pierre Petitjean, and Georgette Levol, 1985. Antibacterial activities of erythromycins A, B, C, and D and some of their derivatives. *Antimicrobial Agents and Chemotherapy*, 28: 630-633.

Jian Wang, Daniel Leung and Steven P. Lenz 2006. Determination of five macrolide antibiotic residues in raw milk using liquid chromatography−electrospray ionization tandem mass spectrometry. *Journal of Agriculture and Food Chemistry*, 54: 2873–2880.

turbo ion spray tandem mass spectrometry for quantitative analysis of a potent motilin receptor agonist, EM574, and its metabolites in human plasma. *J Chromatogr B Biomed Sci Appl.,* 29 (734): 101-112.

Masakazu Horie, Harumi Takegami, Kazuo Toya and Hiroyuki Nakazawa. 2003. Determination of macrolide antibiotics in meat and fish by liquid chromatography–electrospray mass spectrometry. *Analytica Chimica Acta*, 492: 187–197.

Michael P. Schlüsener, Kai Bester and Michael Spiteller, 2003. Determination of antibiotics such as macrolides, ionophores and tiamulin in liquid manure by HPLC-MS/MS. *Analytical and Bioanalytical Chemistry*, 375: 942–947.

Nagla. F. Galal, Safinaz G.M. Ismail, R.H.Khalil and M.K. Soliman, 2005. Studies on *Edwardsiella* infection in *Oreochromis niloticus*. *Egyptian Journal of Aquatic Research*, 31: 460 – 471.

Norouzi, P., Daneshgar, P. and Ganjali, M.R. 2009. Electrochemical evaluation of non-electroactive drug Erythromycin in trace amount at biological samples by continuous cyclic voltammetry. *Materials Science and Engineering: C*, 29: 1281-1287.

Stanley M. Billedeau, Thomas M. Heinze and Paul H. Siitonen, 2003. Liquid chromatography analysis of erythromycin A in salmon tissue by electrochemical detection with confirmation by electrospray ionization mass spectrometry. *Journal of Agriculture and Food Chemistry*, 51: 1534–1538.

Tang HP, Ho C and Lai SS. (2006). High-throughput screening for multi-class veterinary drug residues in animal muscle using liquid chromatography/tandem mass spectrometry with on-line solid-phase extraction. *Rapid Communications in Mass Spectrometry*, 20: 2565 – 2572.

Xiao W, Chen B, Yao S and Cheng Z. 2005. Simultaneous determination of Erythromycin propionate and base in human plasma by high-performance liquid chromatography–electrospray mass spectrometry. *Journal of Chromatography B*, 817: 153–158.

Yong-Xi Li, Kathy Neufeld, Jim Chastain, Alletta Curtis and Poonam Velagaleti, 1998. Sensitive determination of erythromycin in human plasma by LC-MS/MS. *Journal of Pharmaceutical and Biomedical Analysis*, 16: 961-970.

Zierfels G and Petz M. 1994. Fluorimetric determination of Erythromycin residues in

****

foods of animal origin after derivatization with FMOC and HPLC separation. *Z Lebensm Unters Forsch.*, 198: 307-312.